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(19) **United States**(12) **Patent Application Publication**
Gersbach et al.(10) **Pub. No.: US 2021/0002665 A1**(43) **Pub. Date: Jan. 7, 2021**(54) **RNA-GUIDED GENE EDITING AND GENE
REGULATION**(71) Applicant: **Duke University**, Durham, NC (US)(72) Inventors: **Charles A. Gersbach**, Durham, NC
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GA (US); **Joshua B. Black**, Durham,
NC (US)(21) Appl. No.: **16/858,689**(22) Filed: **Apr. 26, 2020****Related U.S. Application Data**(63) Continuation of application No. 14/895,316, filed on
Dec. 2, 2015, now Pat. No. 10,704,060, filed as
application No. PCT/US14/41190 on Jun. 5, 2014.(60) Provisional application No. 61/831,481, filed on Jun.
5, 2013, provisional application No. 61/839,127, filed
on Jun. 25, 2013, provisional application No. 61/904,
911, filed on Nov. 15, 2013, provisional application
No. 61/967,466, filed on Mar. 19, 2014, provisional
application No. 61/981,575, filed on Apr. 18, 2014.**Publication Classification**(51) **Int. Cl.****C12N 15/85** (2006.01)**A61K 38/46** (2006.01)**A61K 48/00** (2006.01)**C12N 9/96** (2006.01)**C07K 14/47** (2006.01)**C12N 9/22** (2006.01)**C12N 15/90** (2006.01)(52) **U.S. Cl.**CPC **C12N 15/85** (2013.01); **A61K 38/465**
(2013.01); **A61K 48/0058** (2013.01); **C12N**
9/96 (2013.01); **C07K 14/4708** (2013.01);
C12N 9/22 (2013.01); **C12N 2840/20**
(2013.01); **C12Y 301/00** (2013.01); **A61K**
48/005 (2013.01); **C07K 2319/71** (2013.01);
C12N 2740/16043 (2013.01); **C12N 2800/40**
(2013.01); **C12N 15/907** (2013.01)

(57)

ABSTRACTDisclosed herein are Clustered Regularly Interspaced Short
Palindromic Repeats (CRISPR)/CRISPR-associated (Cas)
9-based system related compositions and methods of using
said CRISPR/Cas9-based system related compositions for
altering gene expression and genome engineering. Also
disclosed herein are compositions and methods of using said
compositions for altering gene expression and genome engi-
neering in muscle, such as skeletal muscle and cardiac
muscle.**Specification includes a Sequence Listing.**

Fig. 1A

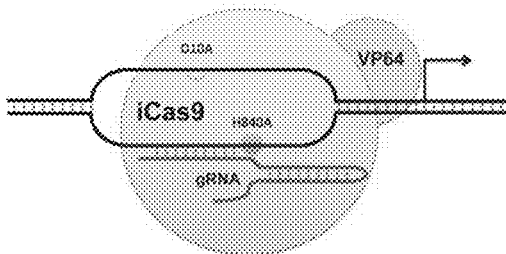


Fig. 1B

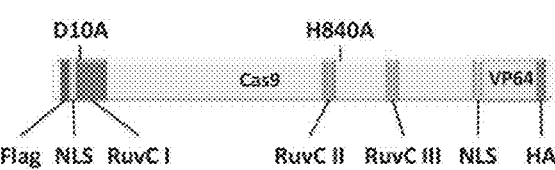


Fig. 1C

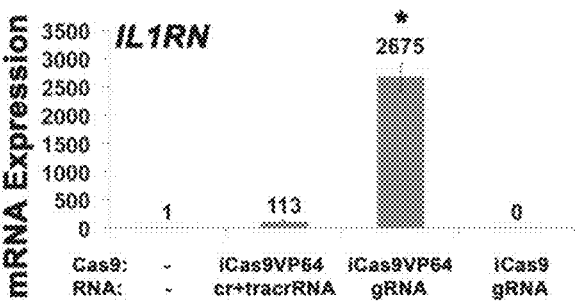


Fig. 1D

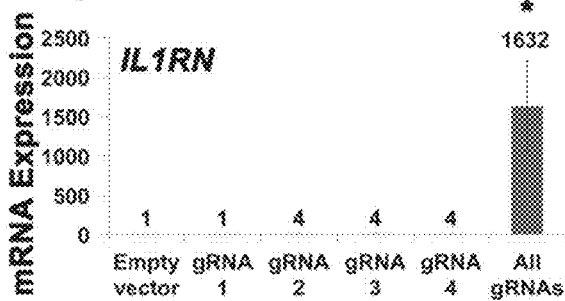


Fig. 1E

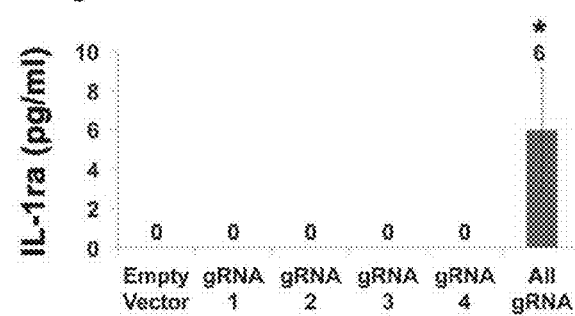
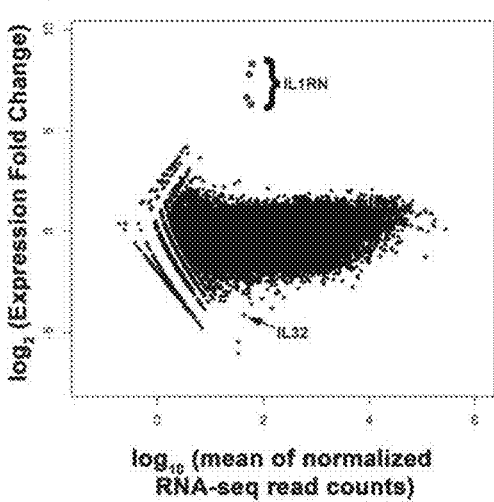


Fig. 1F



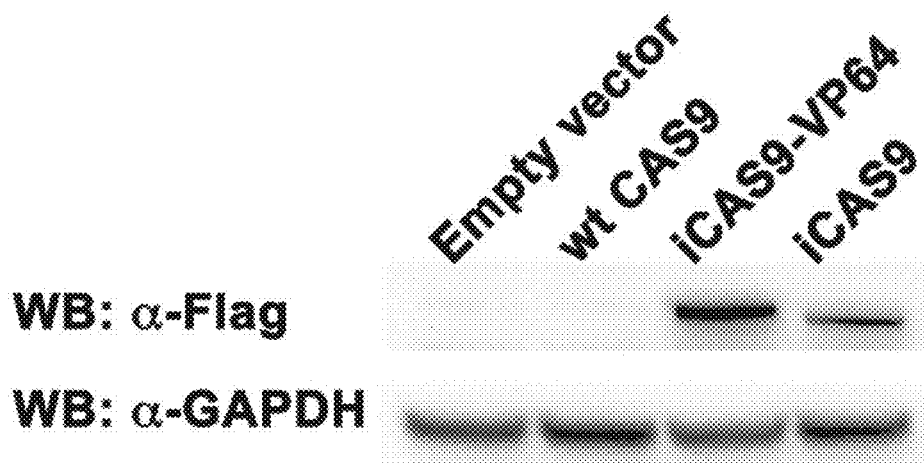
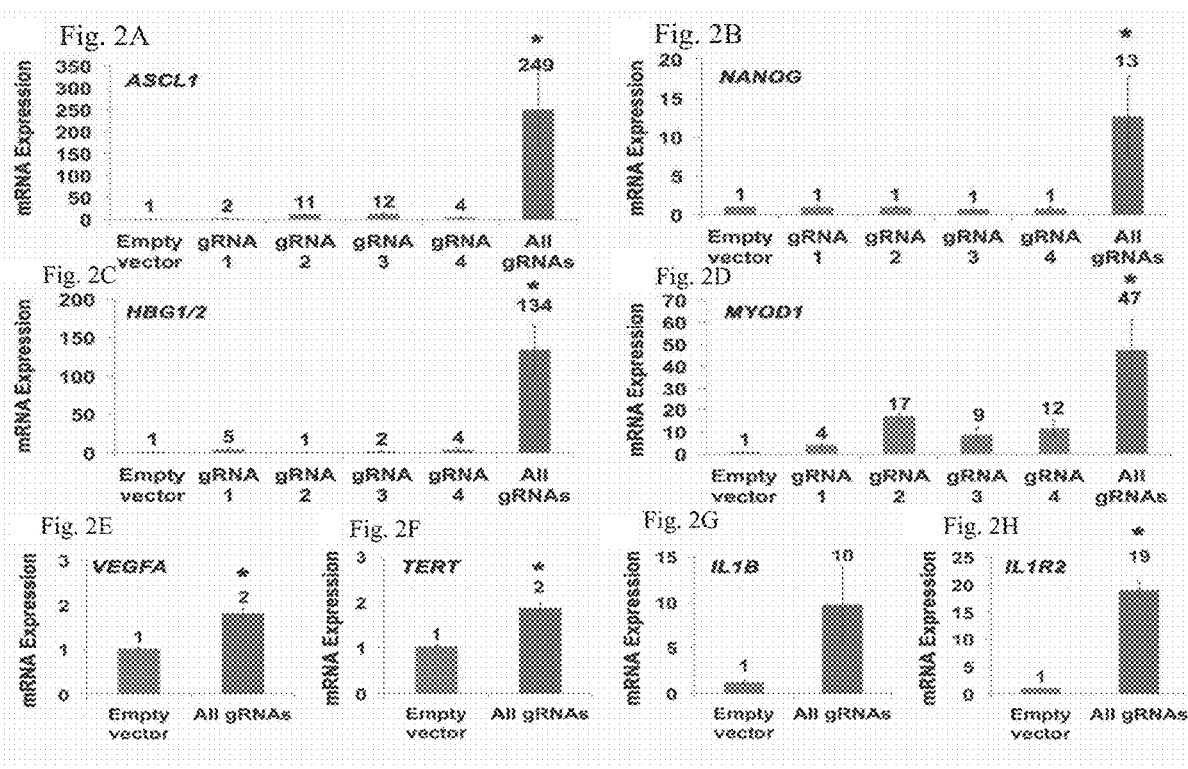
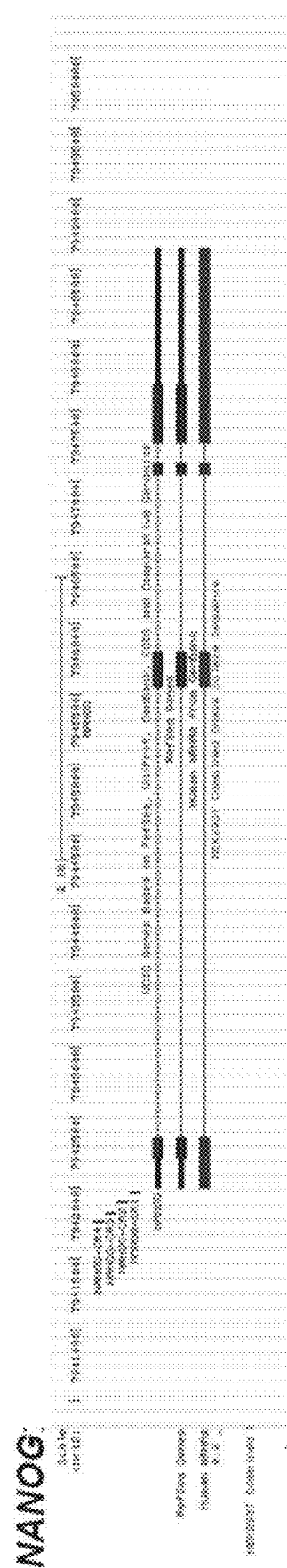
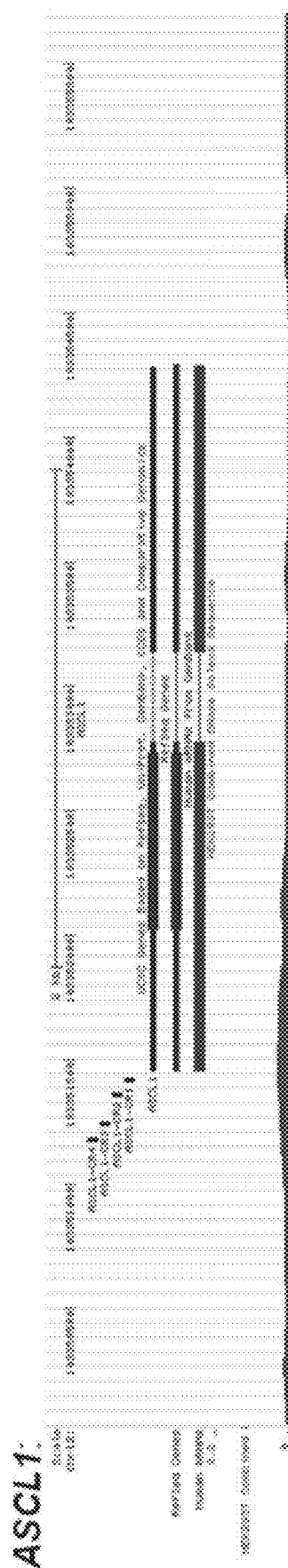
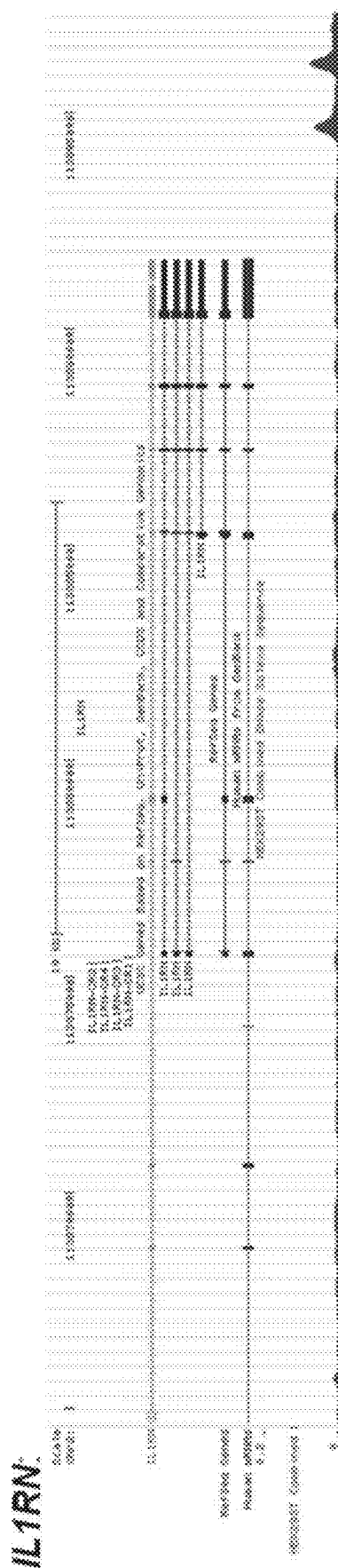
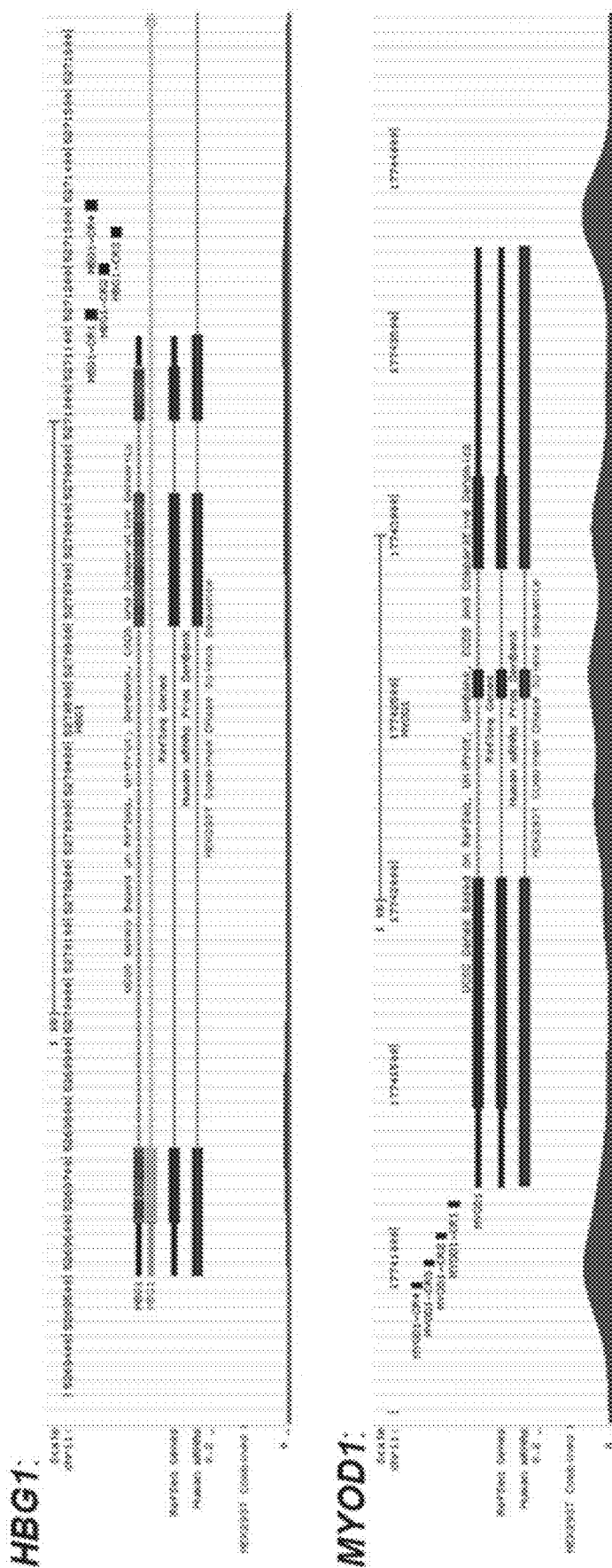


Fig. 3



1000



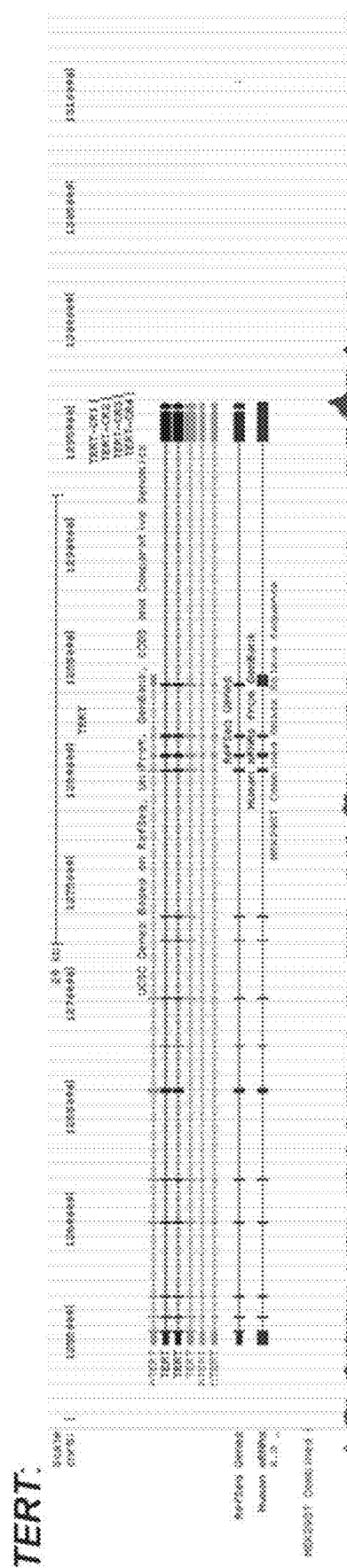
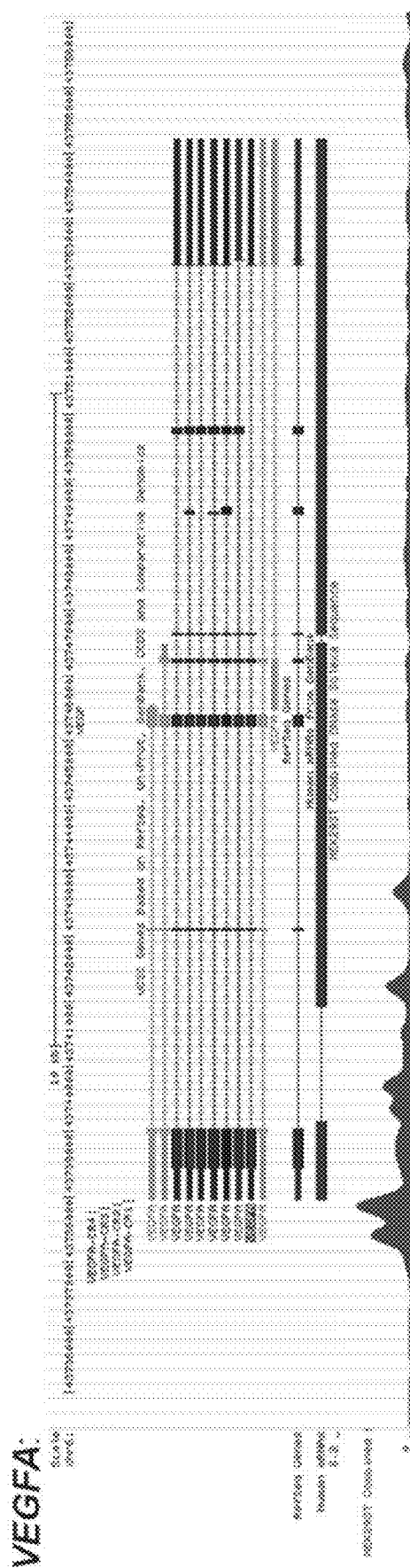


Fig. 4 (Continued)

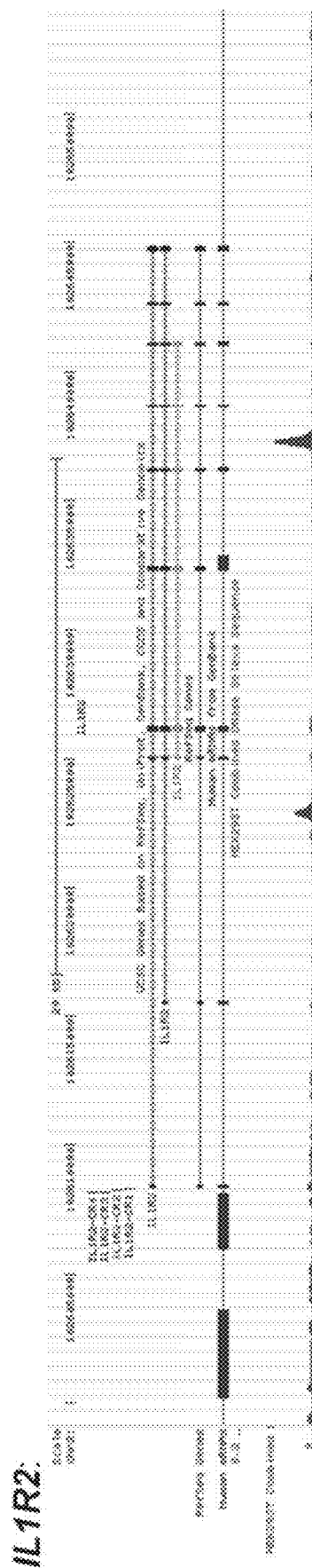
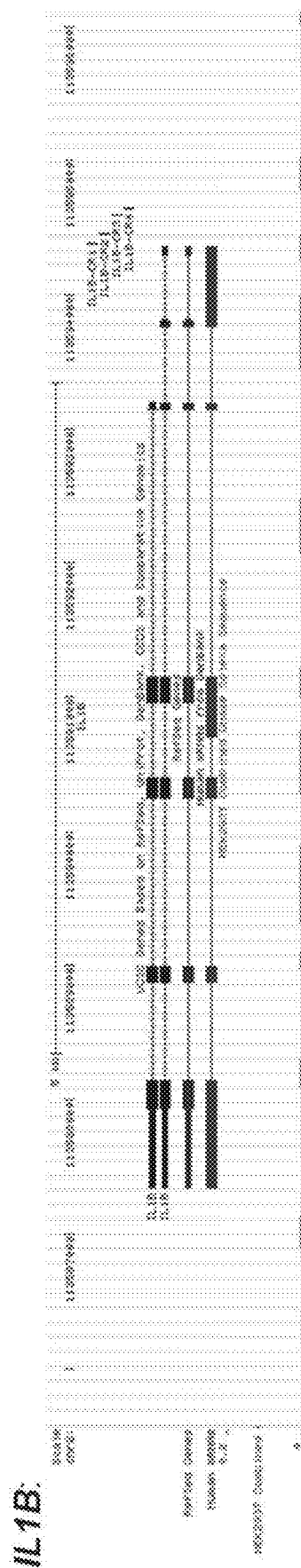


Fig. 4 (Continued)

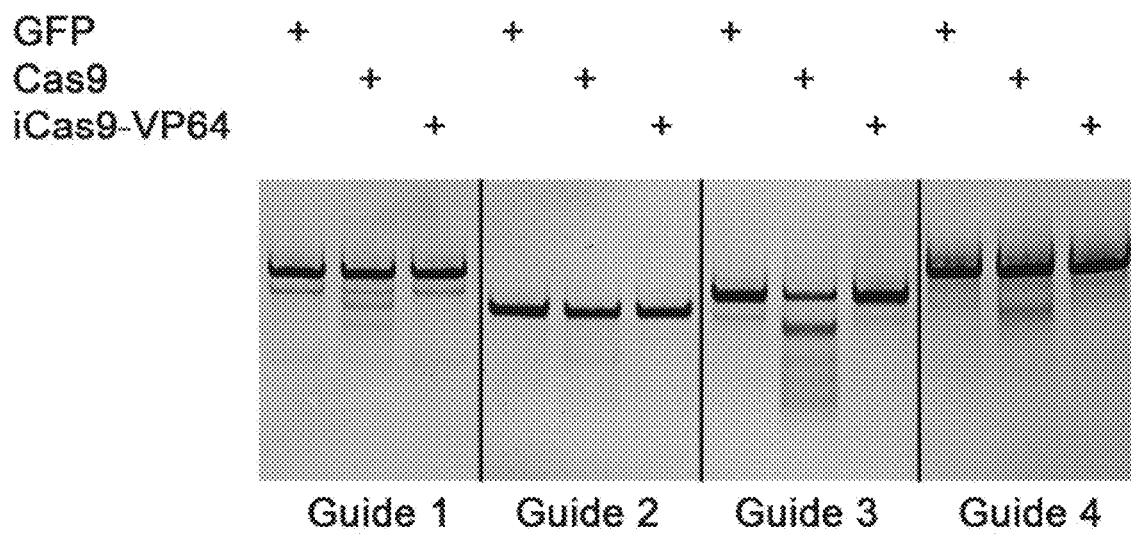


Fig. 5

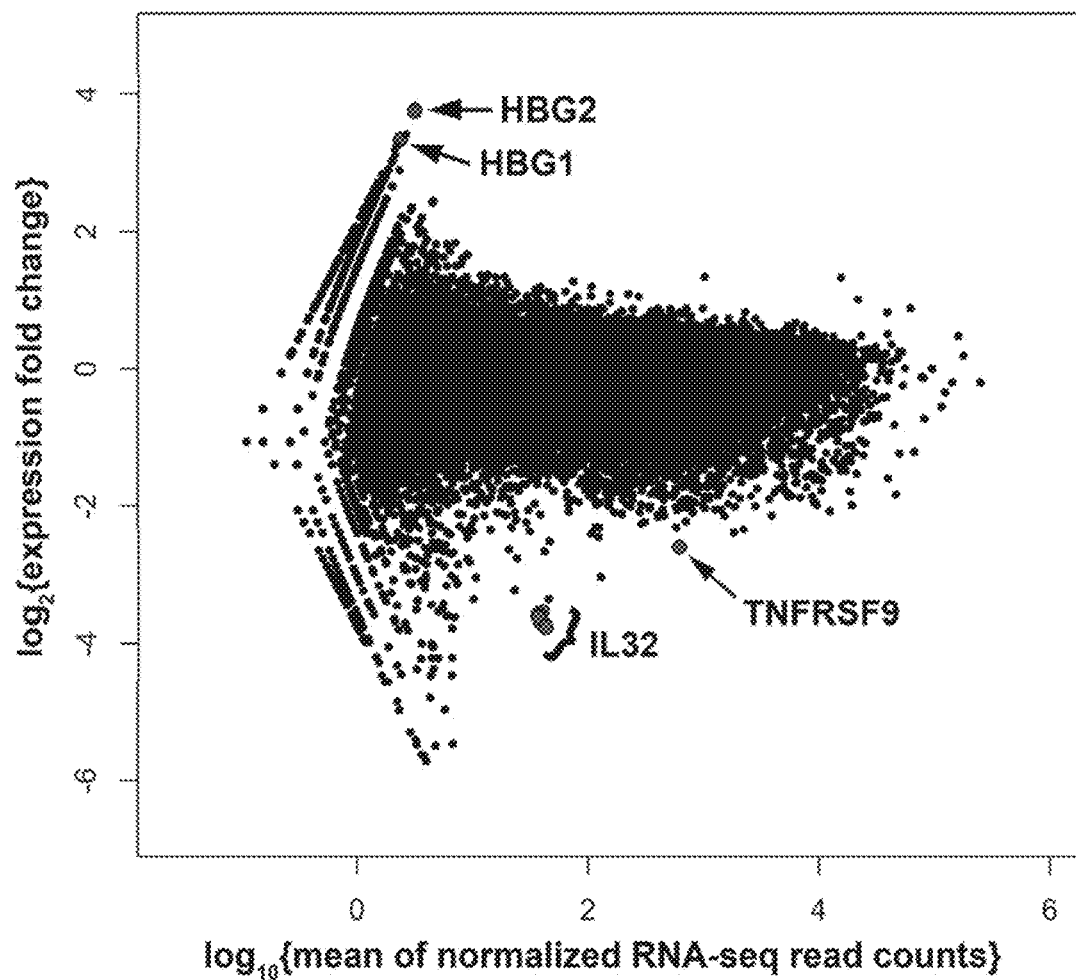


Fig. 6

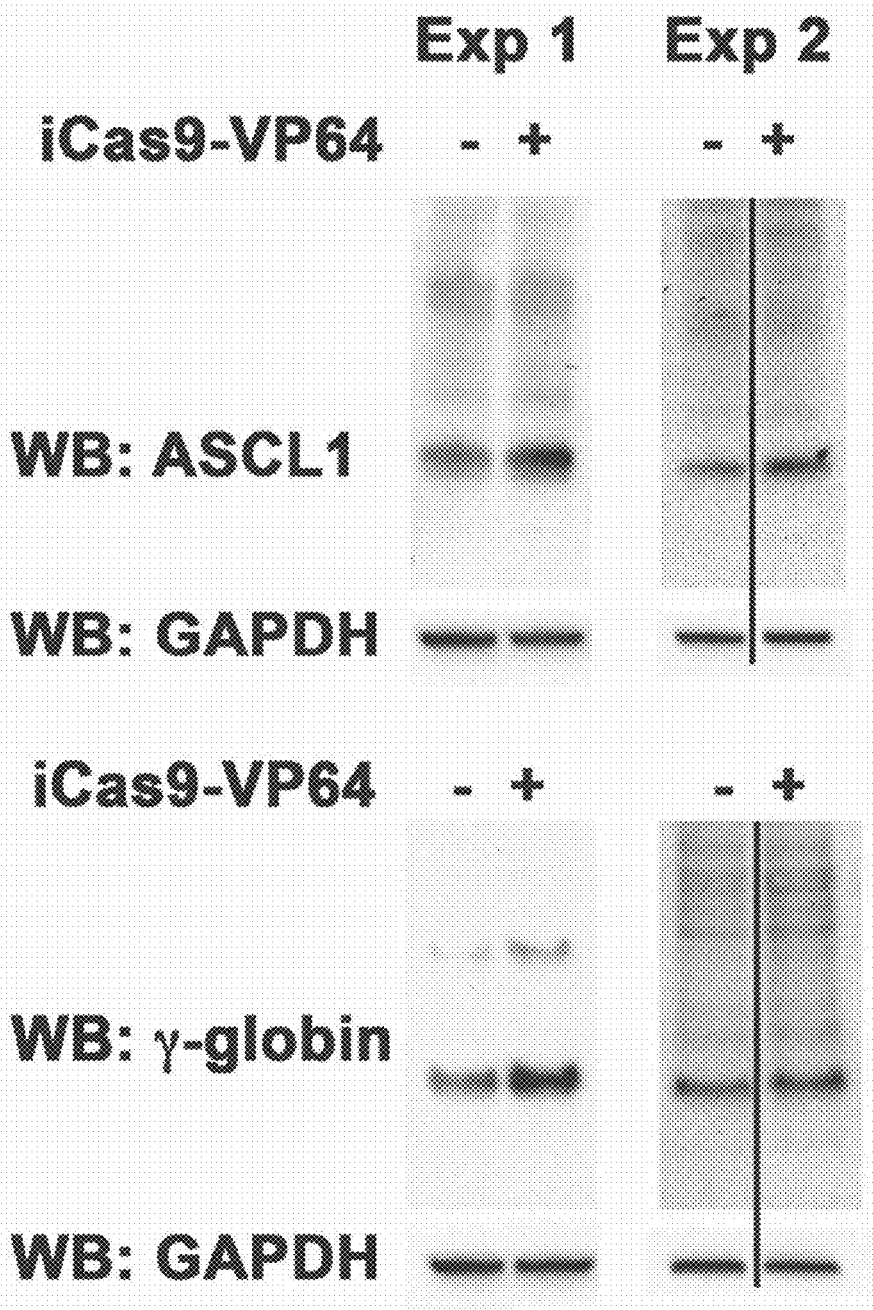


Fig. 7

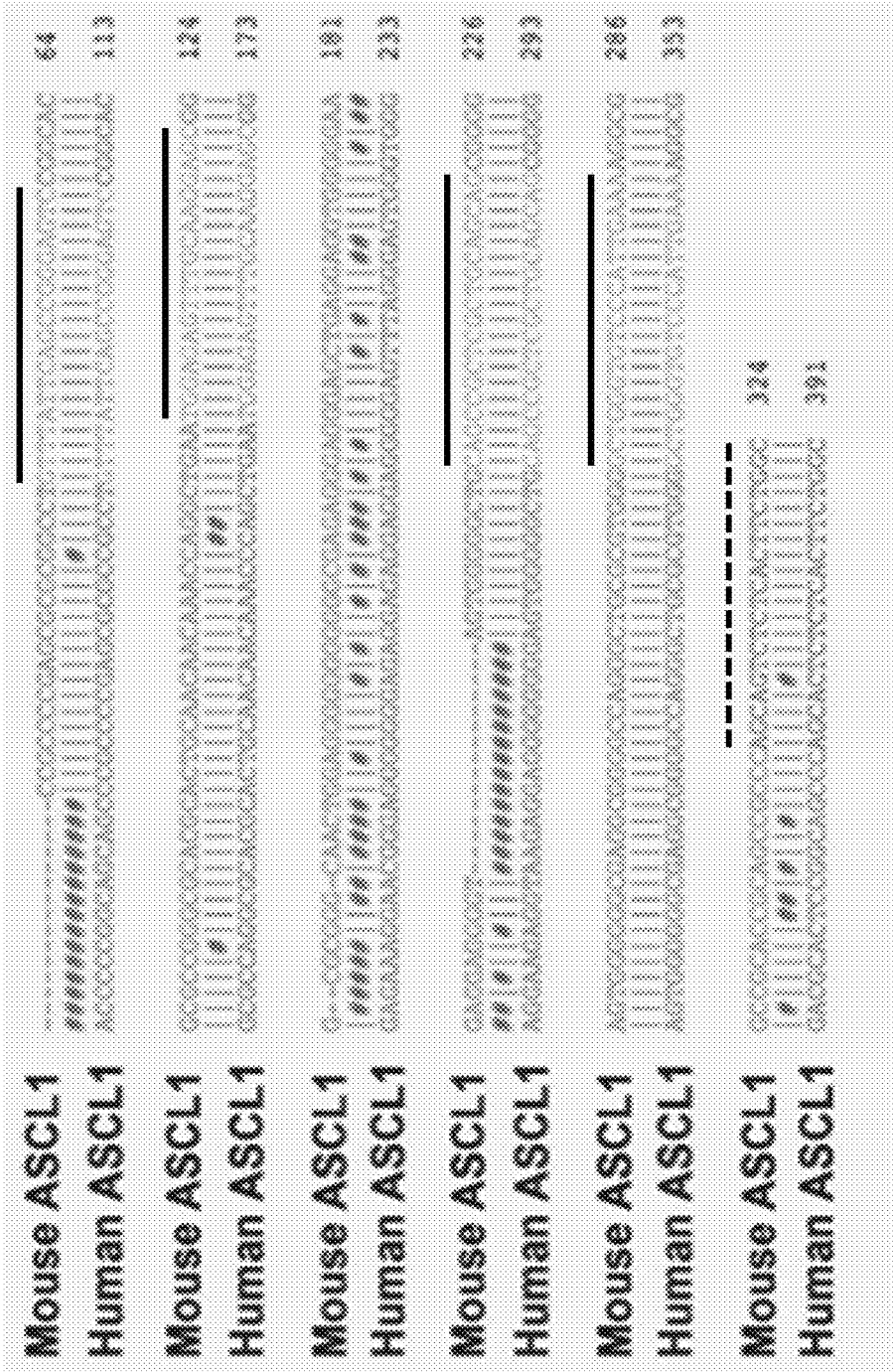


Fig. 8A

Fig. 8B

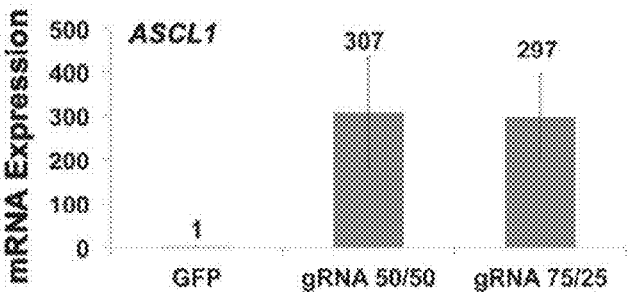


Fig. 8C

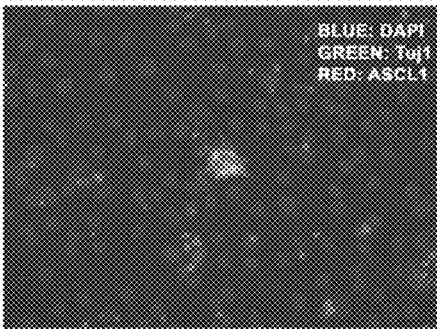


Fig. 8D

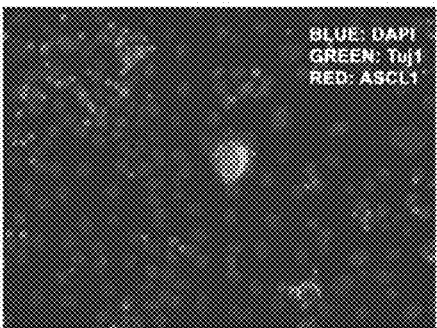


Fig. 8E

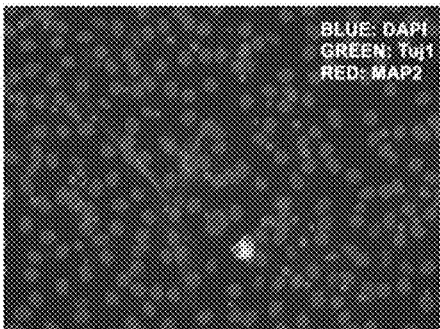


Fig. 8F

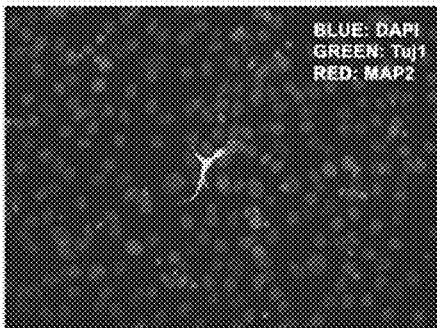


Fig. 8G

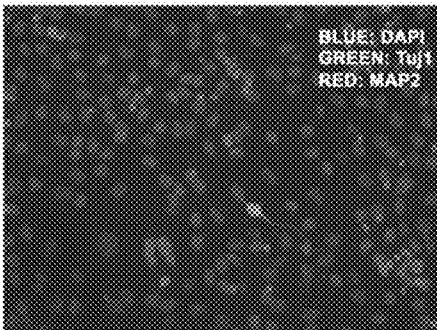
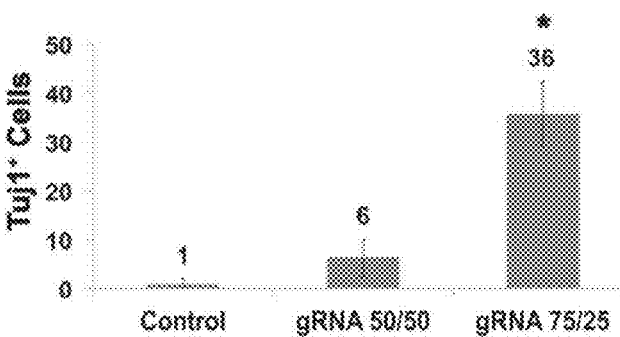


Fig. 8H



MDYKDHDGDYKDHDIDYKDDDDKMAP**PKKKRKV**GRGMDKKYSIGLAIGTNSVGWAVITD
EYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLO
EIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVD
STDKADLRILIYALAHMIKFRGHFLIEGDLNPDNSDVKLFQILVQTYNQLFEEENPINASG
VDAKAILSARLSKSRLENLIAQLPGEKKNLFGNIALSLGLTPNFKSNFDLAEDAKLQL
SKDTYDDDLNLLAQIGDQYADFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEH
HQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE
LLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASQSFIERMTNFDKNLPNEKVL
PKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDY
FKKIECFDSVEISGVEDRFNASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTTLTFEDRE
MIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFAN
RNFMQLIHDDSLTFKEDIQKAQVSGQGDLSLHEHIANLAGSPAIKKGILQTVKVVDDELVKV
MGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEIGIKELGSQILKEHPVENTQLQNE
KLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKMKMNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQIT
KHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAY
LNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTE
ITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESI
LPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIKLPKYSLFELENGRKRMLASAGELQKGNELALPSK
YVNFLYLASHYEKLGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVLS
AYNKHARDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITG
LYETRIDLSQLGGDPIAGSKAS**PKKKRKV**GRADALDDFDL**DMLGSDALDDFDL DMLGS**
DALDDFDL DMLGSDALDDFDL DMLINYPYDVPDYAS (SEQ ID NO: 1)

FLAG epitope tag = italicized
Nuclear localization sequence = bold
Streptococcus pyogenes Cas9 = underlined
VP64 (4x minimal VP16 domain) = italicized and bold
HA epitope tag = italicized and underlined

Fig. 9A

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTG
TTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACA
AAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAA
TTATGTTTTAAATGGACTATCATATGCTTATCGTAACTTGAAAGTATTTCTGA
TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGGTCTTCGAGA
AGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATC
AACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT (SEQ ID NO:2)

U6 promoter = bold

+1 transcription start site = underlined

BbsI restriction sites to clone in guide RNA = italicized and underlined

Chimeric guide RNA sequence = italicized

Poly-T terminator sequence = bold and underlined

Fig. 9B

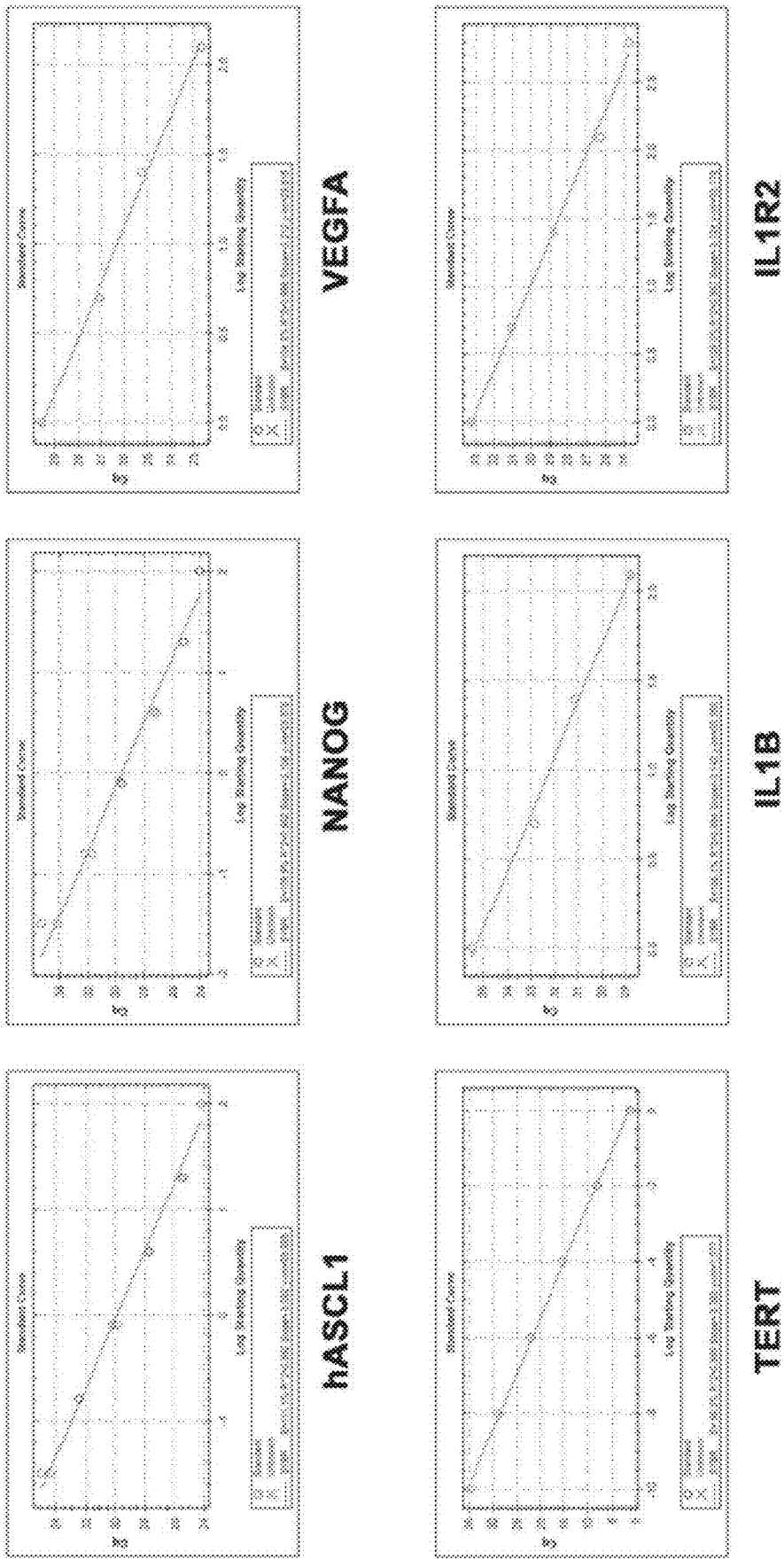
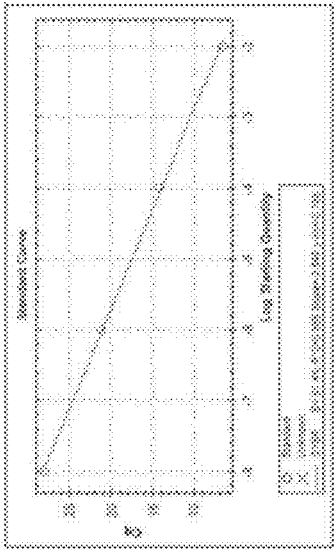
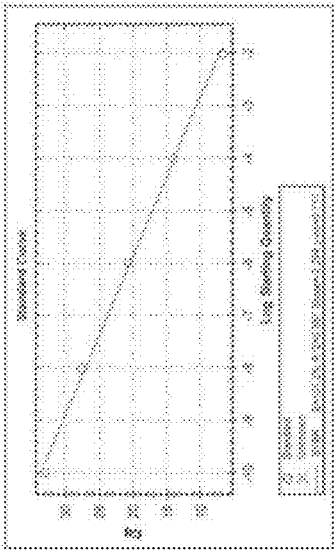


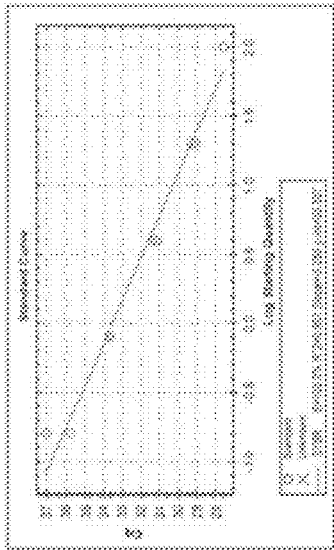
Fig. 10



MYOD1

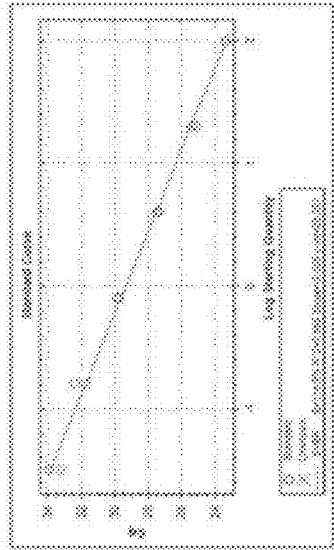


HGB1

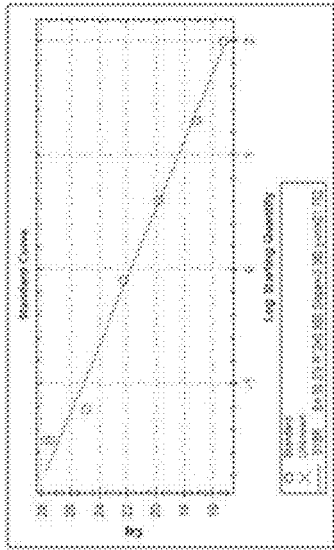


mASCL1

Fig. 10 (Continued)



IL1RN



GAPDH

Fig. 11A

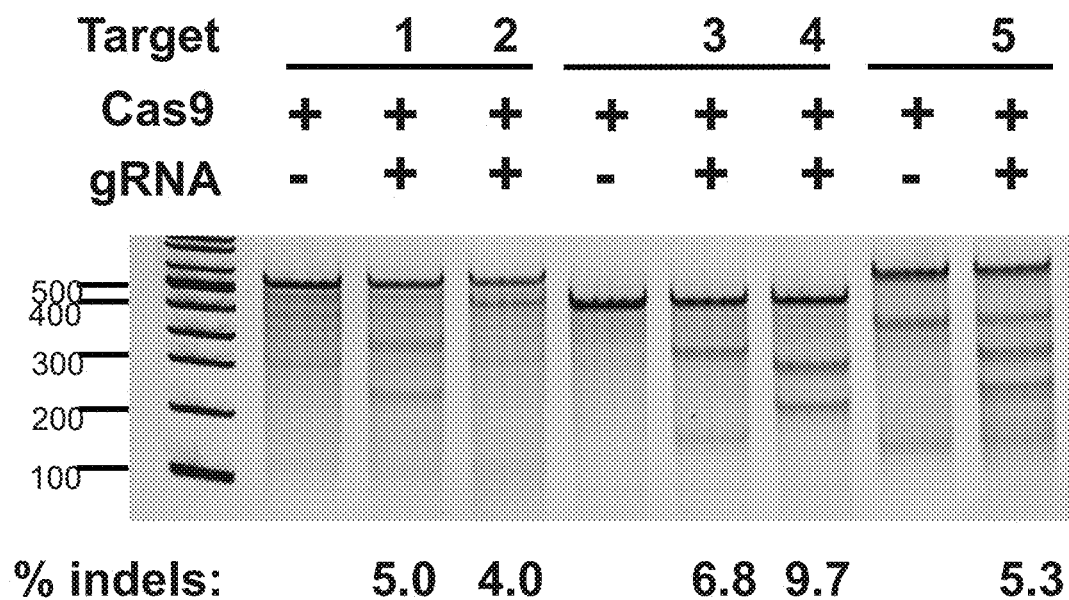


Fig. 11B

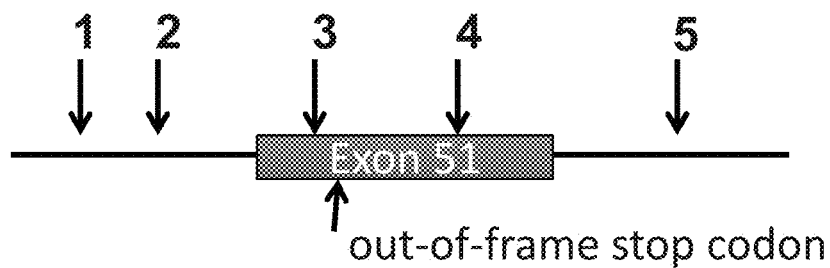


Fig. 11C

Expected cleavage sizes

CR1=352/237

CR2=461/128

CR3=301/150

CR4=272/179

CR5=280/203

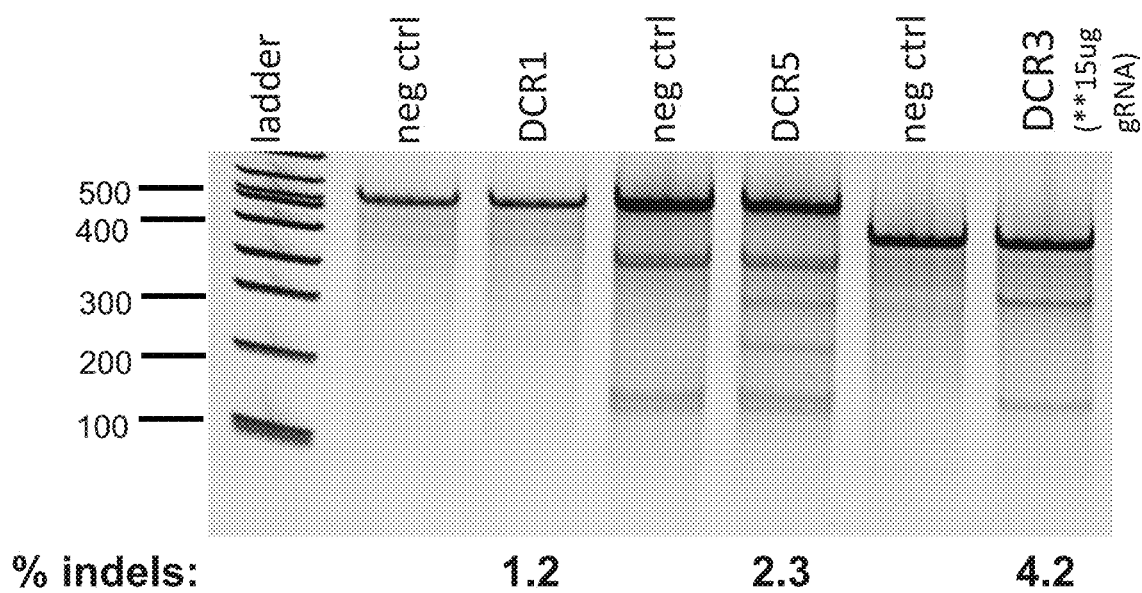


Fig. 12

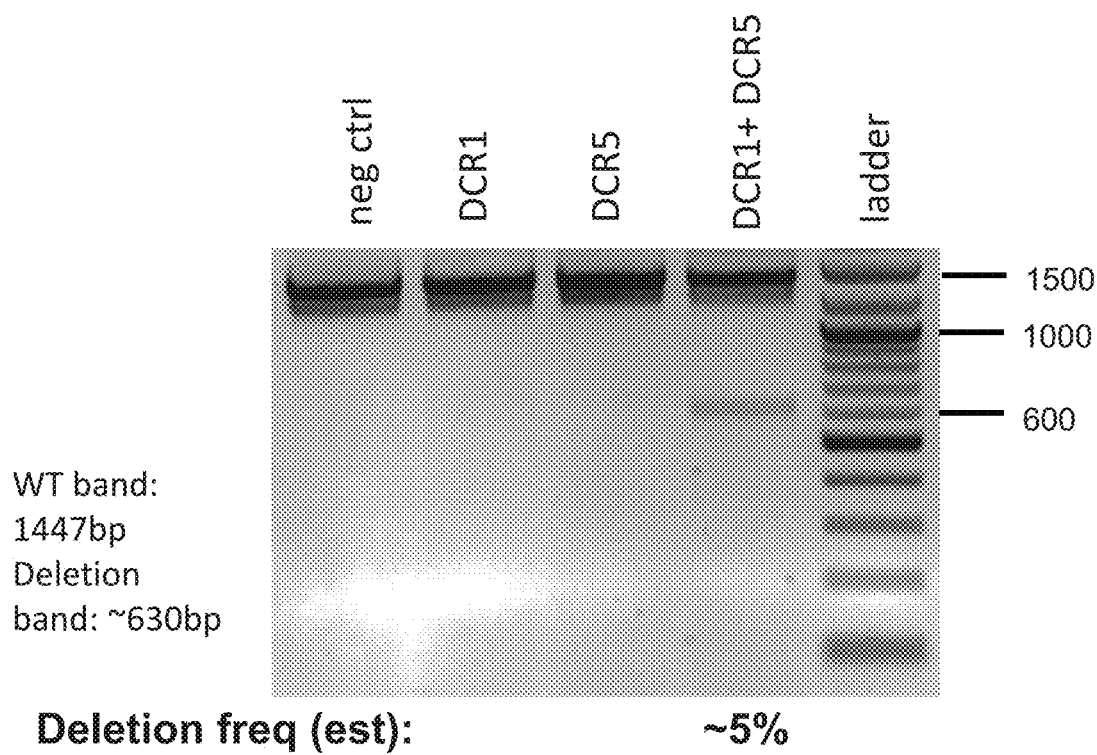


Fig. 13

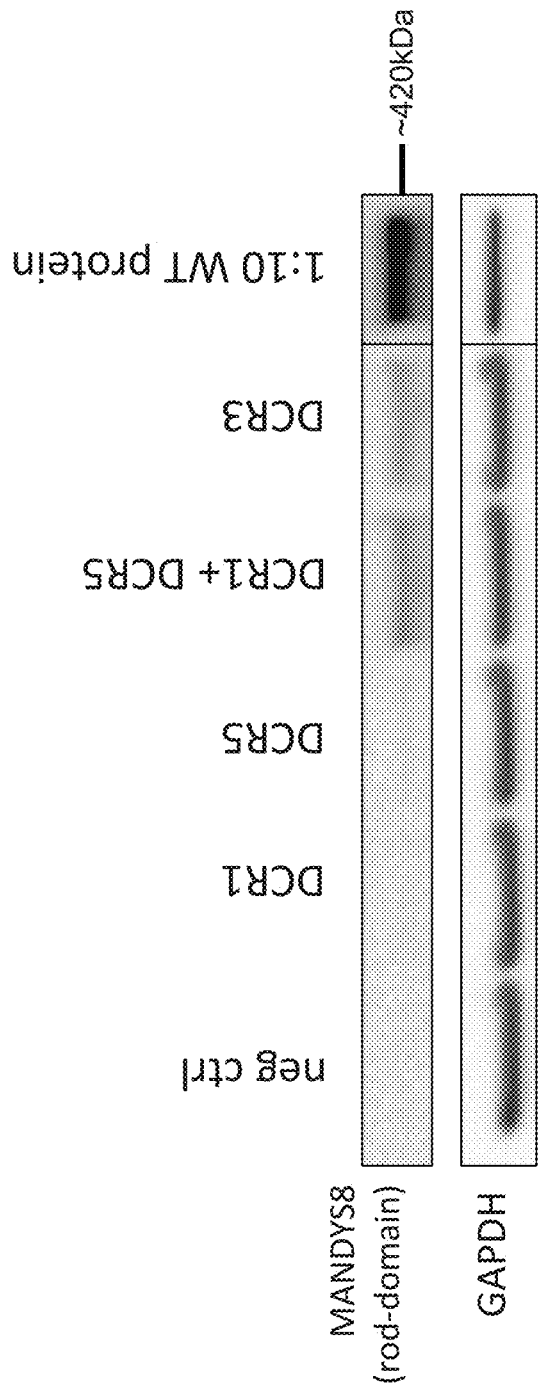


Fig. 14

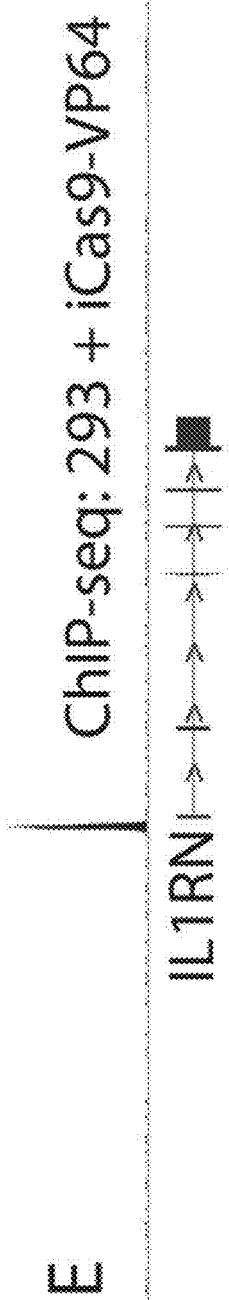


Fig. 15

Fig. 16A

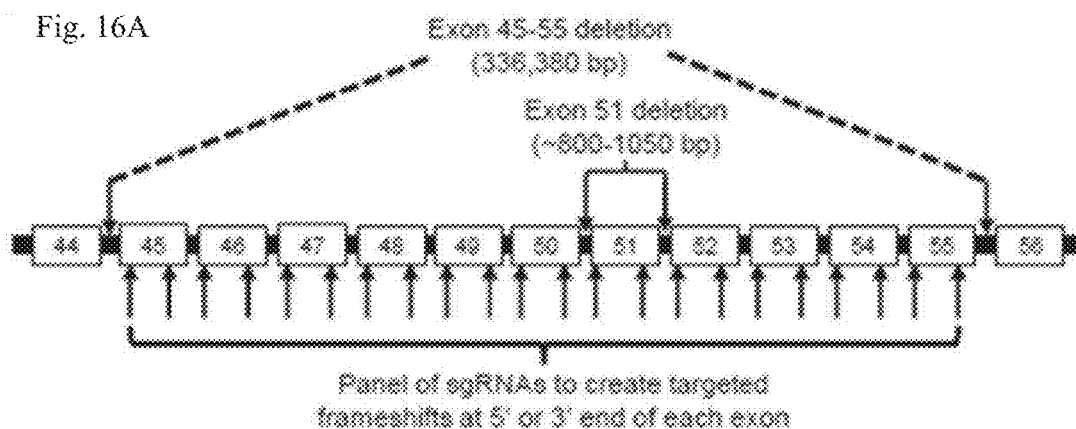


Fig. 16B

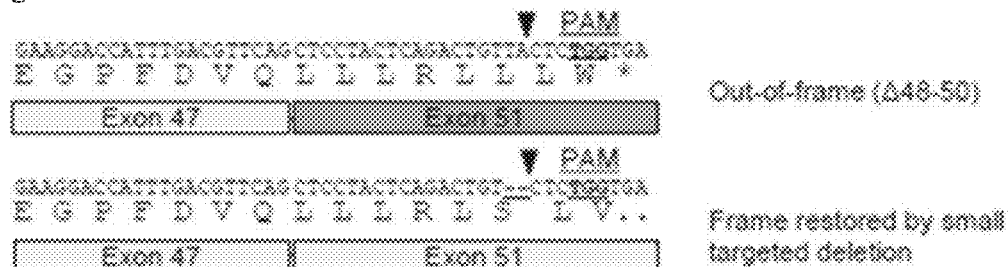


Fig. 16C

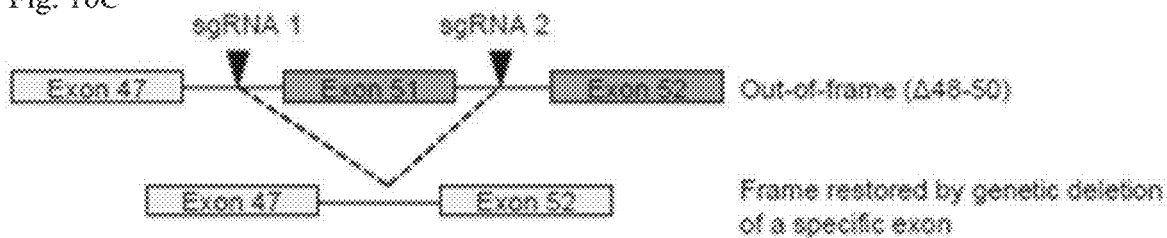
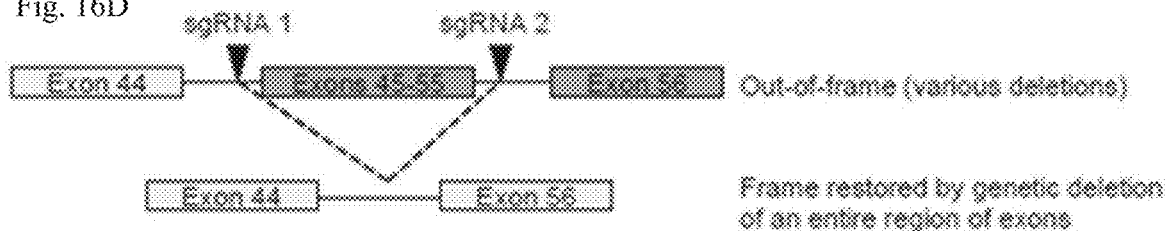


Fig. 16D



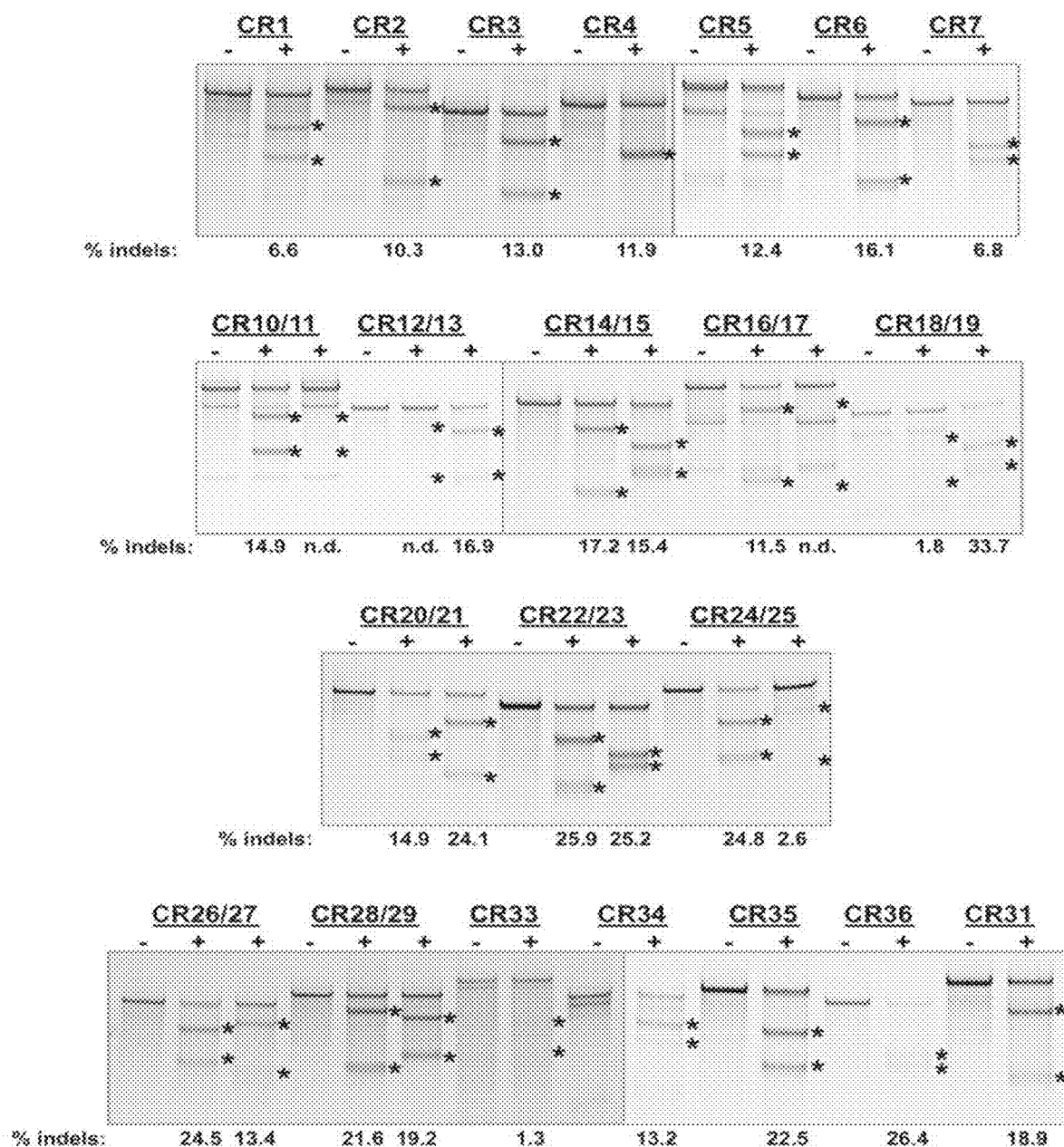


Fig. 17

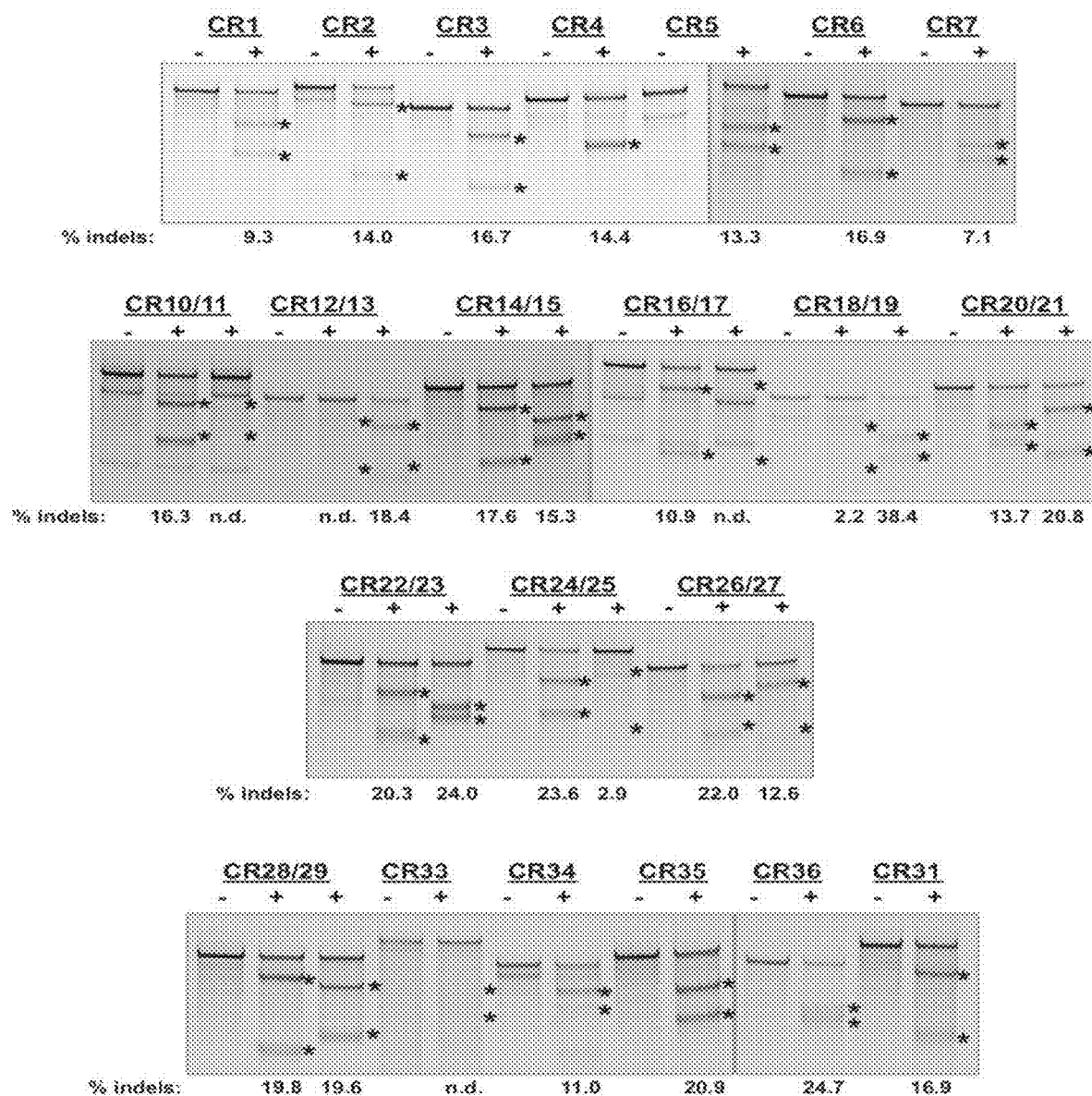


Fig. 18

Fig. 19A

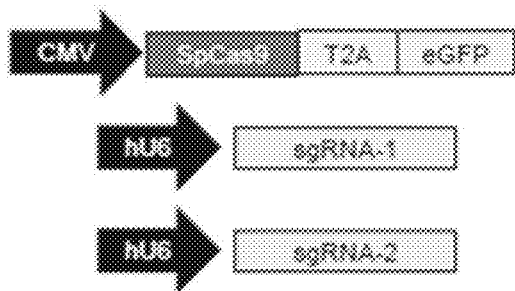


Fig. 19B

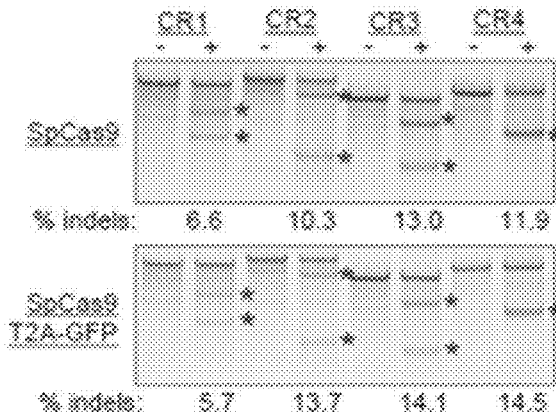


Fig. 19C

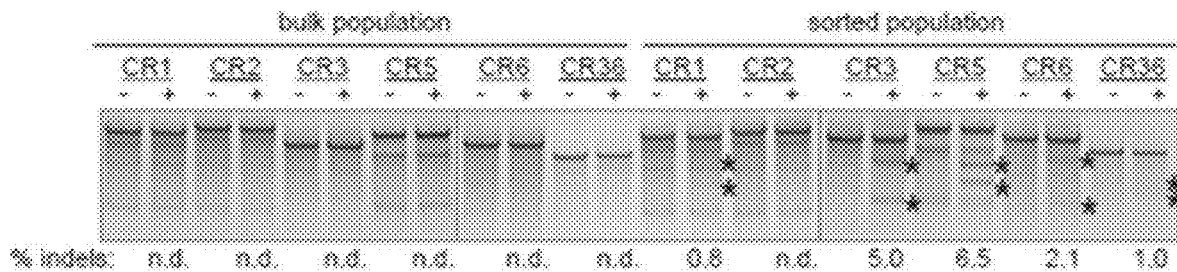


Fig. 19D

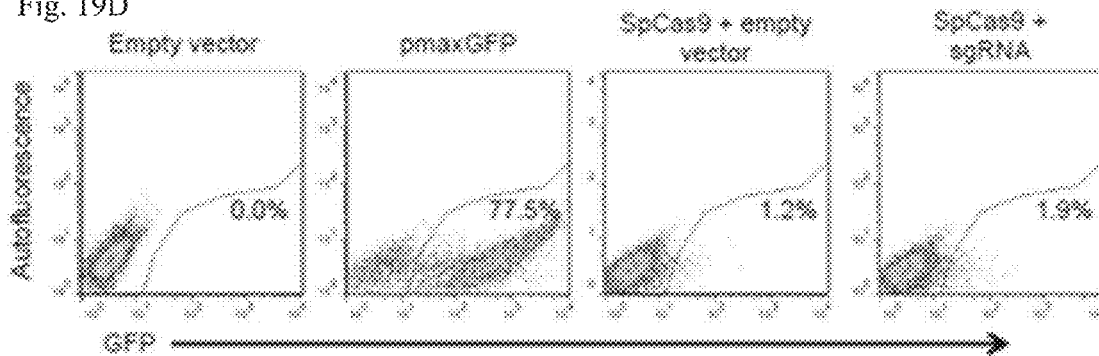


Fig. 20A

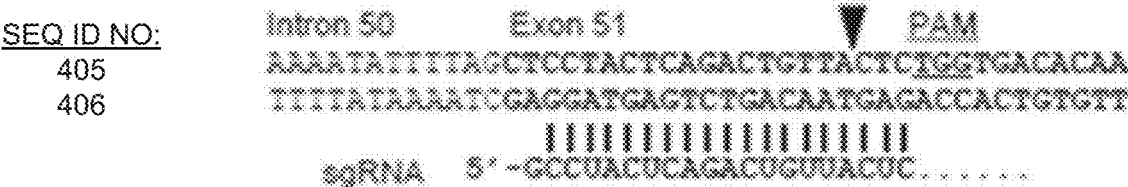


Fig. 20B

SEQ ID NO:	Deletions			
407	TAGCTCCTACTCAGACTGTTACTCTGGTGACACAAAC	(x16)	Length	Frame
408	TAGCTCCTACTCAGACT-----GCTGACCCAAAC		-8	+2
409	TAGCTCCTAC-----TCTGGTGACACAAAC		-12	+3
410	TAGCTCCTACTCAGAC-----TGGTGACACAAAC	(x2)	-8	+2
411	TAGCTCCTACTCAGAC-----		-21	+3
412	TAGCTCCTACTCAGACTGTT-----ACACAAAC		-9	+3
413	TAGCTCCTACTCAGACTG-----TGGTGAGGTGAC		-6	+3
414	TAGCTCCTACTCAGAC----TCTCTGGTGACACAAAC		-4	+1
415	TAGCTCCTACTCAGA----GCTCTGGTGACACAAAC	(x2)	-5	+2
416	TAGCTCCTACTCAGACTG-----TCTGGTGACACAAAC		-4	+1
417	TAGCTCCTACTCAGACT---ACTCTGGTGACACAAAC		-3	+3
418	TAGCTCCTACTCAGAC-----TGGTGACACAAAC		-8	+2
419	-----CTGGTGACACAAAC		-56	+2
420	TAGCTCCTACTCAGACTGTTA-----GACACAAAC		-7	+1
421	TAGCTCCTACTCAGACT---GCTCTGGTGACACAAAC		-3	+3
	Insertions			
422	CAGAC-----TGTACTCTGGTGAC	(x16)	Length	Frame
423	CAGACCACTGTGGTCTCCTA-----CTGGTGAC		+9	+3

Fig. 20C

Total events: 17/33 (52%)

+1 Frame: 3/17 (18%)

+2 Frame: 7/17 (41%)

+3 Frame: 7/17 (41%)

Fig. 20D



Fig. 21A

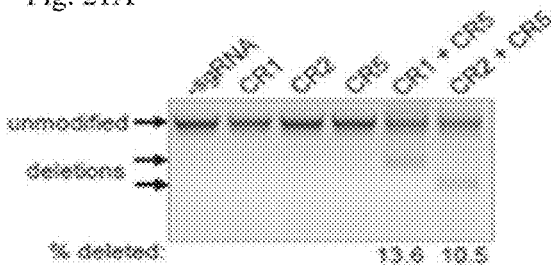


Fig. 21B



Fig. 21C

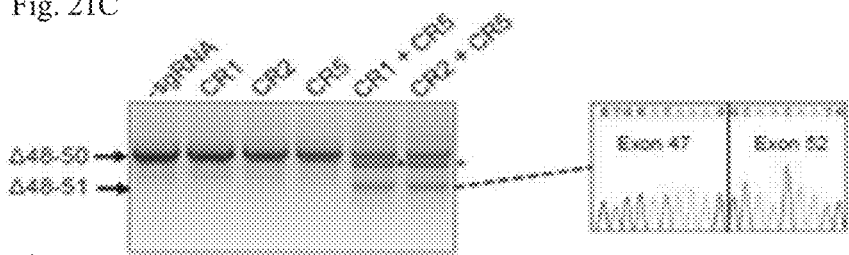
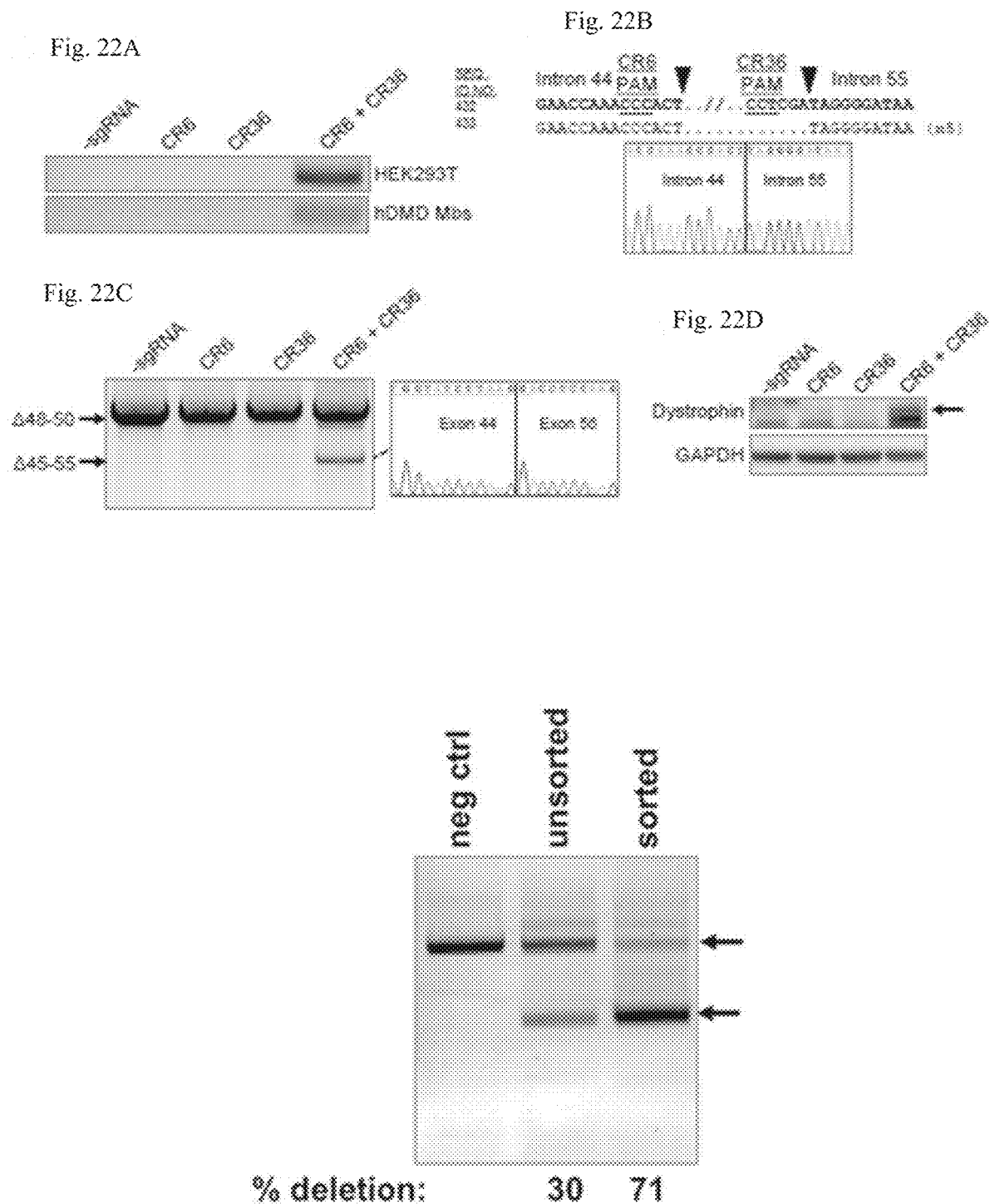


Fig. 21D





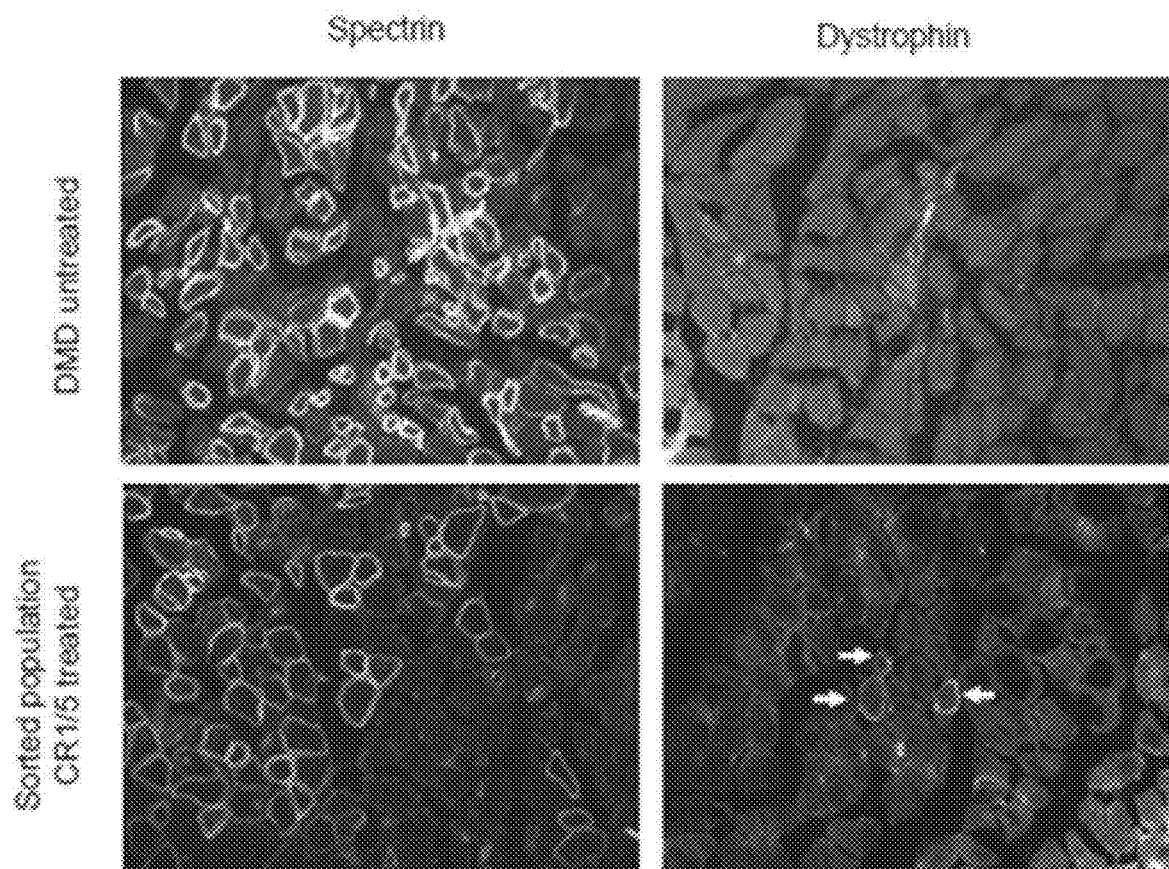


Fig. 24

Fig. 25A

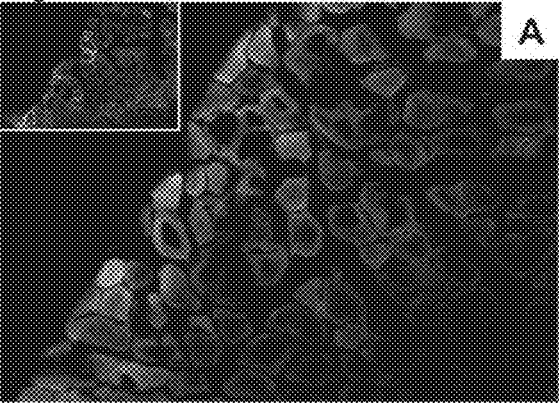


Fig. 25B

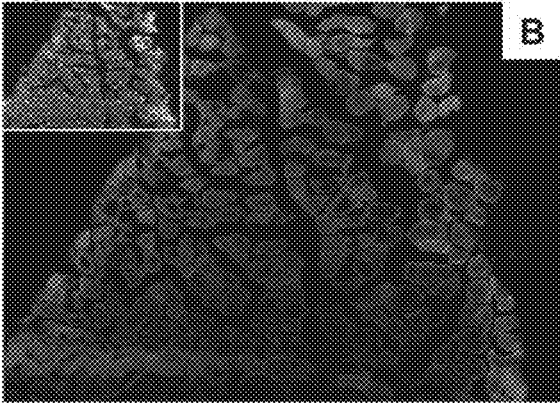


Fig. 25C

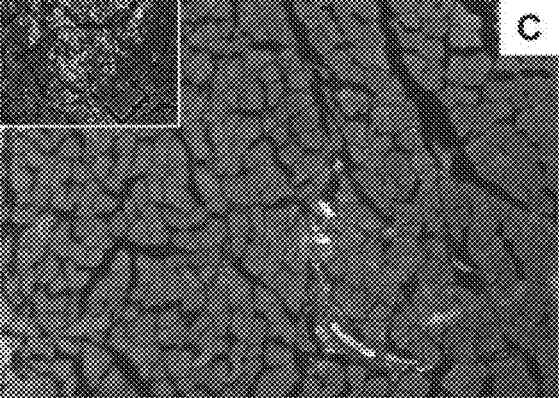


Fig. 25D

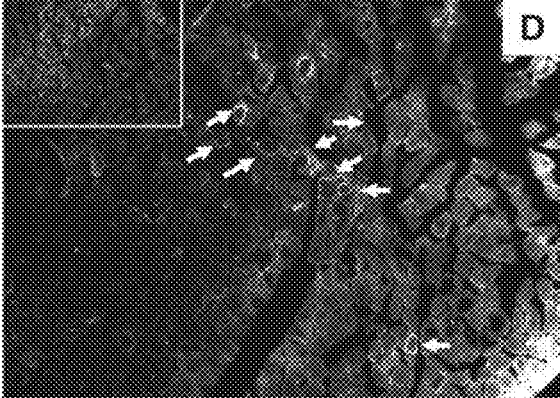


Fig. 25E

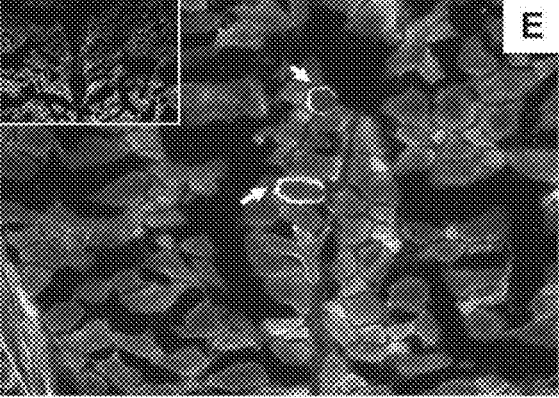
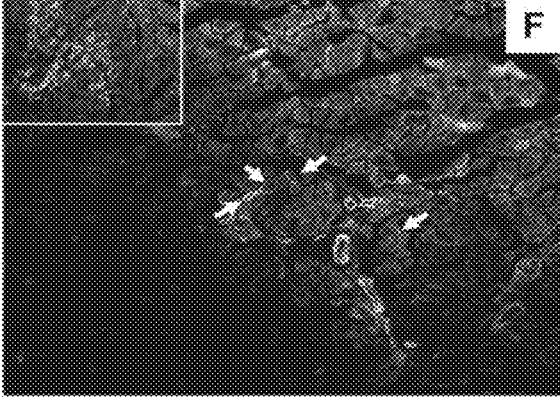


Fig. 25F



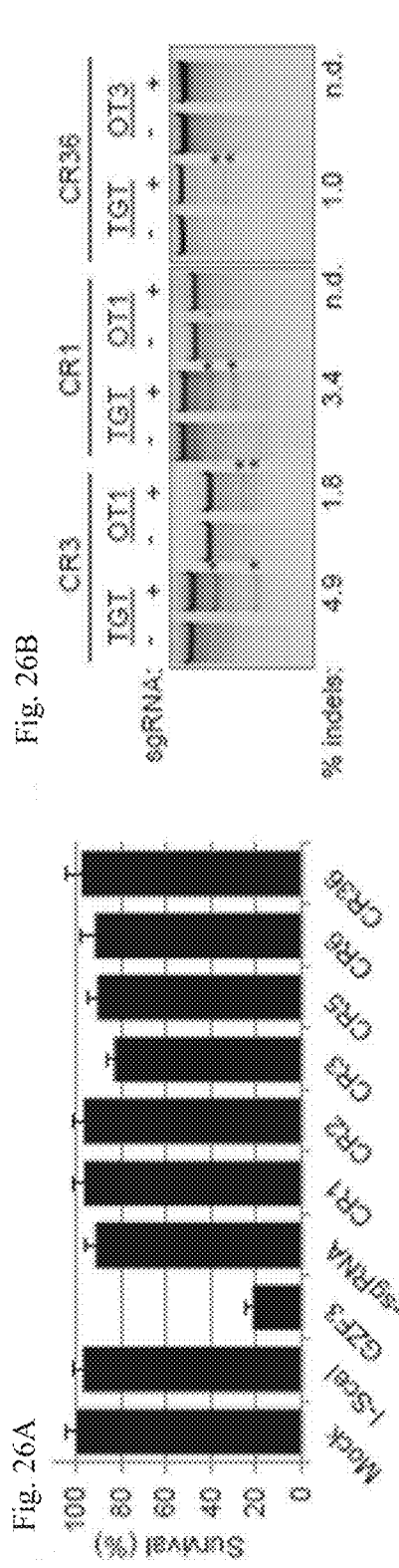


Fig. 26B

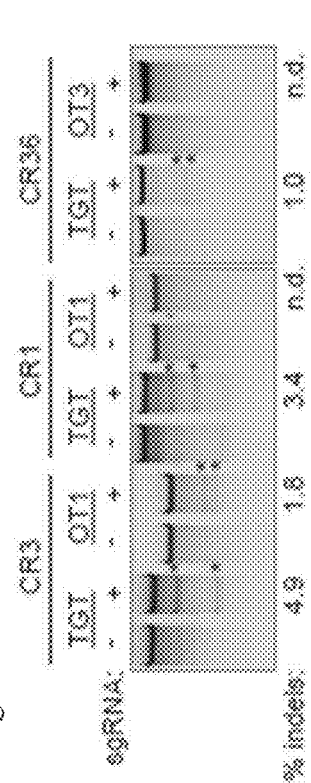


Fig. 26C

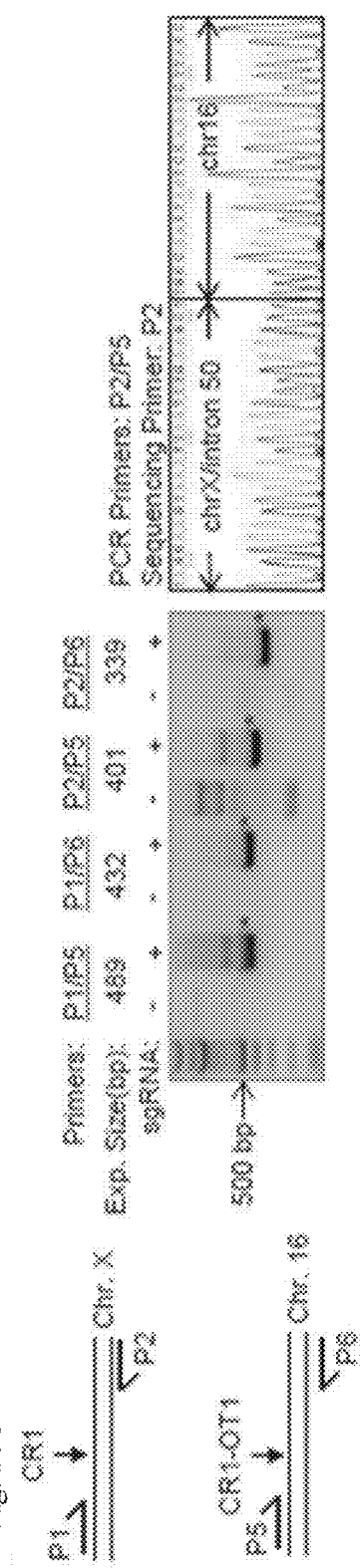
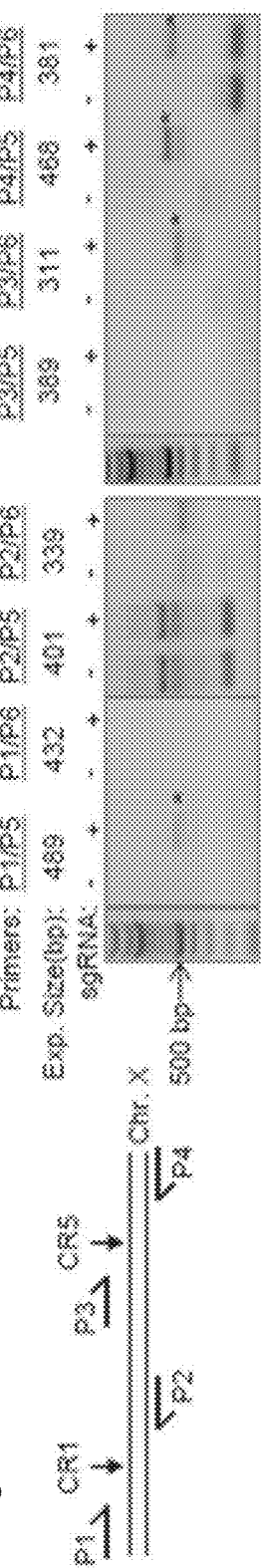


Fig. 26D



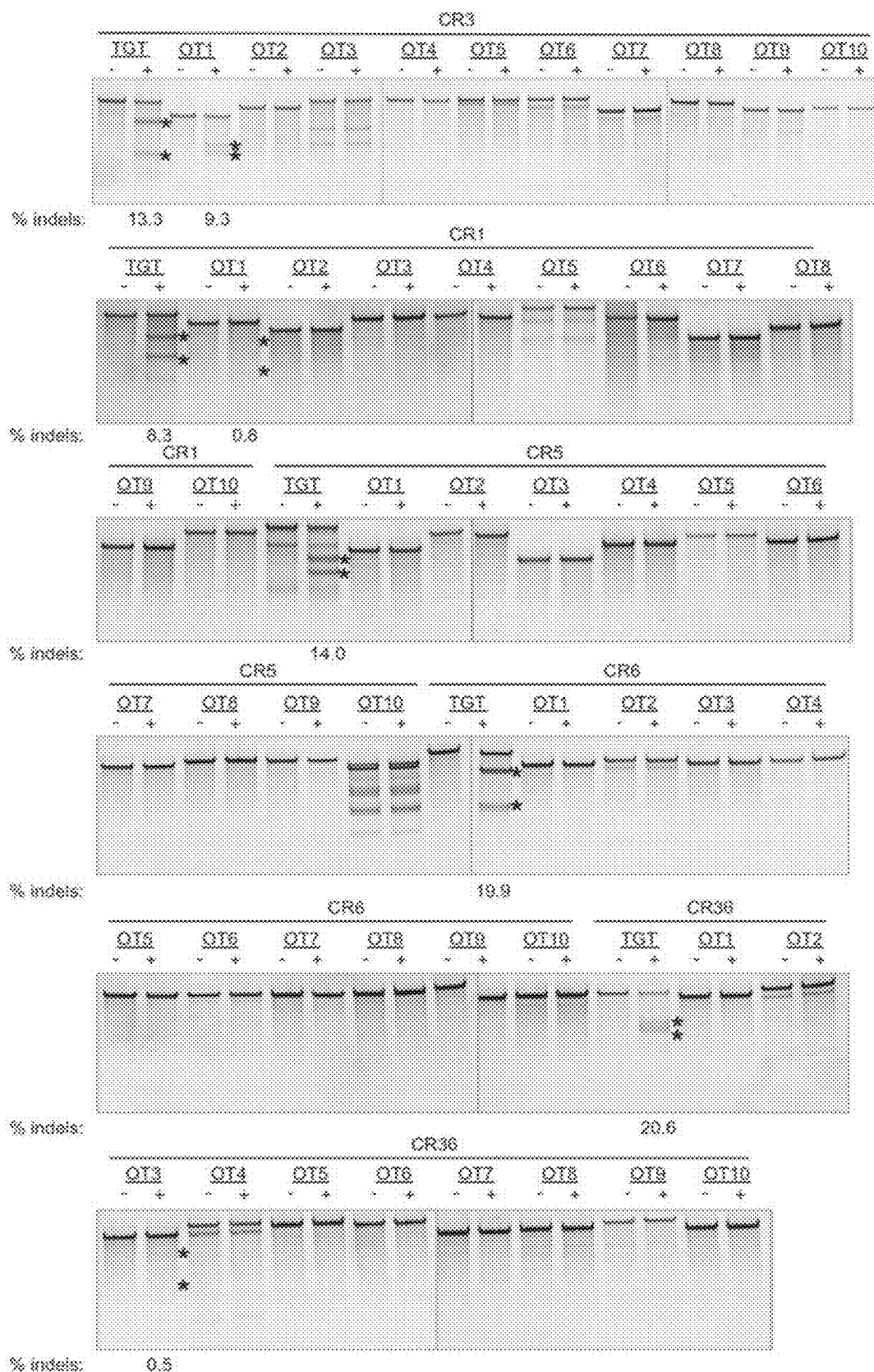


Fig. 27

Fig. 28A

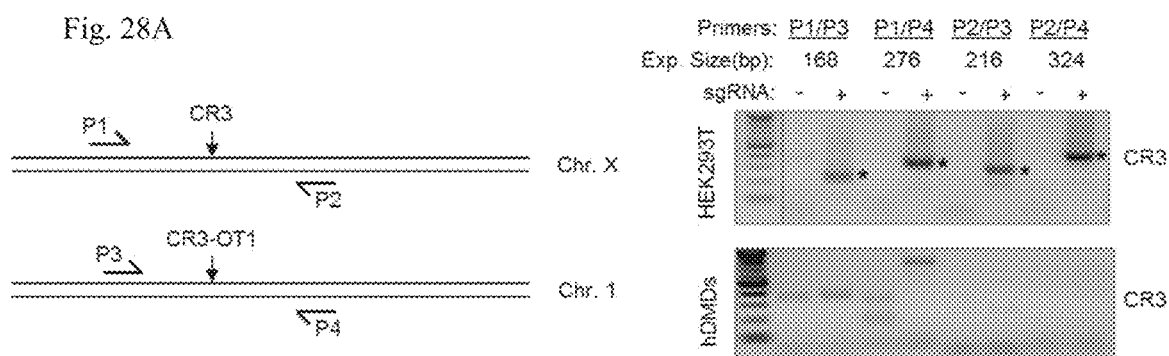


Fig. 28B

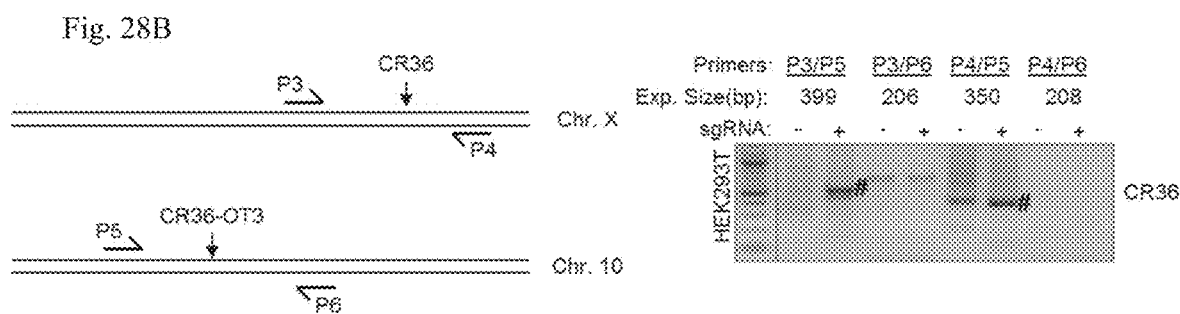
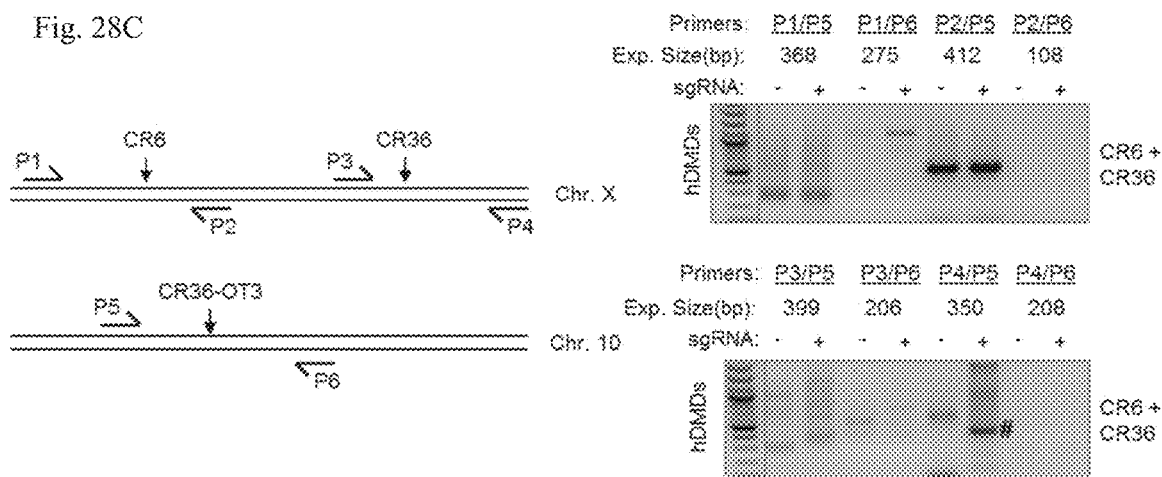


Fig. 28C



P1/P3 band

Forward primer



Reverse primer



P1/P4 band

Forward primer

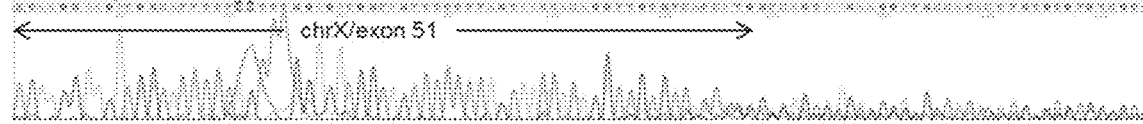


Reverse primer



P2/P3 band

Forward primer



Reverse primer



P2/P4 band

Forward primer



Reverse primer



Fig. 29

P1/P5 band

Forward primer



Reverse primer



P1/P6 band

Forward primer



Reverse primer -- no priming

P2/P5 band

Forward primer -- no priming

Reverse primer



P2/P6 band

Forward primer



Reverse primer



Fig. 30

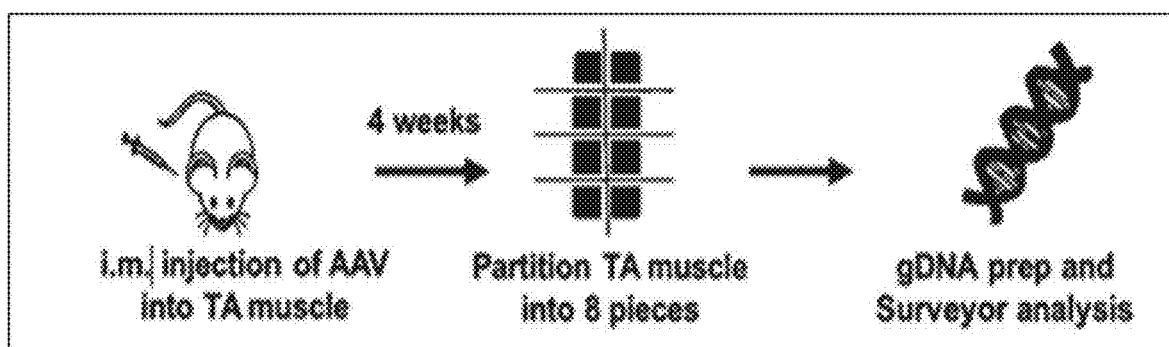


Fig. 31

Fig. 32A

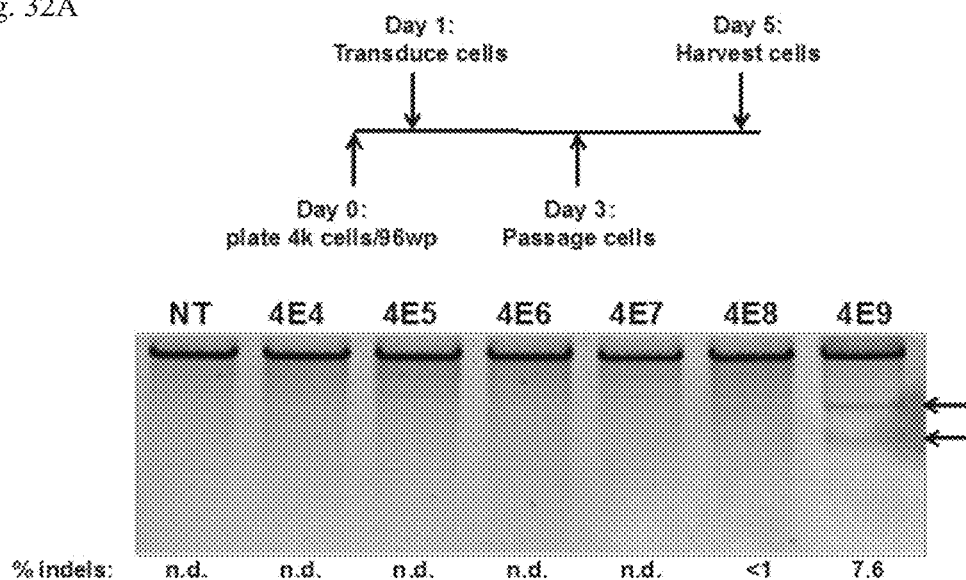


Fig. 32B

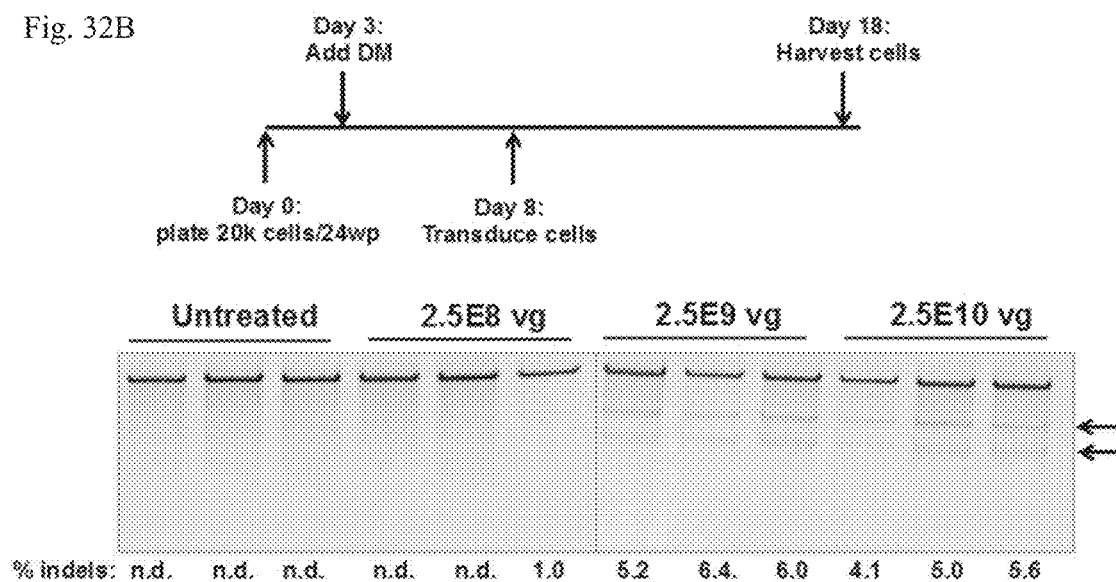
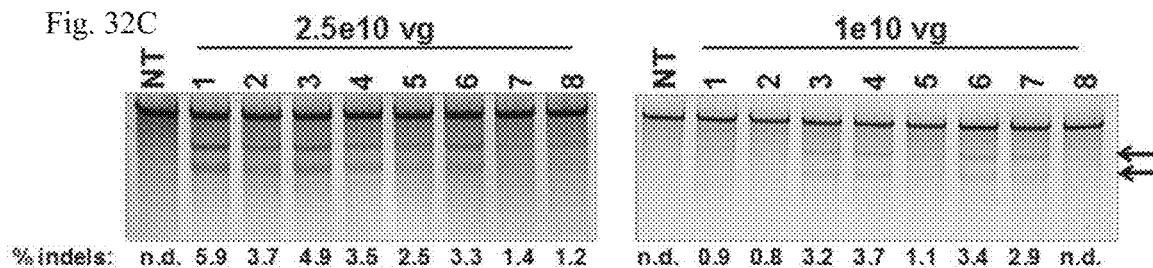


Fig. 32C



Rosa T2A opt DNA sequence (SEQ ID NO: 434)

ATGAGGTCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGAT
GGCCCCCAAGAAGAAGaggaaggtgggccclogagCCCCGGAGAAAAACCGTACAAGTGCCTGAGTGCCTGGAAATCAT
TCTCCGACCCCTGGGGCGCTCGTCCGGCACCAAAAGGAACGCATACAGGGGAAAAAGCCGTATAAGTGCCTCGAGTGTGGA
AAGAGCTTCTCGCAGAGAGGCCACCTTGAAACGACACCAAGAACACACACTGGTGAGAAAACCTATAAGTGTCCAGA
GTGCGGGCAAAATCGTTTAGCAGATCCGATGACTTGGTGCGCCACCAGCGGACACACACGGGTGAAAAGCCCTACAAAT
GCCCGGAGTGTGGGAAGTCGTTTTCAAGGTCCGATCATCTGACTACCCATCAGCGCACCCATACGGGAGCGggcgcc
cgcgccctGGTGAAGACCGAGCTGGAGGAGAAAGTCCGAGCTGCCGACAAAGCTGAAGTACGTGCCCCACGAGTA
CATCGAGCTGATCGAGATCGCCAGGAACCCACCCAGGACCGGCATCCCTGGAGATGAAGGTGATGGAGTTCTTCATGA
AGGTGTACGSSCTACAGGGGAGAGCACCTGCGCGGAAGCAGAAAGCCTGACSSGCGCATCTATACAGTGGGCGACCCCC
ATCGATTACGGCGTGATCGTGGACACAAAGGCCTACAGCGCGCGCTACAATCTGCCTATCGGCCAGGCCGACGAGAT
GCAGAGATACGTGAAGGAGAACAGACCCGGAATAAGCAGATCAACCCAGCGAGTGGTGGAAAGGTGTACCTAGCA
GCGTGACCGAGTTCAAGTTCCTGTCTGTGAGCGGCCACTTCAAGGCCAACTACAAGGCCAGCTGACGAGGCTGAAC
CGCAAAACCAACTGCAATGCGCCCGTCTCTGAGCGTGGAGGAGCTGCTGATCGGCGGCGAGATGATCAAGGCCGGCAC
CTTGACACTGGAGGAGGTGCGCGCAAGTTCAACAACGGCGAGATCAACTTCGAGGGCGAGAGGAAGTCTTCTAACAT
GCGGTGACGTGGAGGAGAAATCCCGGCCCTAGatctTGACTACAAAGACCATGACGGTGTATTATAAAGATCATGACATC
GATTACAAAGATGACGATGACAGATGGCCCCCAAGAAGAAGaggaaggtgggccclogagccGGGAGAGAAAGCCGTA
CAAGTGTCCCCAATGTGGAAGAGCTTCTCACAGTCCGGGGACCTTCGGCCGCCACGAGCGACACACTGGTGAAA
AGCCGTATAAGTGTCCAGAATGTGCAAAATCATTTCTCCACATCAGGGAGCGCTGTTGAGGCACCGGGAACCCACACG
GTTGAGAAAGCCCTATAAGTGGCCCGAATCGGGGAAGTCTTTTTGCGAGAGAGCCCACTTGGAGAGGCCACAGAGGAC
CCATACGGGGGAGAAACCTTACAAAGTGCCTGAAATGCGGAAAGTCTGTTCTCGACCCATCTGGATCTCATCAGACATC
AGAGAACGCACACTGGAGAGAAACCTTACAAATGTCCCGAGTGTGGGAAGTCTTTAGCCGAAAGGACAATCTCAAA
AACCATCAACGGACACACACGGGTGAAAAACCATACAATGCCCGGAGTGCGGCAAAATCGTTTTCCCAACTTGGCGCA
CTTGGCGGGCACACCAACGCAACGCATCTGAGAGCGGCCGccgcccCTGGTGAAGAGCGAGCTGAGAGAGAAGAAGT
CCGAGCTGCGGCACAAAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTEATCGAGATCGCCAGGAACCCACCCAG
GACCGCATCCTGGAGATGAAGGTGATGGAGTTCTTTCATGAAGGTGTACGGCTACAGGGGAGAGCACCTGGCGGAAG
CAGAAAGCCCTGACGGCGGCCATCTATACAGTGGGCGAGCCCACTCGATTACGGCGTGATCGTGGACACAAAGGCCTACA
GCGCGGCTACAACTCGCTATCGCCACGCGGACGAGATGGAGAGATACGTGGAGCAGAACCCAGACACGGGATGAAG
CACTCAACCCCAACGAGTGGTGGAAAGGTGTACCTAGCAGAGCTGACCGAGTTCAAGTTCCTGTCTGTGAGCGGCCA
CTTCAAGGGCAACTACAAGGCCAGCTGACCAAGGTGAACACATCACCAACTGCAATGCGCCCGTCTCTGAGCGTGG
AGGAGCTGCTGATCGCGGGGAGATGATCAAGGCCGGCACCTGACACTGGAGGAGGTGCGGCGCAAGTTCAACCAAC
GGCGAGATCAACTTCTGA

Rosa T2A opt protein sequence (SEQ ID NO: 435)

MRSDYKDHWDGUYKDHUIIDYKDDDDIMAFKKRKVGLPGEKPYKCECGKSFSPFGALVRHQRTHTGEKPYKCECG
KSFSPQRAHLERHQRTHTGEKPYKCECGKSFSPSSDDLVRHQRTHTGEKPYKCECGKSFSPSDHLTFHQRTHTGAAA
KALVKSELEKKSELRHKLKYPHEYLIELIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGRKFPDGAITYTVGSF
IDYGVIVDTKAYSGGYNLFIGQADEMQRYVKENQTRNKHINPNEWWKVYESSVTEFKFLFVSGHFEGNYKAQLTRLN
RKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFFNNGEINFEGRGSLLTCGDVEENPGPRSDYKDHWDGUYKDHUI
DYKDDDDIMAFKKRKVGLPGEKPYKCECGKSFSPSSDLVRHQRTHTGEKPYKCECGKSFSPSSGLVRHQRTHT
GEKPYKCECGKSFSPQRAHLERHQRTHTGEKPYKCECGKSFSPSTHLDLIRHQRTHTGEKPYKCECGKSFSPKDNLK
NHQRTHTGEKPYKCECGKSFSPQLAHLRAHQRTHTGAAARALVKSELEKKSELRHKLKYPHEYLIELIARNPTQ
DRILEMKVMEFFMKVYGYRGEHLGGRKFPDGAITYTVGSFIDYGVIVDTKAYSGGYNLFIGQADEMERYVEENQTRDK
HLNPNNEWWKVYSSVTEFKFLFVSGHFEGNYKAQLTRLNHTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFFNN
GEINF

Fig. 33

SASTG capsid sequence (SEQ ID NO: 436)

tggccaactccctctatgcgcaactcgctcgctcggtggggcctggcgacccaaaggtcgccagaggaagtgctttgca
cgtccggccccaccgagcgagcgagtgccatagagggagtgggccaaactccatcactagaggtatggcagtgaccta
acgcgaagcgccggaagcgagaccacgcctaccagctcgctcagcagtcaggtgaccttttgggacagtttggcgac
accacgtggcgctgaggggtatataattctcgagtgagcggaaccaggaggtccatthtgacogcgaaatttgaacgag
cagcagccatgcgggggttctacgagattgtccctgaaggtcccgagtgacctggacgagcactgcccgggcatttct
aactcgtttgttaactgggtggcgagaaaggaatgggagctgcgcgcggattctgacatggatccgaatctgattga
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gcagcttccgaactgggttcgcggtgacccaaacgcgaatggcgccggggggcggaacaaggtggtgacgaactgct
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aggtcaccaaaacaggaagtaaaaggaacttttccggtgggttccgatcagctgactgaogtggctcatgagttctac
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ctgaacagaaacgcaaggaaacaaacctctggaacaaacaaacaaatcagggctgcttttagccaggctgggcctcagtc
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ccagctatggccagtcacaaggacgatgaagaaaaattttccctatgcacggcaatctaatatttggcaaaaggy
gacaacggcaagtaacgagaattagataatgtaatgattaoggtgaagaaagagattcgtaccacaaatctgtgg
caacagagcagtatggaactgtggcaataacttgcagagctcaaatcagctcccacgactagaactgtcaatgat
cagggggcccttacctggcatggtgtggcaagatcgtgacgtgtaccttcaaggacctatctgggcaaaagattcctca
cacggatggacactttcatctctctctgatgggaggtttggactgaaacatccgctctctcaaatcatgatca
aaaatactccggtacccgcaaatcctccgacgactttcagcccgcccaagtttgccttcaatttatcactcagtaactc

Fig. 34

actggacaggtcagcgtggaaattgagtgaggagctacagaaagaaaacagcaaacggttgyaatccagagattcagta
cacttccaactacaacaagtcgtttaatgtggactttactgtagacactaatggtgtttatagtgaaacctcgcccta
ttggaacccggtatctcacacgaaaacttgtaactctggttaatcaataaacggtttaattcggttcagttgaacttt
ggtctcttggtgaactcttattatcttctgtttccatgggtactgogtagataagcagcggcctgogggogcttgcyct
logoggtttacaastgctggttaabatttaactctcyccataacctotagtgatggagttgycactccctctahgog
cactogctogctoggtggggccggagctgcaaagcaogtcoogtctggcagacctttggtogccagggccccaccgagcg
agcgagtgogcatagagggagtgggccaa

SASTG capsid peptide sequence (SEQ ID NO: 437)

MAADGYLPDWLEDNLSEGIREWVALKPGVPQPKANQQHQDNRRGLVLPQYKYLGPNGNGLDKGEFVNEADAAALEHBN
AYDQQLKAGENPYLKYNHADAEPQERLQEDTSFEGGNLGRAVFOAKKRILEPLGLVEEAAKTAPGKKRFVVDQSPQEPD
SSSGVGKSKQPKARKLNFGQTGDSSESVDPFQFLGEPFAAPTS LGSNTMASGGGAPMADNNEGADGVGNSSGNWHCD
SQWLGDVITTTSTRTNALFTYNNHLYKQISSASTGASNDNHYPGYSTFWGYFDENREHCHFSFRLWQRLINNNWGR
PKKLSFKLFNIQVKEVTQNDGTTIANNLSTTVQVFTDSEYQLPYVLGSAHQGCLPFFPADVFVVPQYGYLTNNGS
QAVGRSSFYCLEYFPSQMLRTGNFPQFSYTFEDVFFHSSYARSQSLBRLMNPLIDQYLYILNPTQSTTS GTTNQSR
LFSQAGPQSMSLCARNWLPGPCYPQQRLSKTANDNNNSNFWIAASKYHLNGRDSLVNFGFAMASHNDDEEKFFPMH
GNLIPGNEGTTASNAELDNVMITDEEIRTTNFPVATEQYGTVANNLQSSNTAFTTFTVNDQGALPGMVWQDRDVYLQ
GPIWAKIPHTUGHFHPSPLMGGFGLKHPPPQIMIKNTEVPANPPTTFSPAKFAFITQYSTGQVSVEIEWELQKENS
KRWNPFIQYTSNINKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

Fig. 34 (continued)

DZF16 2FN target site (SEQ ID NO:442):

5'-CAAACTAGAAATGCCATCTTCCTTGATGTTGGAGGTACCTGC-3'
3'-GTTTGATCTTTACGGTAGAAGGAACTACAACCTCCATGGACG-5'

DZF16-L6 left full amino acid sequence (SEQ ID NO:443)

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKKRVGRLEPGEKPYKCPECGKSFSR~~KD~~ALRGHQRTHTGEKPY
KCPECGKSFS~~SH~~RTTLTNHQRTHTGEKPYKCPECGKSFSQ~~RNAL~~AGHQRTHTGEKPYKCPECGKSFS~~SH~~KNA
LQNHQRTHTGEKPYKCPECGKSFS~~SD~~PGHLVRHQRTHTGEKPYKCPECGKSFS~~TS~~SGNLVRHQRTHTGAAAR
ALVKSELEKKSEL~~RH~~KLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAI
YTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMER~~Y~~VEENQTRDKHLNPN~~EW~~WKVYPSSVTEFKFLFVSGH
FKGN~~Y~~KAQLTRLNHNITNCNGAVLSVEELLIGGEMIKAGTLTLEEVR~~R~~KNFNNGEINF*

DZF16-R6 right full amino acid sequence (SEQ ID NO:444)

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKKRVGRLEPGEKPYKCPECGKSFS~~Q~~Q~~RS~~LVGHQRTHTGEKPY
KCPECGKSFS~~SK~~KDLTRHQRTHTGEKPYKCPECGKSFS~~TS~~SGHLVRHQRTHTGEKPYKCPECGKSFS~~Q~~RAH
LERHQRTHTGEKPYKCPECGKSFS~~TS~~SGSLVRHQRTHTGEKPYKCPECGKSFS~~TS~~SGNLVRHQRTHTGAAAR
ALVKSELEKKSEL~~RH~~KLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAI
YTVGSPIDYGVIVDTKAYSGGYNLPIGQADEM~~Q~~RYVKENQTRNKHINPN~~EW~~WKVYPSSVTEFKFLFVSGH
FKGN~~Y~~KAQLTRLNRKTN~~C~~NGAVLSVEELLIGGEMIKAGTLTLEEVR~~R~~KNFNNGEINF*

Fig. 35

E51C3 target site (SEQ ID NO:445):

5'-(t)ATCTGCCCCATGACTGSCGCAGGG(a)-3'
3'-(a)TAGACGGGTACTGACCCCGTCCC(t)-5'

E51C-3L left full amino acid sequence (SEQ ID NO:446)

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKKRVGRLEPGE~~RP~~FQCRICMRNFS~~SK~~QALAVBTRTHTGEK~~PF~~
QCRICMRNFS~~Q~~STTLKRHLRTHTEK~~PF~~QCRICMRNFS~~RS~~DHLSLHLKTHL~~GS~~QLVKSELEKKSEL~~RH~~
KLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYTVGSPIDYGVIVDTK
AYSGGYNLPIGQADEM~~Q~~RYVKENQTRNKHINPN~~EW~~WKVYPSSVTEFKFLFVSG~~B~~FKGN~~Y~~KAQLTRLNRKTN
CNGAVLSVEELLIGGEMIKAGTLTLEEVR~~R~~KNFNNGEINF*

E51C-3R right full amino acid sequence (SEQ ID NO:447)

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKKRVGRLEPGE~~RP~~FQCRICMRNFS~~RR~~ABLQNHTEK~~PF~~
QCRICMRNFS~~Q~~STTLKRHLRTHTEK~~PF~~QCRICMRNFS~~DS~~GGLTBHLKTHL~~GS~~QLVKSELEKKSEL~~RH~~
KLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYTVGSPIDYGVIVDTK
AYSGGYNLPIGQADEM~~Q~~RYVEENQTRDKHLNPN~~EW~~WKVYPSSVTEFKFLFVSG~~B~~FKGN~~Y~~KAQLTRLNHNIT
NCNGAVLSVEELLIGGEMIKAGTLTLEEVR~~R~~KNFNNGEINF*

Fig. 36

DZF15 target site (SEQ ID NO:448):

5'-ACTAGAAATGCCATCTTCCTTGATGTTGGAGGTACCTGCTCT-3'
 3'-TGATCTTTACGGTAGAAGGAACACTACAACCTCCATGGACGAGA-5'

DZF15-L6 left full amino acid sequence (SEQ ID NO:449)

MDYKDHDG DYKDDDI DYKDDDDKMAPK KKRKVGRLEPGEKPYK CPECGKSFS SRRTTLTNNHQRTH TGEKPY
 KCPECGKSFS SQENALAGHQRTH TGEKPYK CPECGKSFS SHENALQNHQRTH TGEKPYK CPECGKSFS DPGH
 LVRHQRTH TGEKPYK CPECGKSFS STSGNLVRHQRTH TGEKPYK CPECGKSFS QSNNLVRHQRTH TGAAR
 ALVKSELEKKSEL RHKLKYVPHEY IELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAI
 YTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMEERYVEENQTRDKHLNPNNEWKVPSSVTEFKELFVSGH
 FKGNYKAQLTRLNHI TNCGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF*

DZF15-R6 right full amino acid sequence (SEQ ID NO:450)

MDYKDHDG DYKDDDI DYKDDDDKMAPK KKRKVGRLEPGEKPYK CPECGKSFS SQENALAGHQRTH TGEKPY
 KCPECGKSFS QQRSLVGHQRTH TGEKPYK CPECGKSFS DKKDLTRHQRTH TGEKPYK CPECGKSFS STSGH
 LVRHQRTH TGEKPYK CPECGKSFS QRAHLERHQRTH TGEKPYK CPECGKSFS STSGSLVRHQRTH TGAAR
 ALVKSELEKKSEL RHKLKYVPHEY IELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAI
 YTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKH INPNNEWKVPSSVTEFKELFVSGH
 FKGNYKAQLTRLNKTNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF*

DZF15-L5 left full amino acid sequence (SEQ ID NO:451)

MDYKDHDG DYKDDDI DYKDDDDKMAPK KKRKVGRLEPGEKPYK CPECGKSFS SQENALAGHQRTH TGEKPY
 KCPECGKSFS SHENALQNHQRTH TGEKPYK CPECGKSFS DPGHLVRHQRTH TGEKPYK CPECGKSFS STSGN
 LVRHQRTH TGEKPYK CPECGKSFS QSNNLVRHQRTH TGAARALVKSELEKKSEL RHKLKYVPHEYIEL
 IEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQ
 ADEMEERYVEENQTRDKHLNPNNEWKVPSSVTEFKELFVSGHFKGNYKAQLTRLNHI TNCGAVLSVEEL
 LIGGEMIKAGTLTLEEVRKFNNGEINF*

DZF15-R5 right full amino acid sequence (SEQ ID NO:452)

MDYKDHDG DYKDDDI DYKDDDDKMAPK KKRKVGRLEPGEKPYK CPECGKSFS QQRSLVGHQRTH TGEKPY
 KCPECGKSFS DEKDLTRHQRTH TGEKPYK CPECGKSFS STSGHLVRHQRTH TGEKPYK CPECGKSFS QPAH
 LERHQRTH TGEKPYK CPECGKSFS STSGSLVRHQRTH TGAARALVKSELEKKSEL RHKLKYVPHEYIEL
 IEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQ
 ADEMQRYVKENQTRNKH INPNNEWKVPSSVTEFKELFVSGHFKGNYKAQLTRLNKTNCNGAVLSVEEL
 LIGGEMIKAGTLTLEEVRKFNNGEINF*

Fig. 37

E51C4 target site (SEQ ID NO:453):

5' - (t) GCCATCTTCTCTTGATGTTGGAGGT (a) -3'

3' - (a) CGGTAGAAGGAACCTACAACCTCCA (t) -5'

E51C-4L left full amino acid sequence (SEQ ID NO:454)

MDYKDHDGDDYKDHDIDYKDDDDKMAPKKKRVGRLEPGERPFQCRICMRNFSSEPSKLARHTRTHTGEKPF
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RHKLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGSSRKFDGAIYTVGSPIDYGVIVD
TKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGBFKGNKYAQLTRLNR
KTNCGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF*

E51C-4R right full amino acid sequence (SEQ ID NO:455)

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RHKLKYVPHEYIELIEIARNPTQDRILEMKVMEFFMEVYGYRGEHLGSSRKFDGAIYTVGSPIDYGVIVD
TKAYSGGYNLPIGQADEMERYVEENQTRDKHLNPNNEWWKVYPSSVTEFKFLFVSGBFKGNKYAQLTRLNH
ITNCGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF*

Fig. 38

"Single vector, multiplex CRISPR system"



"Dual vector, multiplex CRISPR system"



"Single vector, single gRNA system"

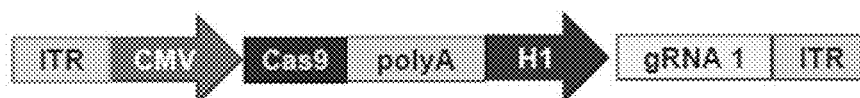


Fig. 39

SaCas9-NLS (NLS underlined) (SEQ ID NO: 459)

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SaCas9 gRNA (SEQ ID NO: 460)

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Fig. 40

NmCas9 (NLS 1 underlined NLS 2 underlined/bold, HA tag bold) (SEQ ID NO: 451)

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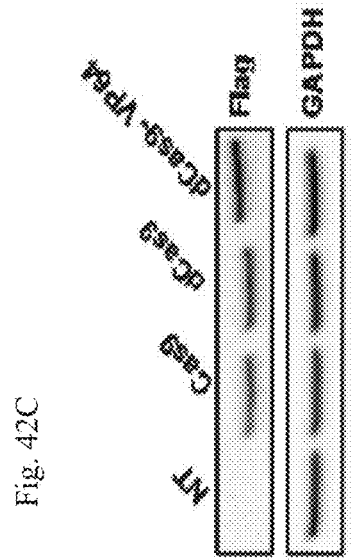
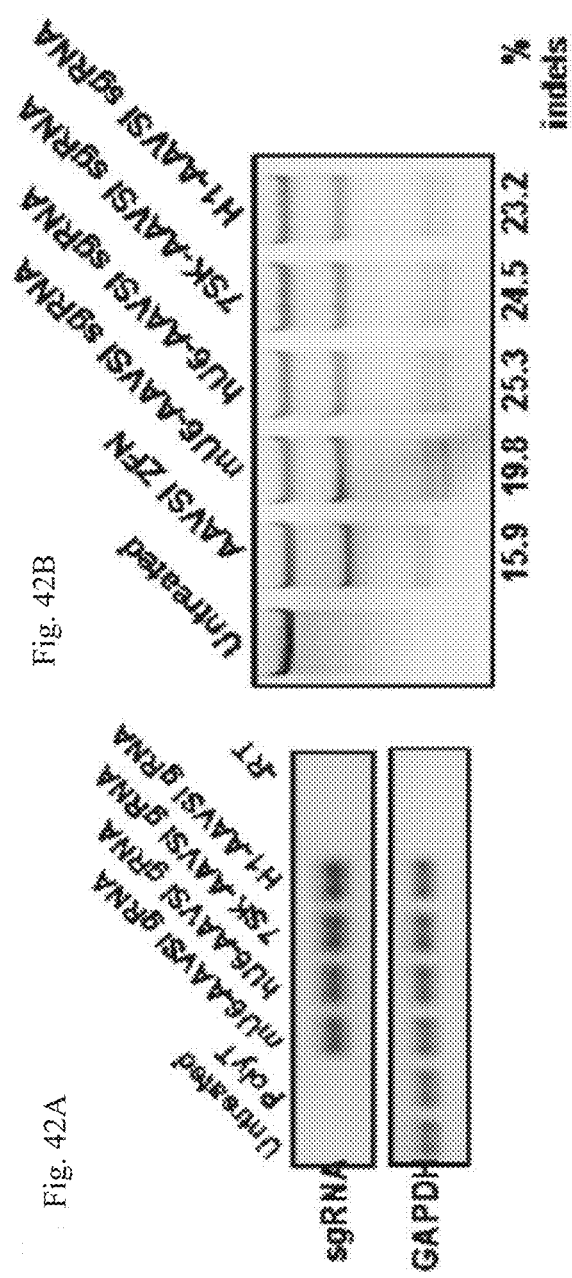
NmCas9 short hairpin from Thomson FNAS 2013 (SEQ ID NO: 462)

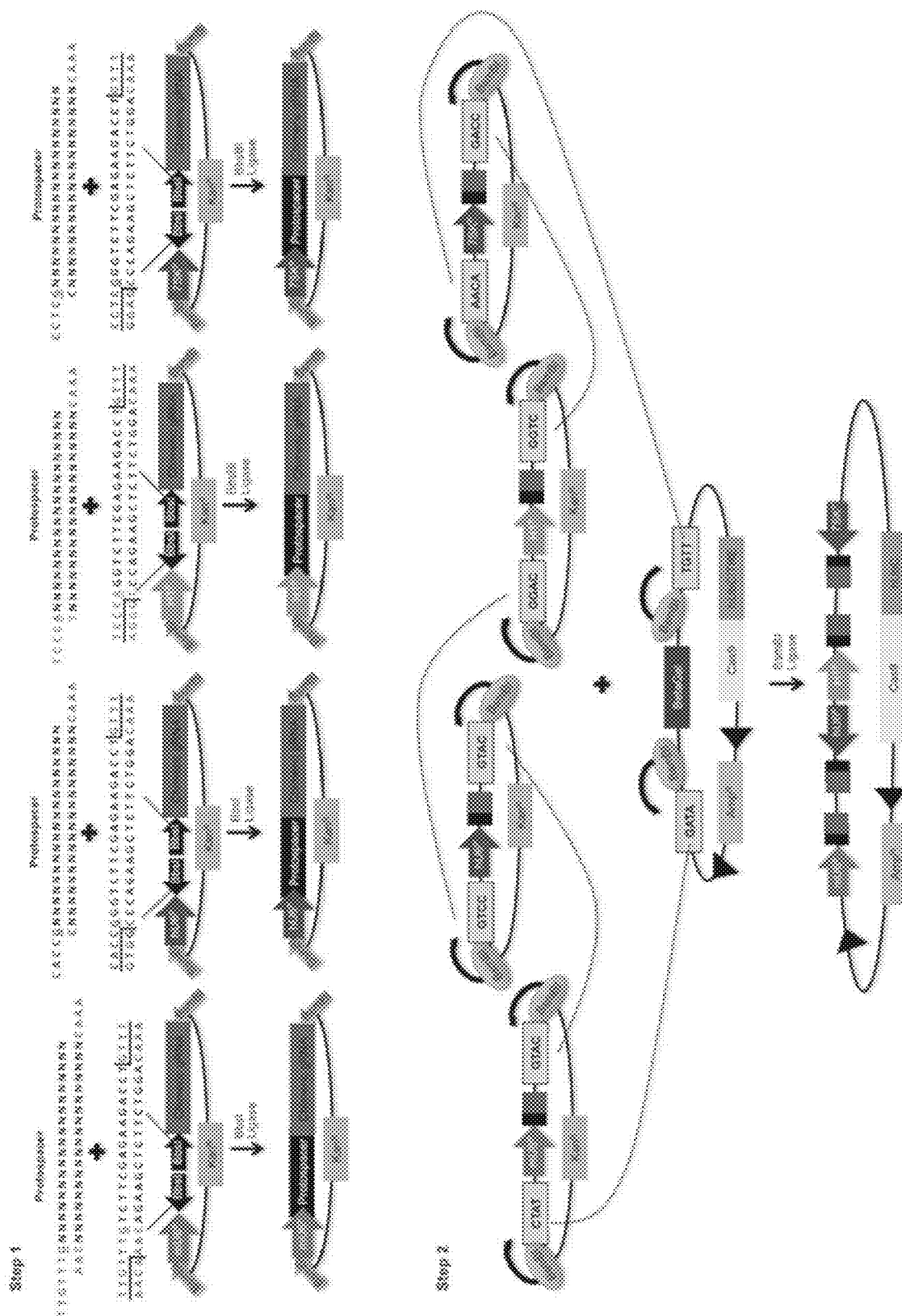
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TT

Fig. 41





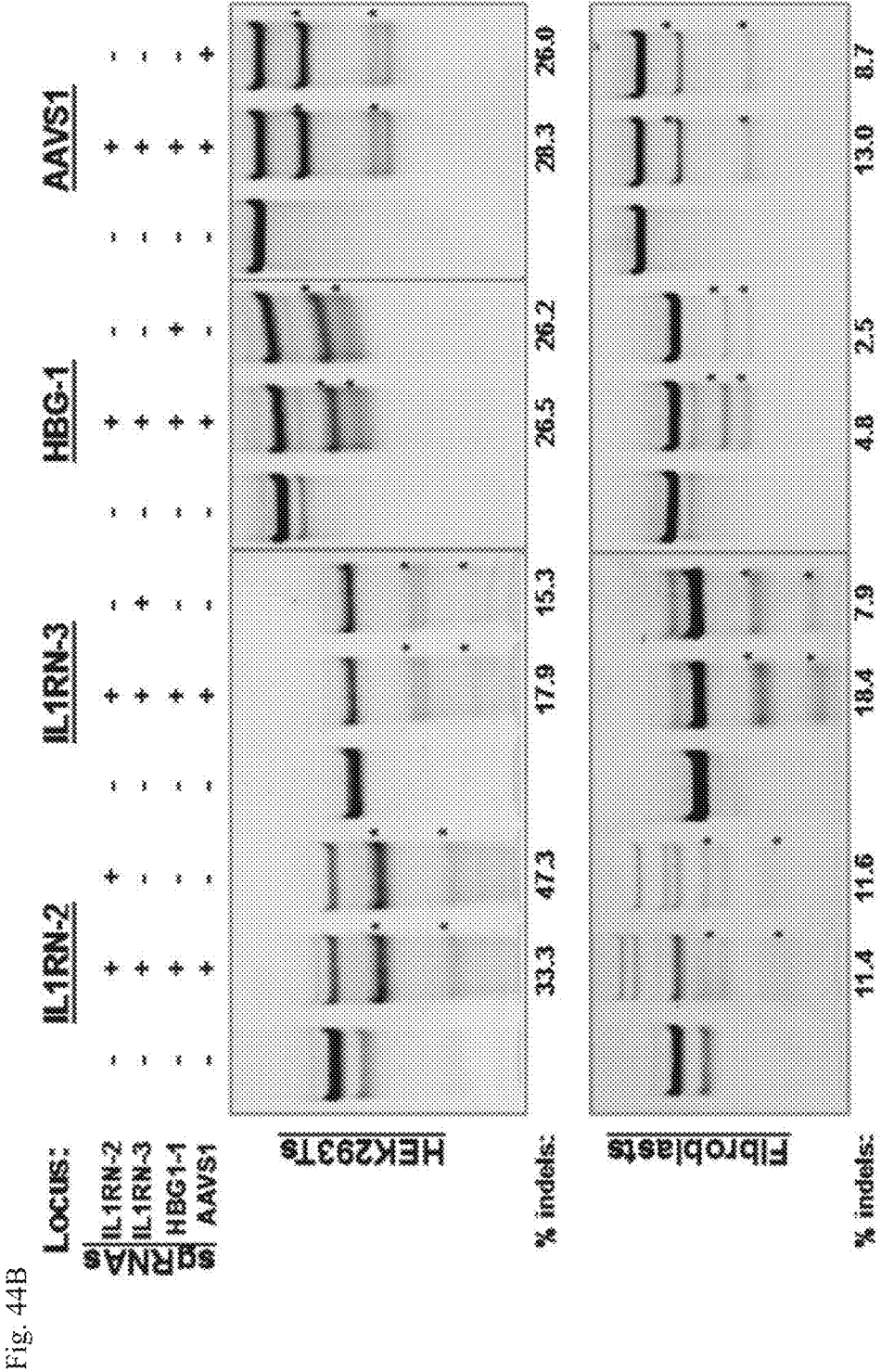
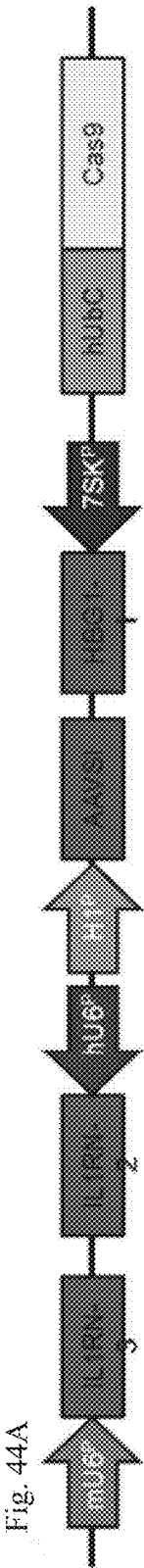


Fig. 45B

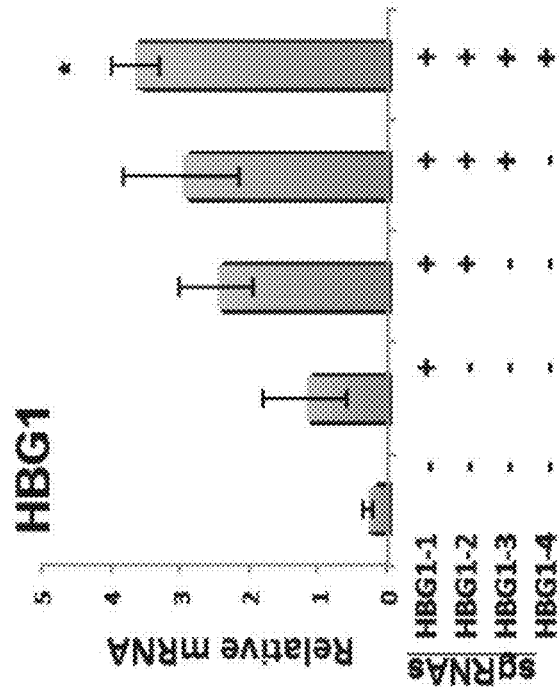


Fig. 45A

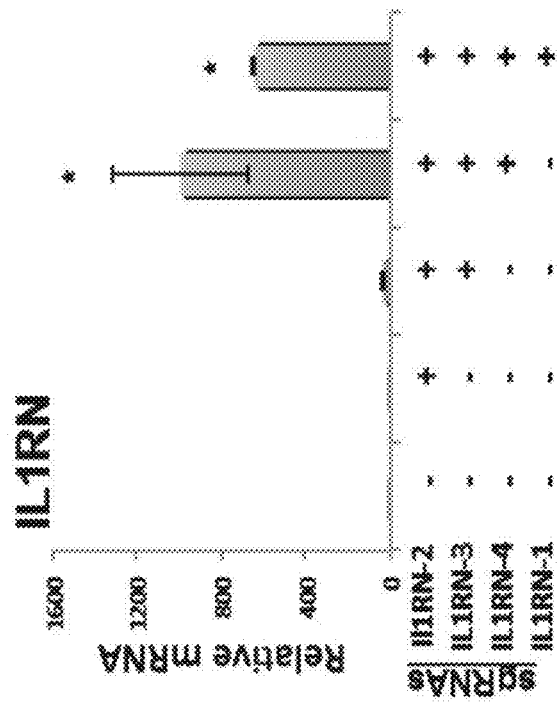


Fig. 45D

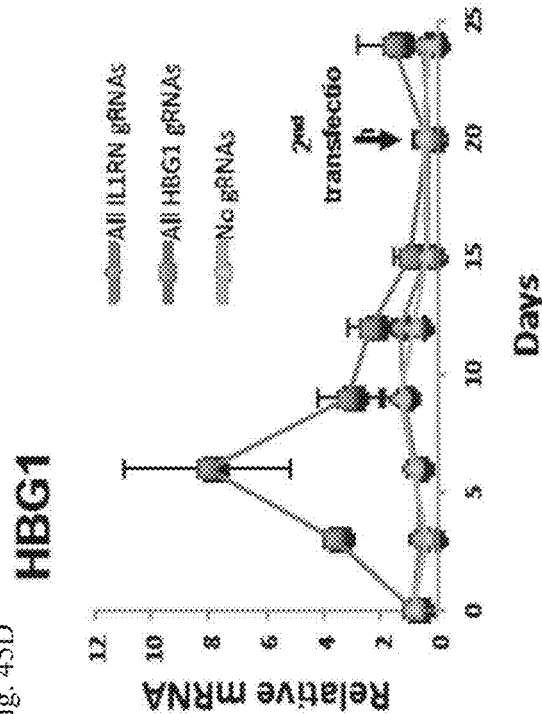
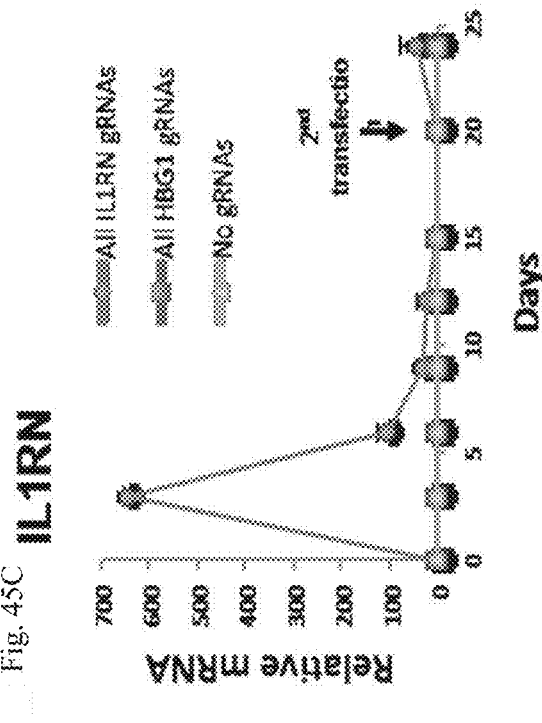


Fig. 45C



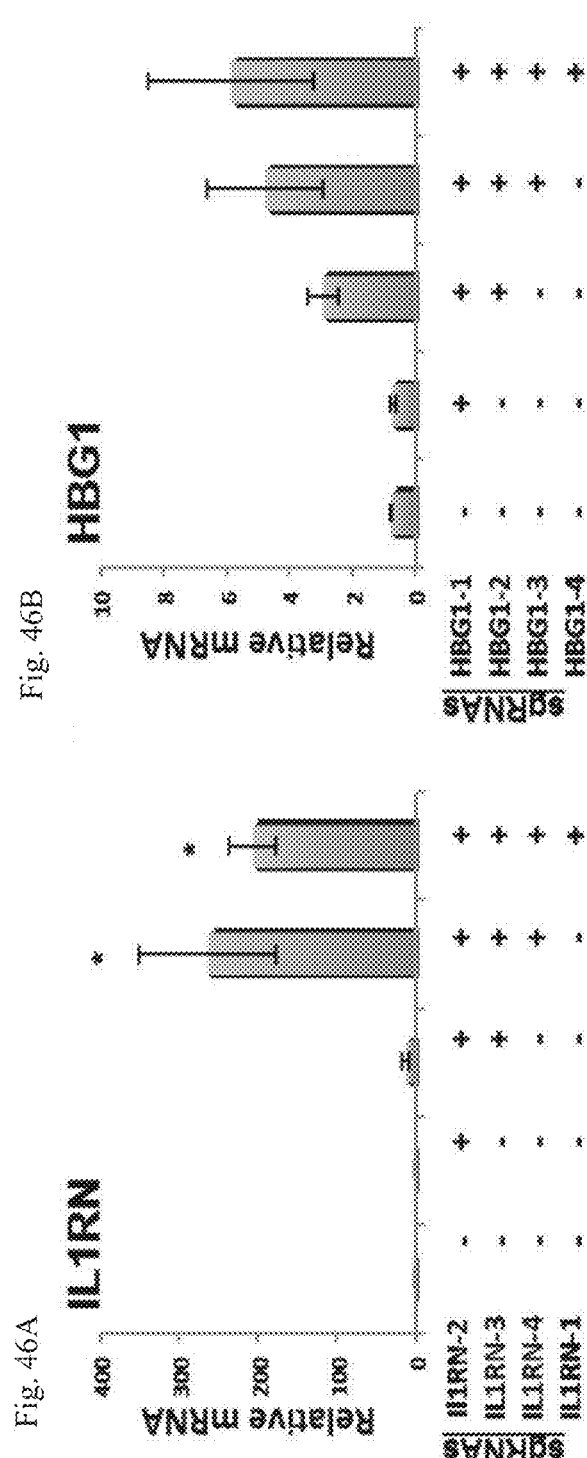
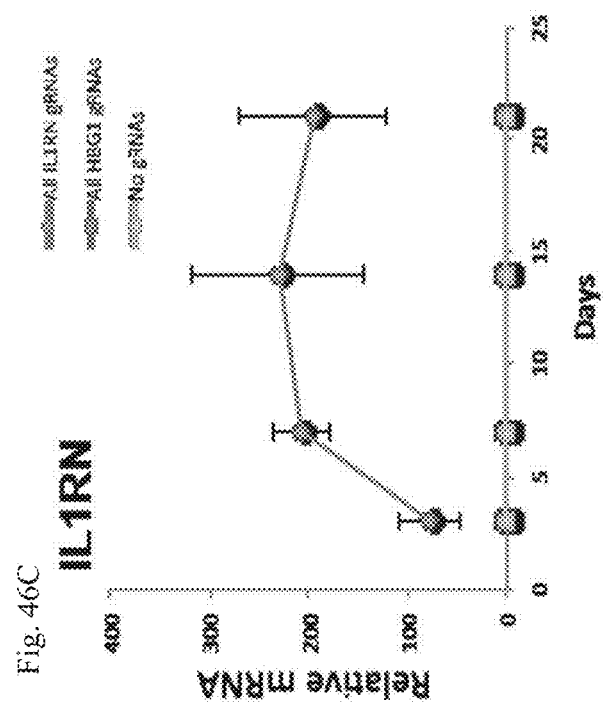
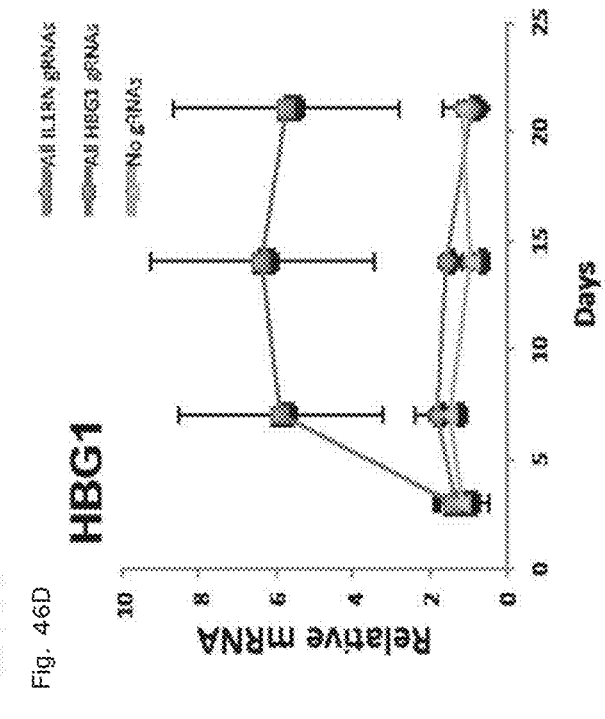
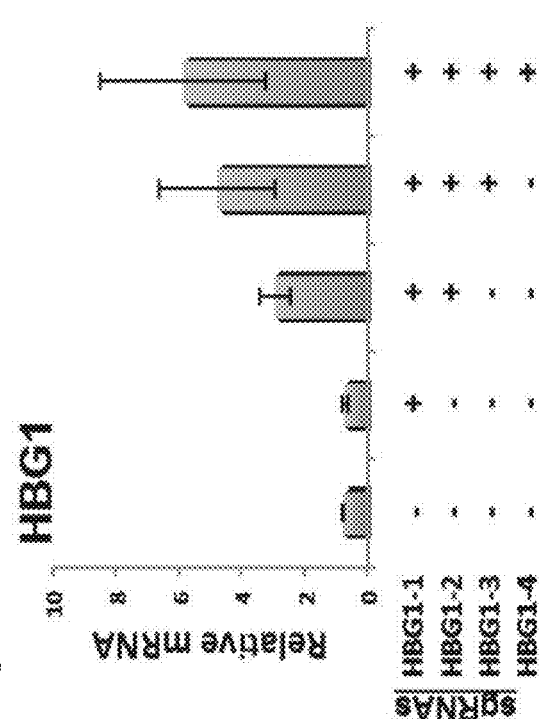


Fig. 46B



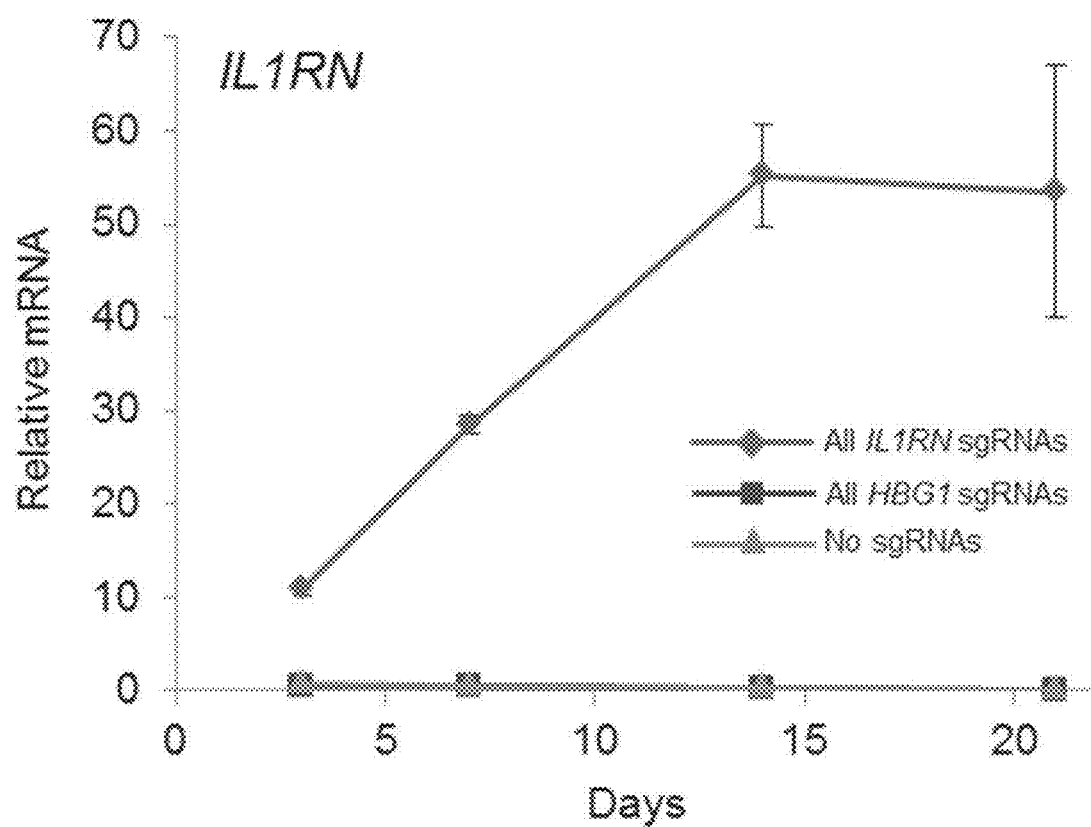


Fig. 47

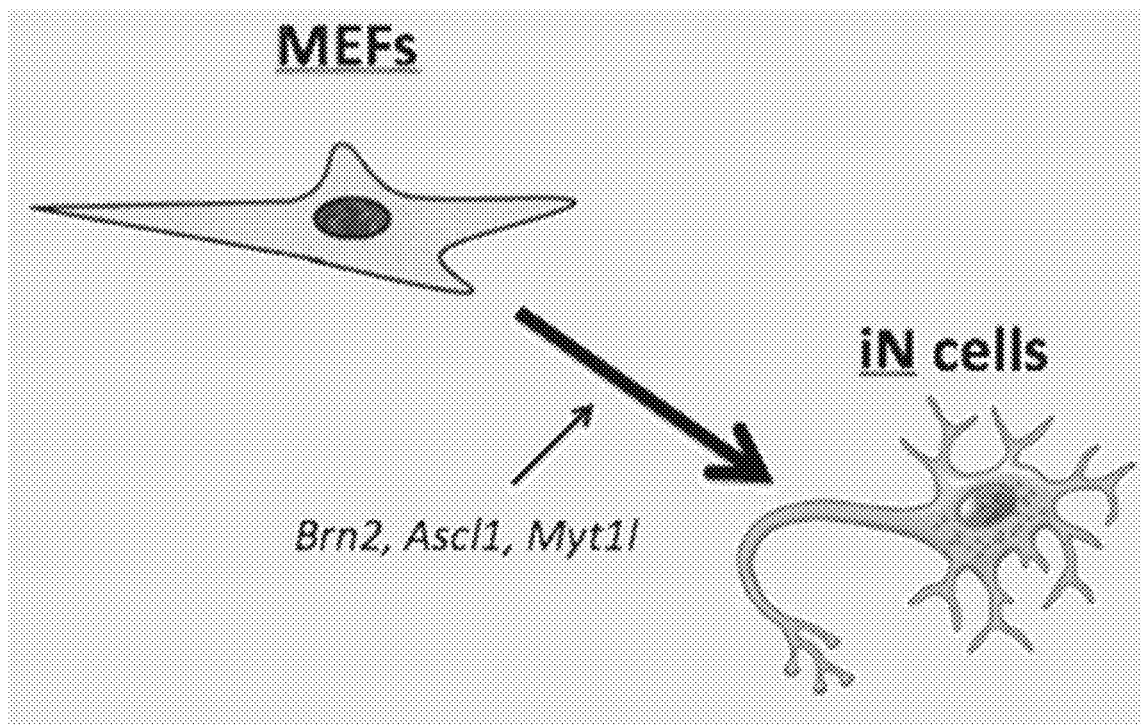


Fig. 48

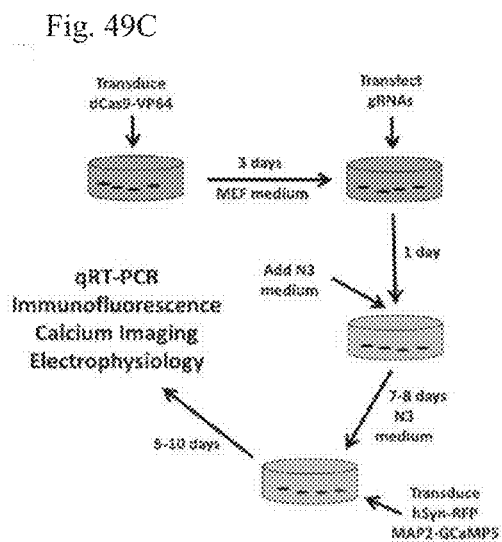
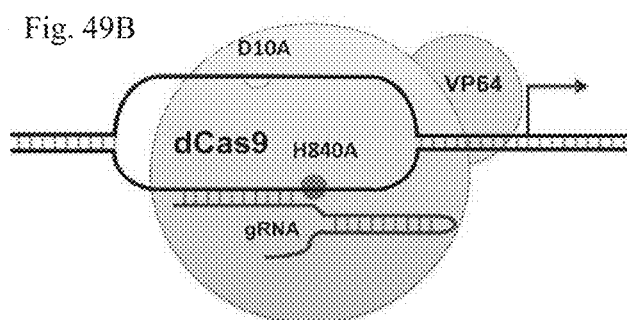
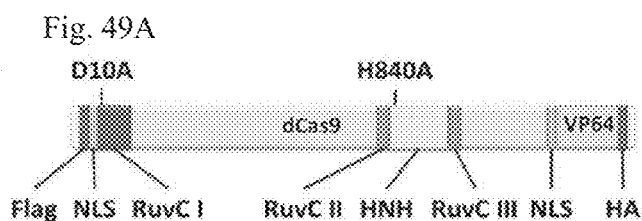


Fig. 50A

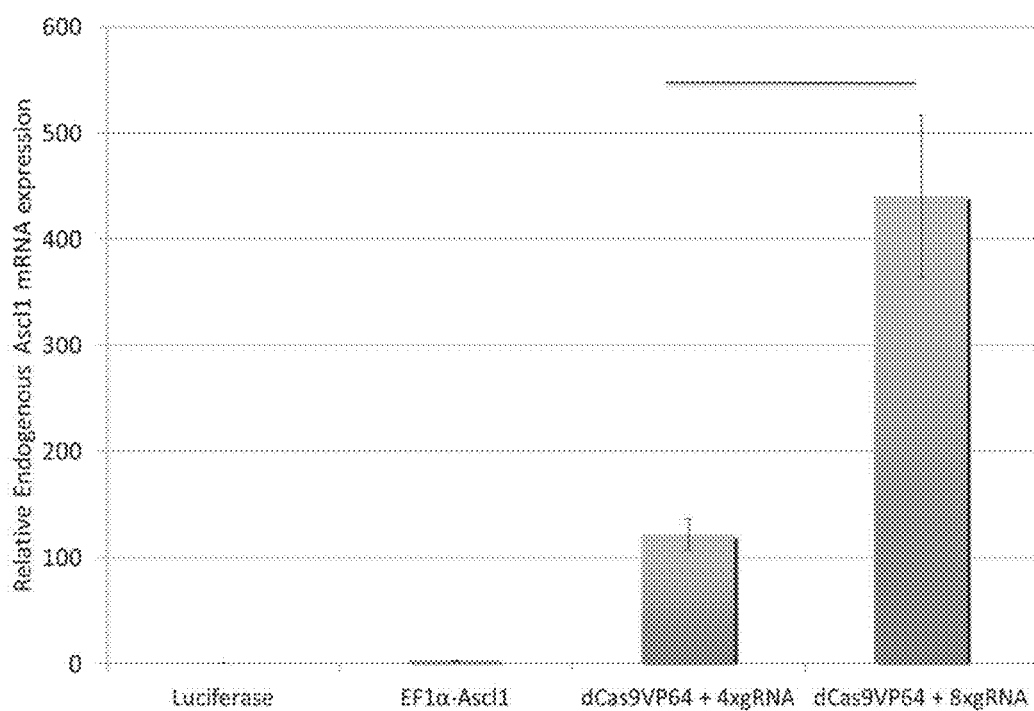


Fig. 50B

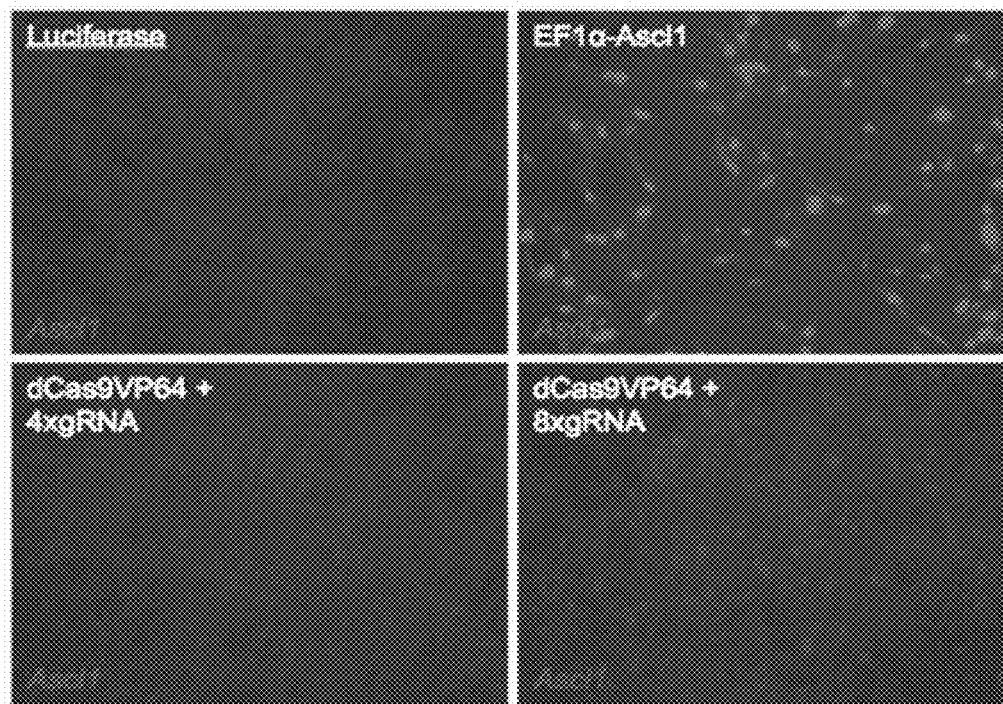


Fig. 51A

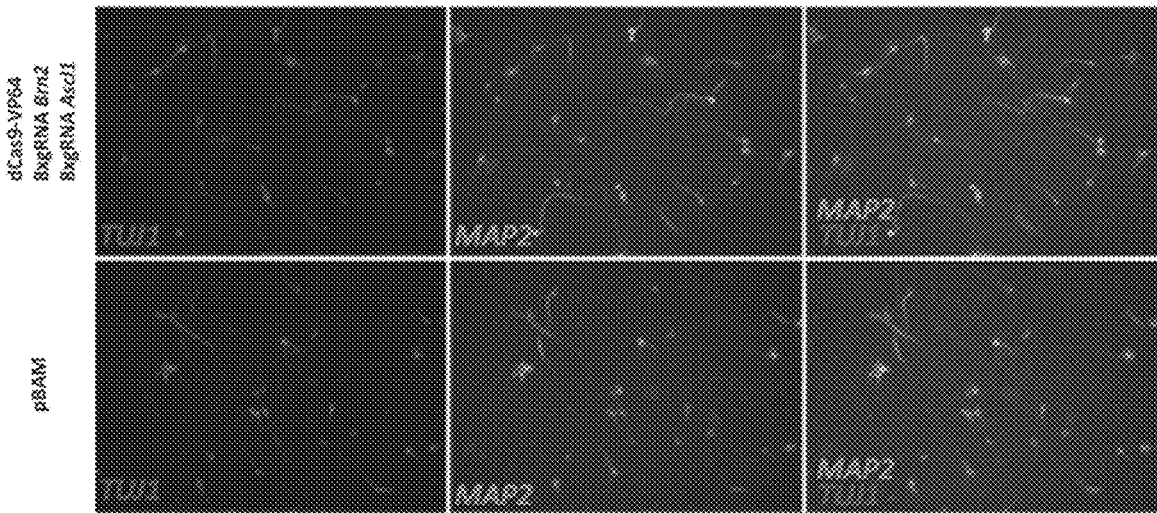


Fig. 51B

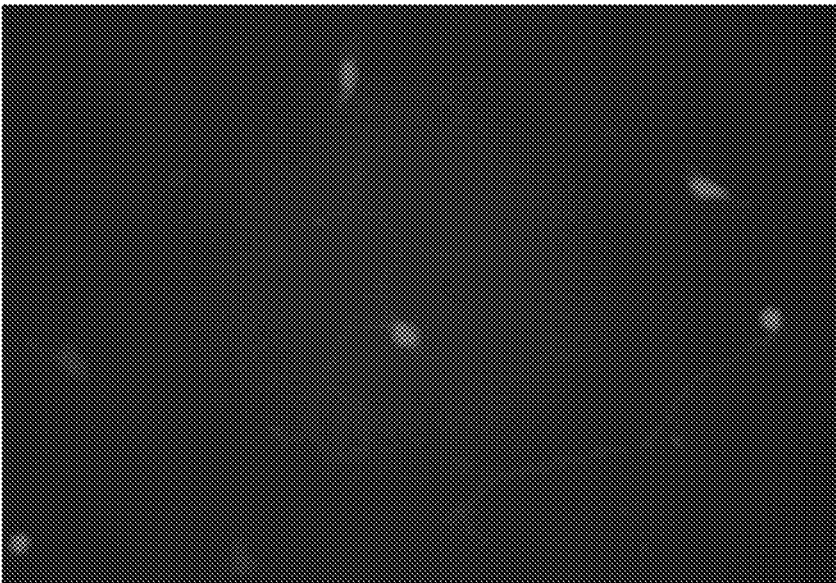


Fig. 52A

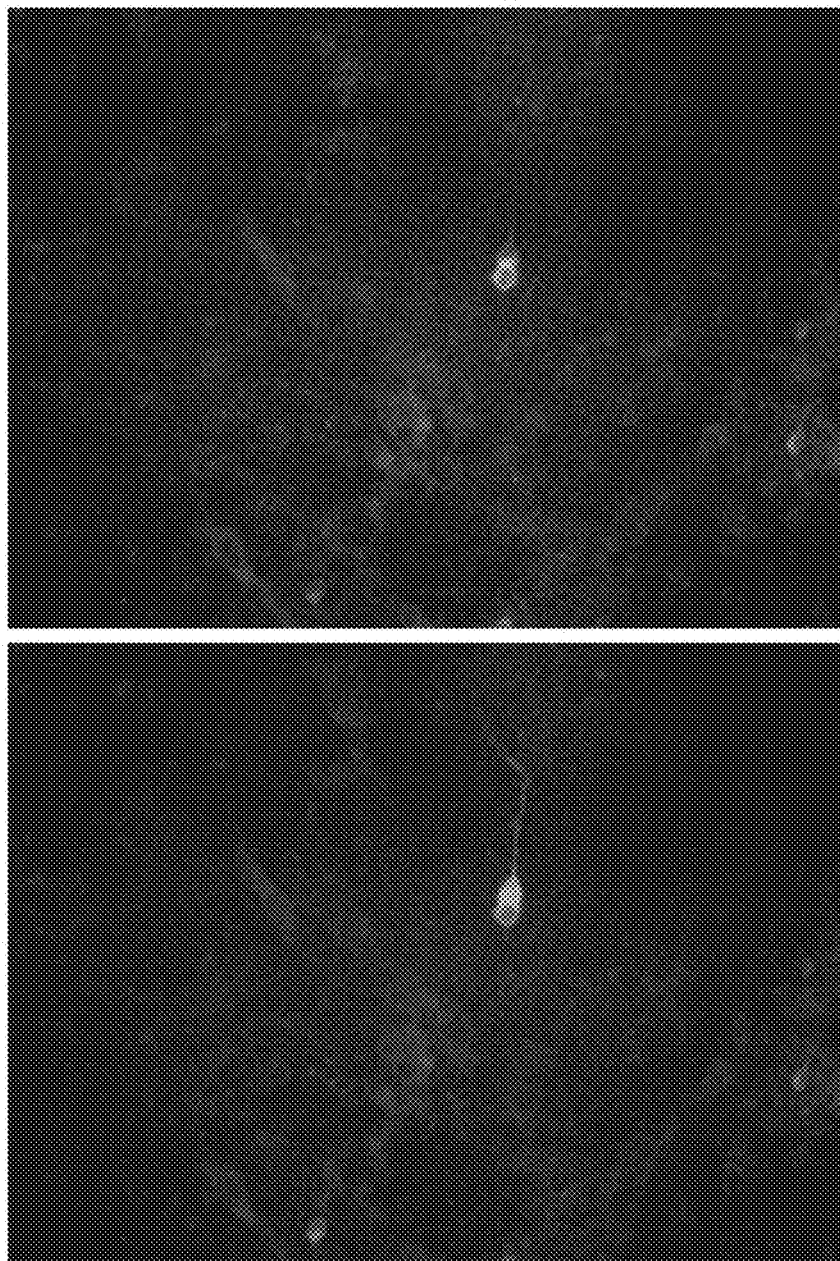


Fig. 52B

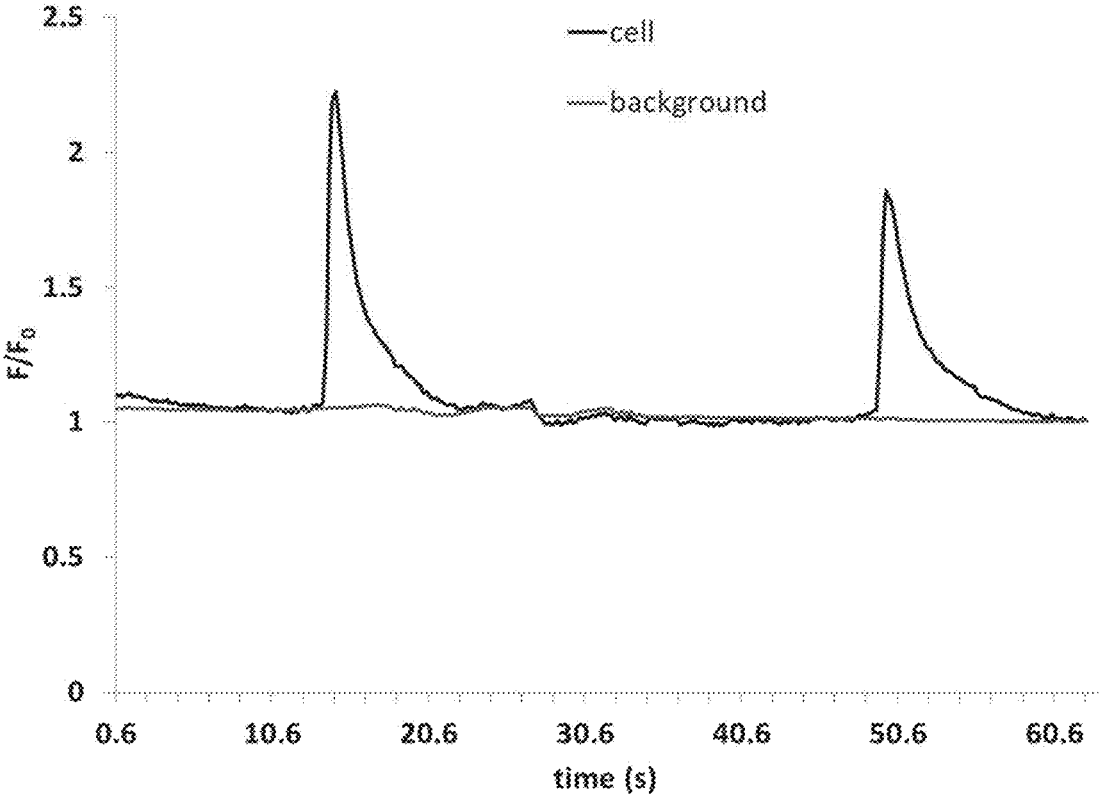


Fig. 53A

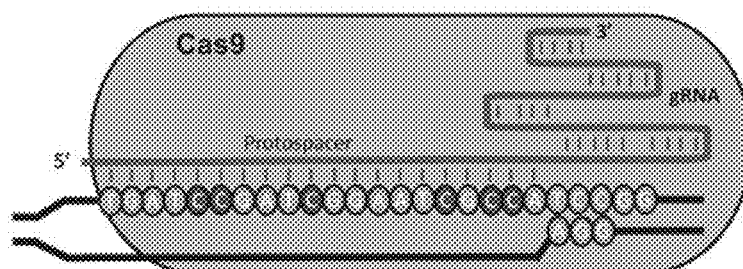


Fig. 53B

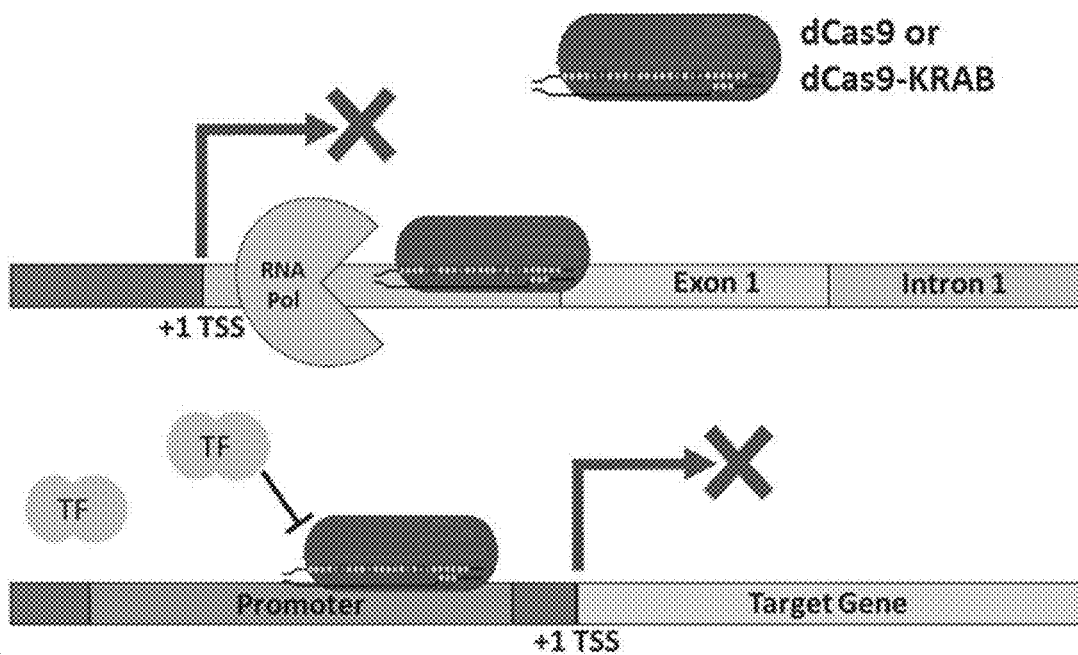


Fig. 53C



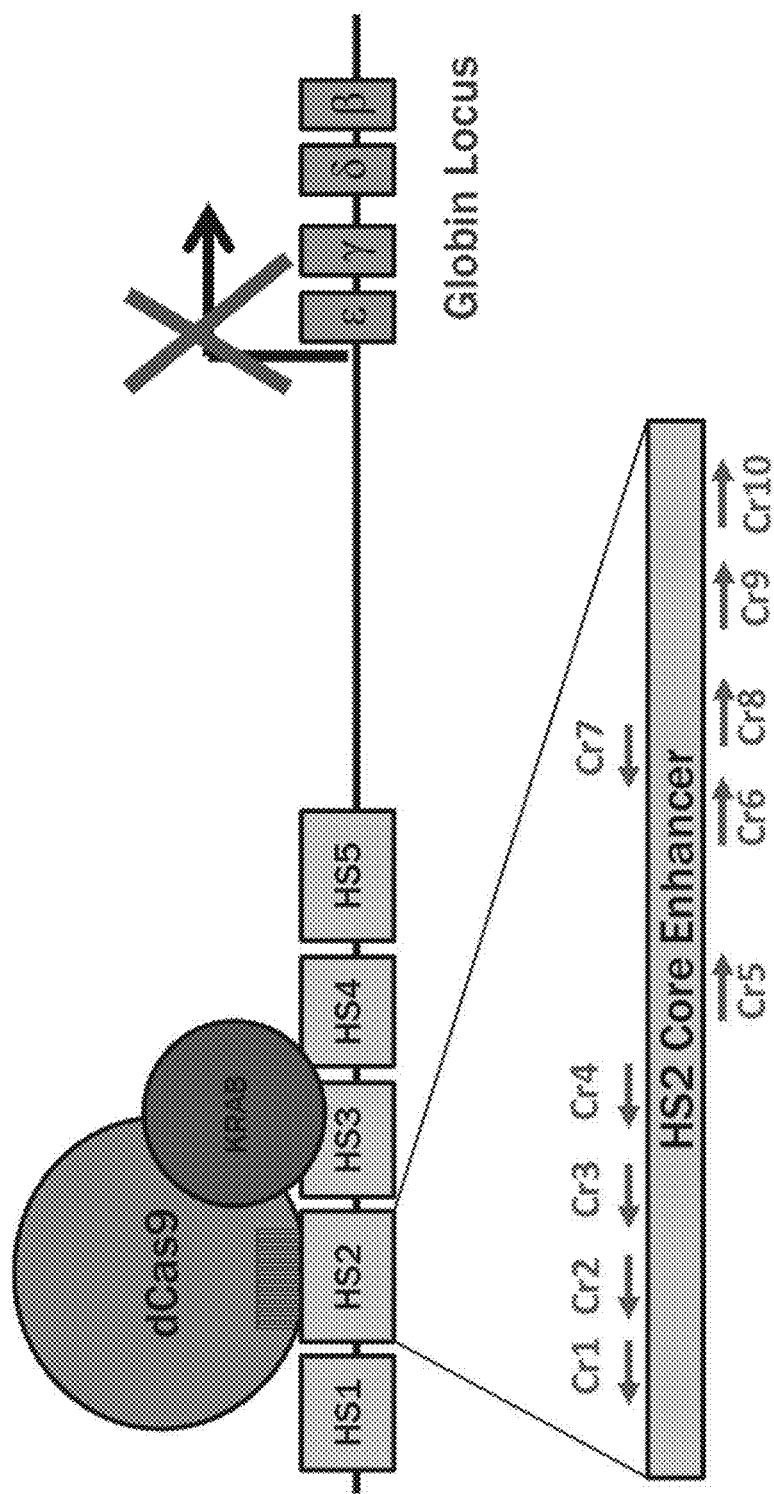
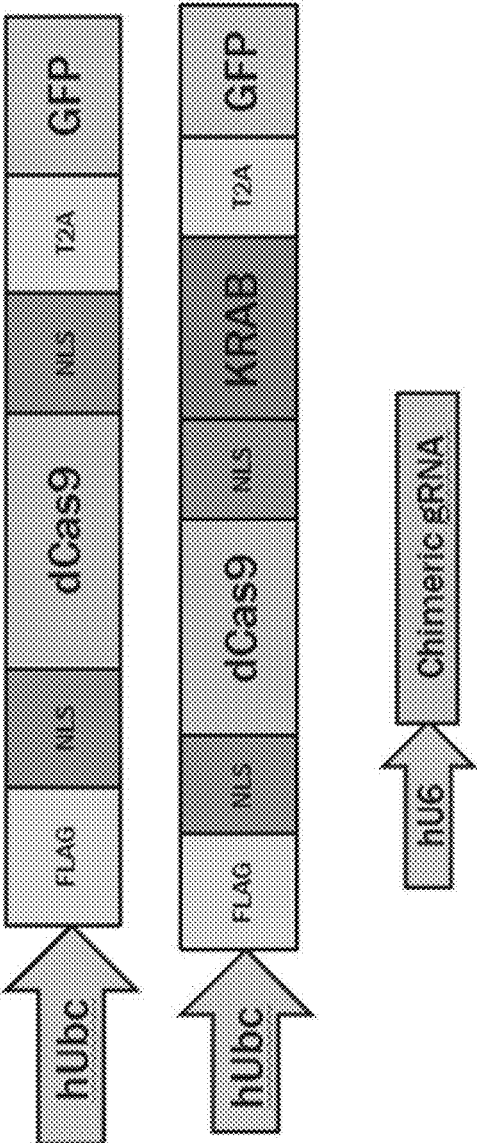
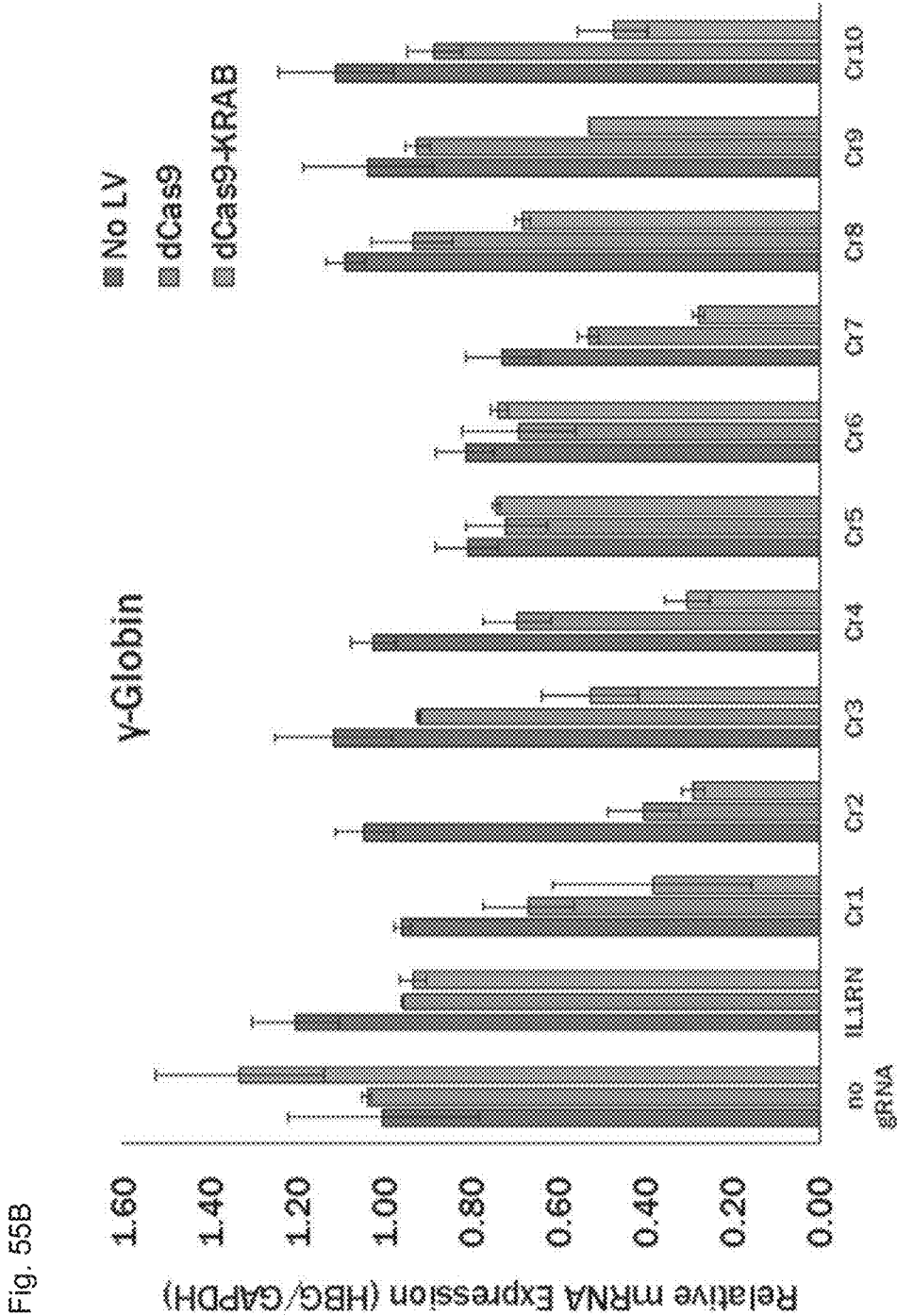


Fig. 54

Fig. 55A





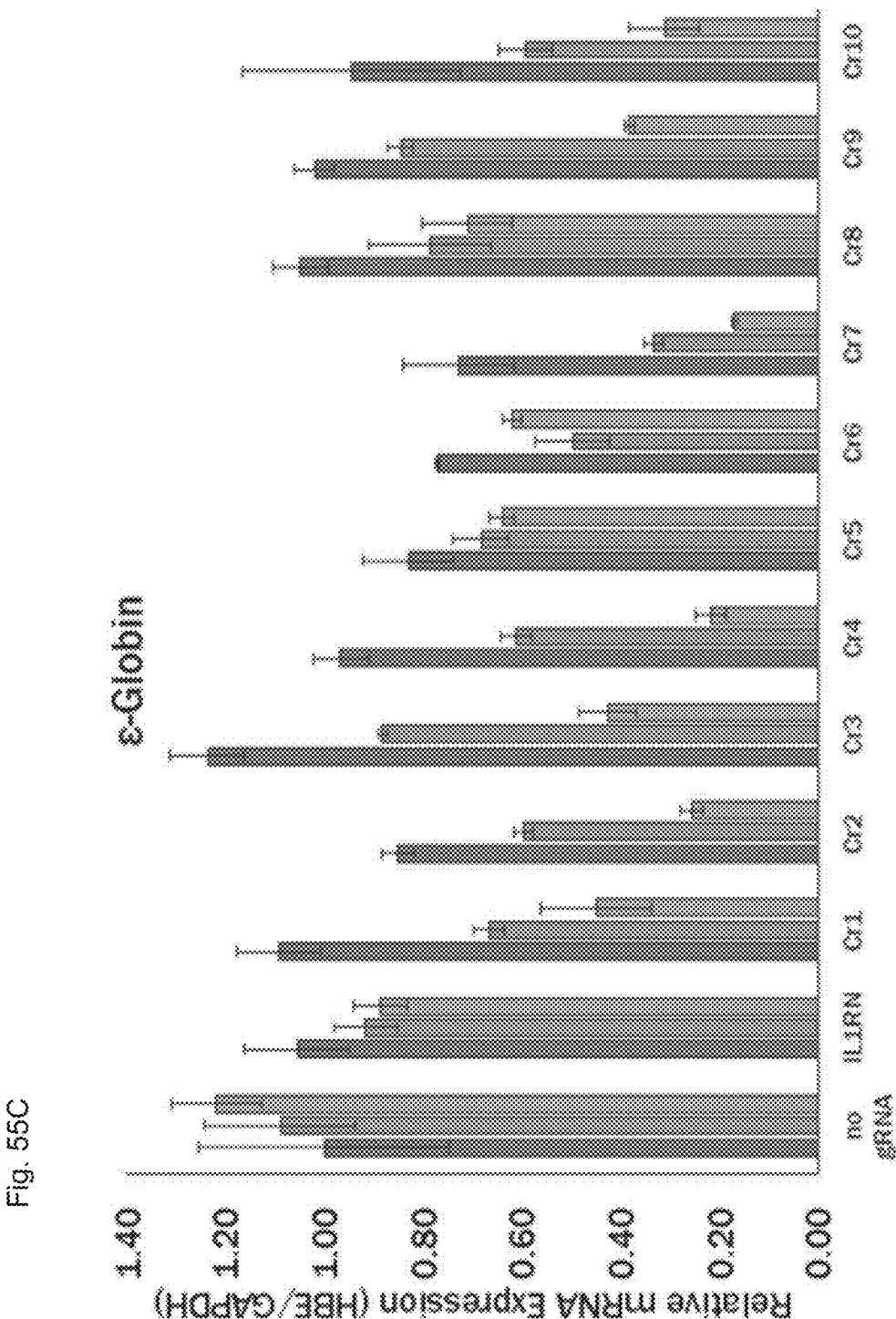
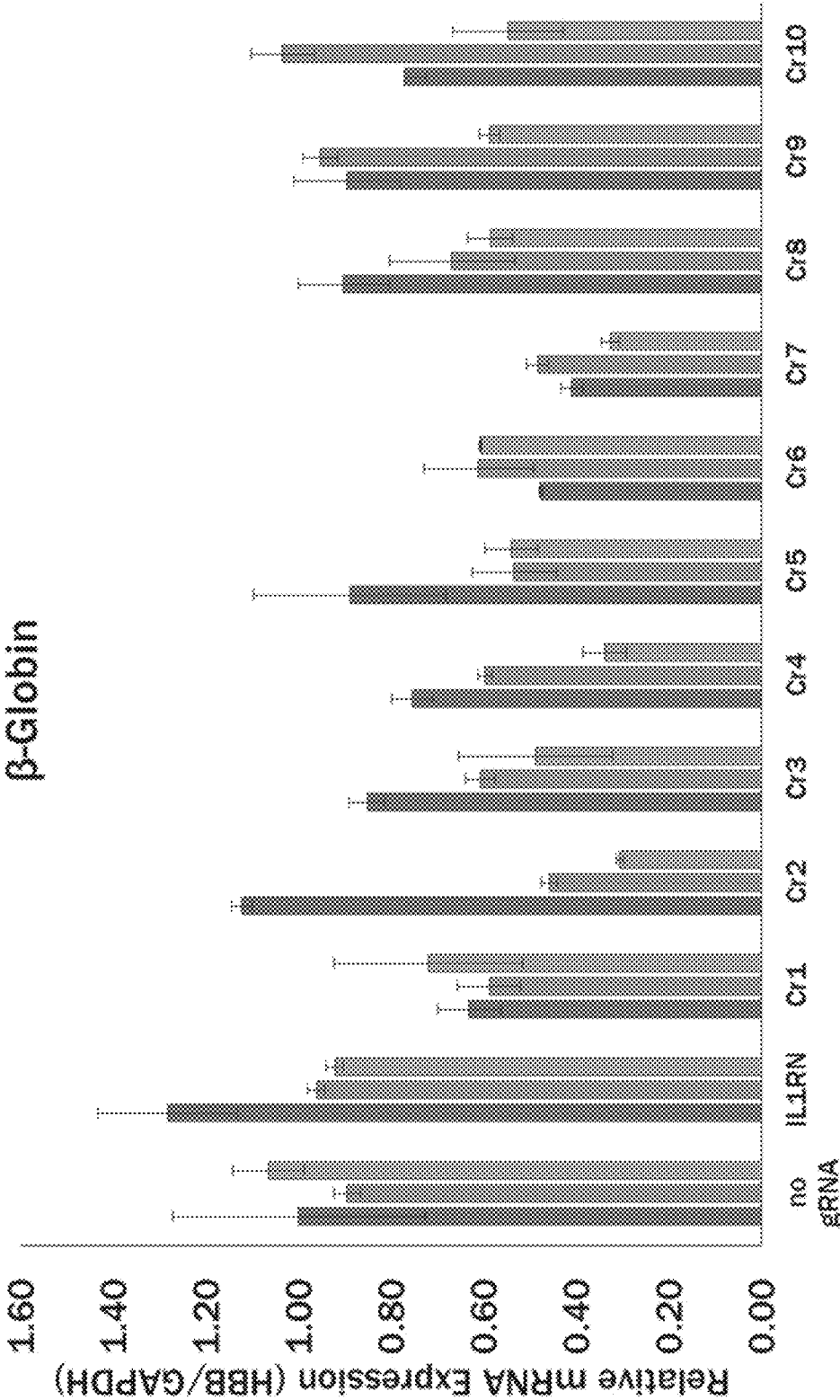


Fig. 5SD



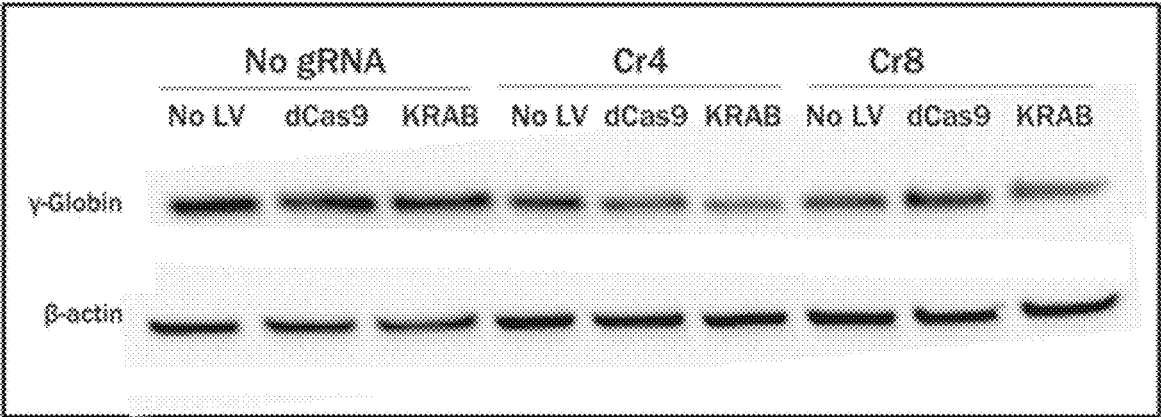


Fig. 55E

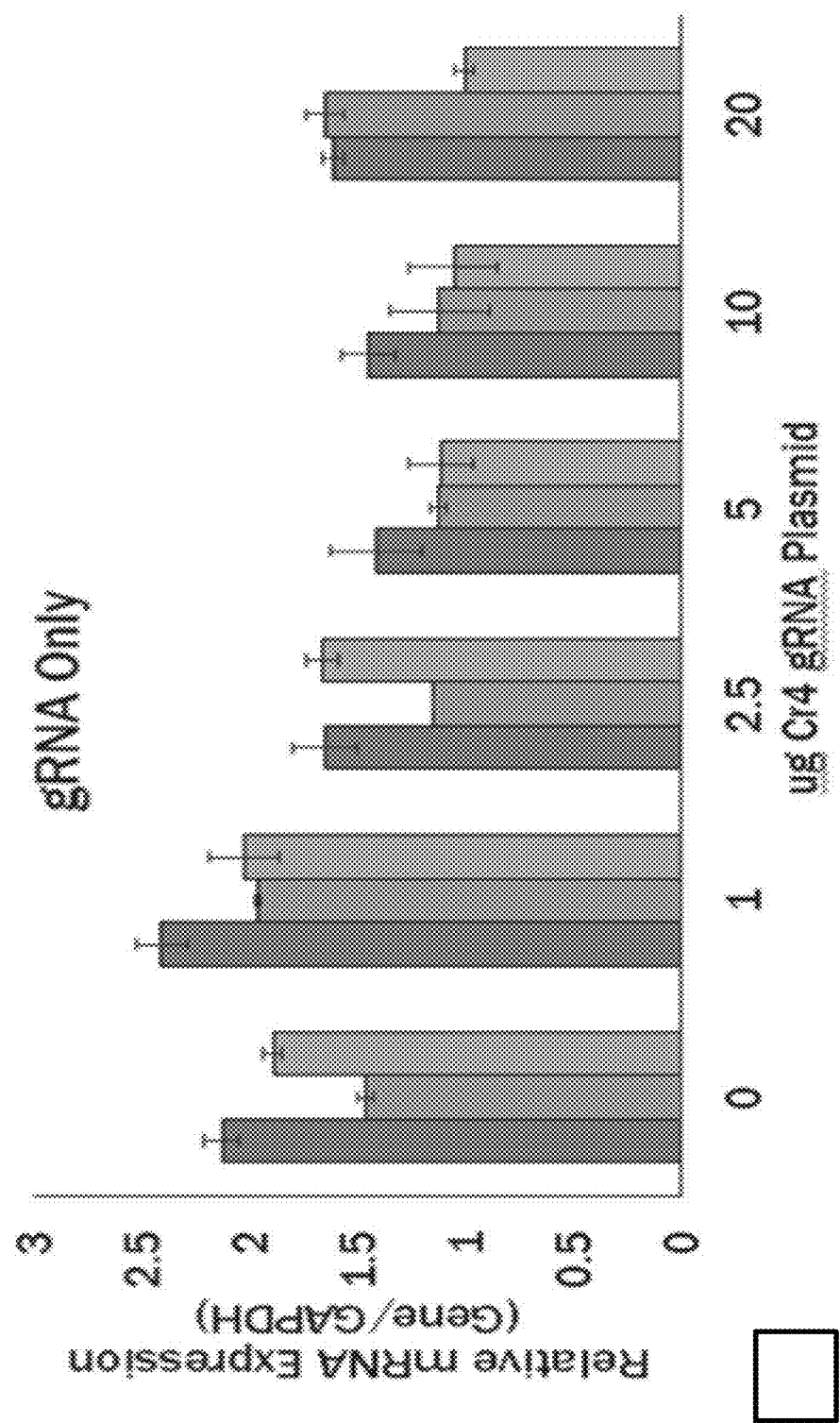


Fig. 56A

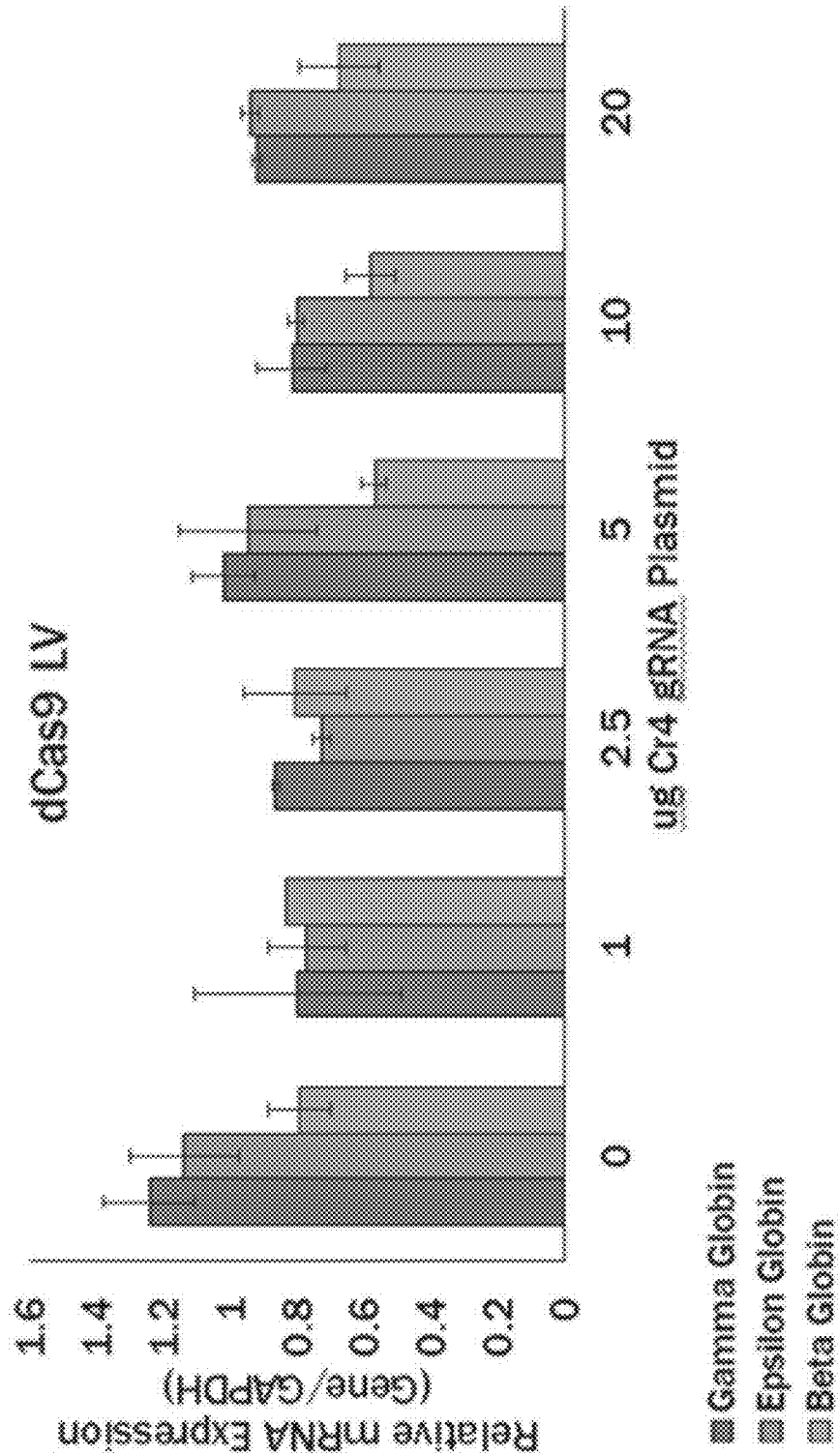


Fig. 56B

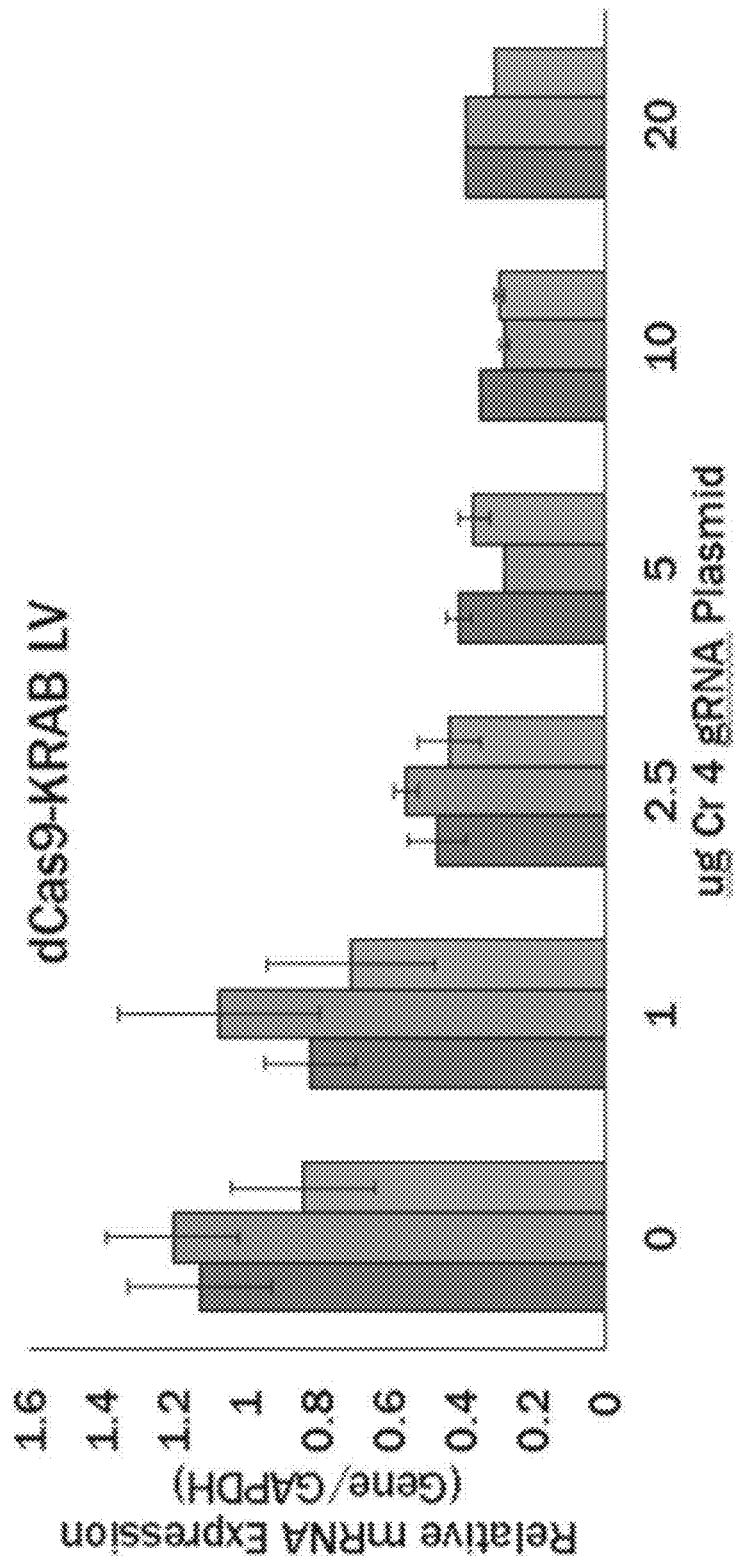


Fig. 56C

Fig. 57A

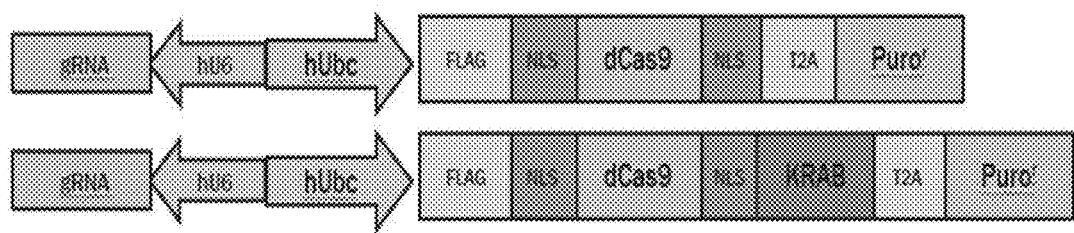


Fig. 57B

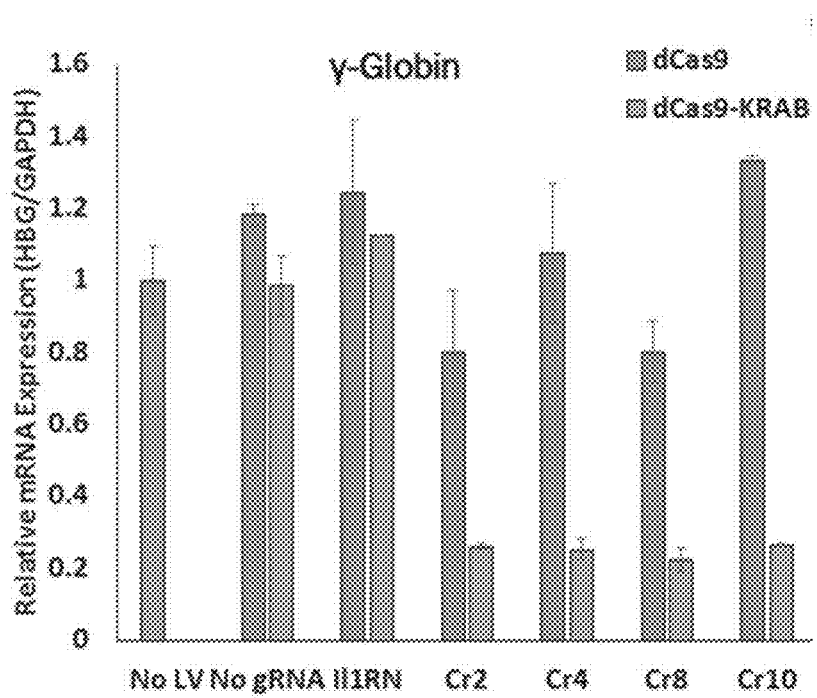


Fig. 57C

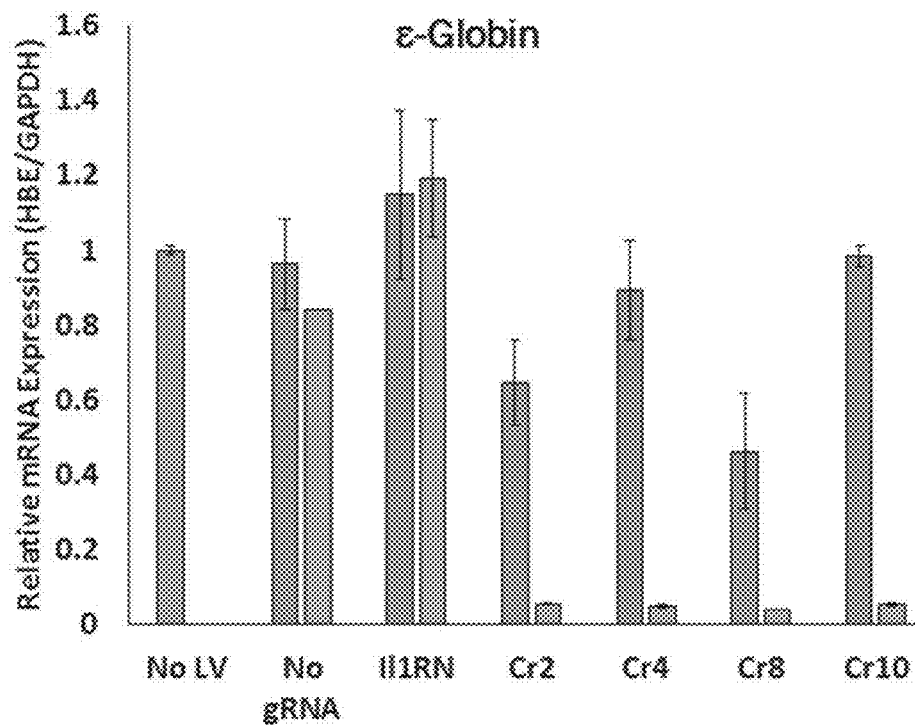
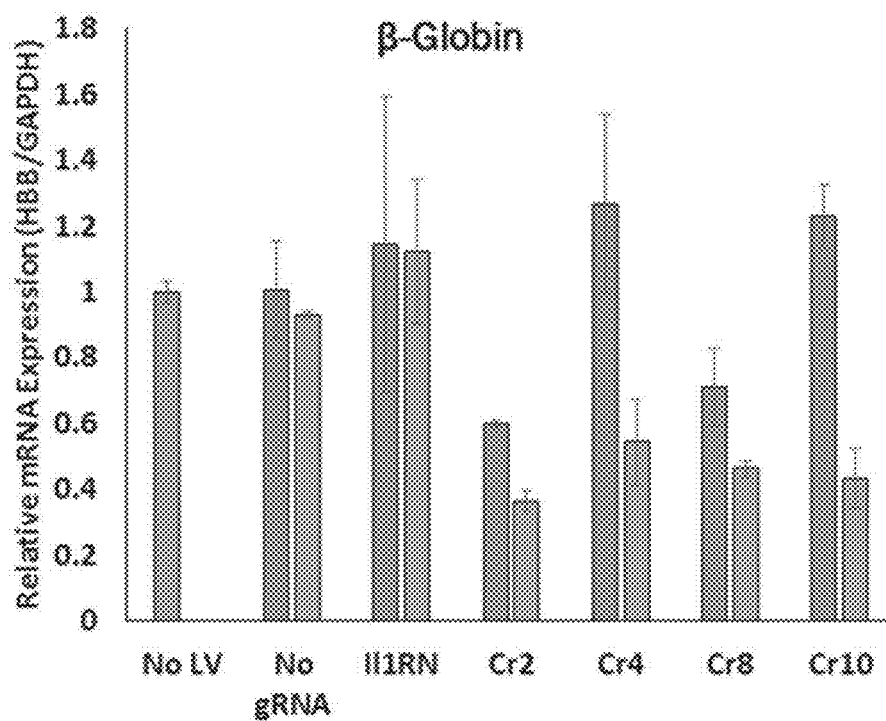


Fig. 57D



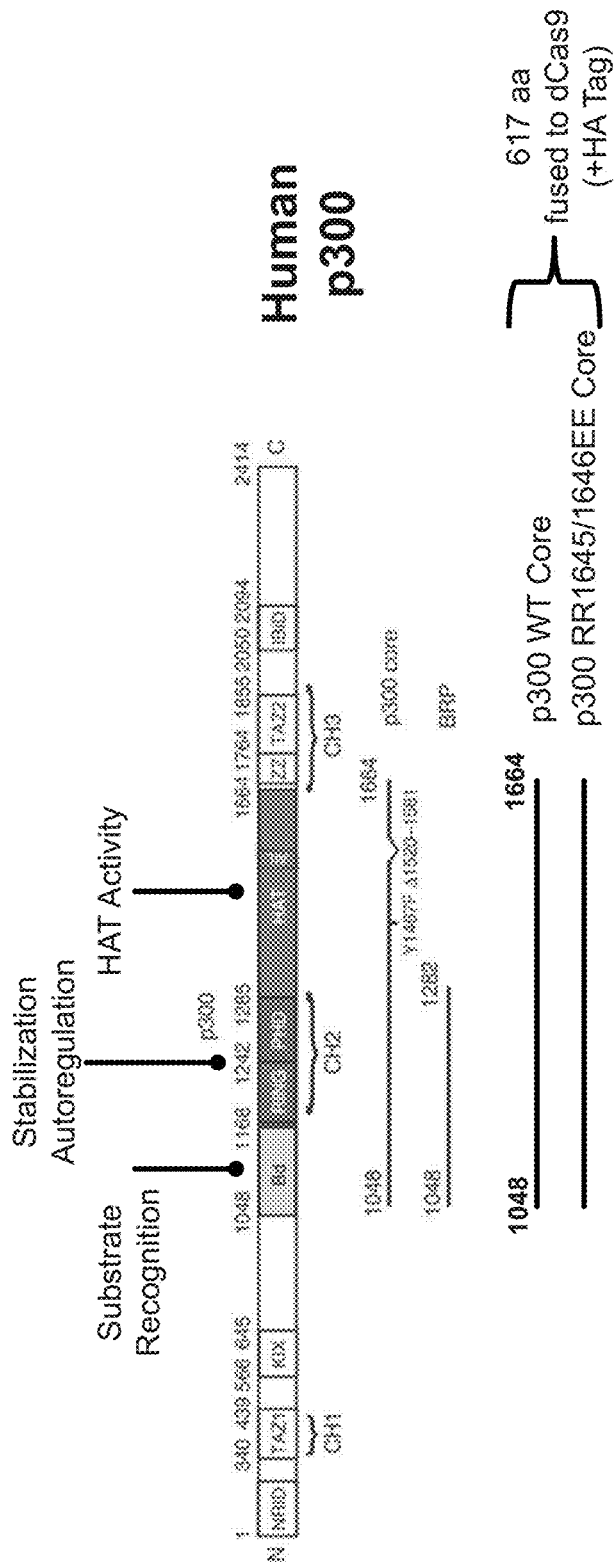


Fig. 58

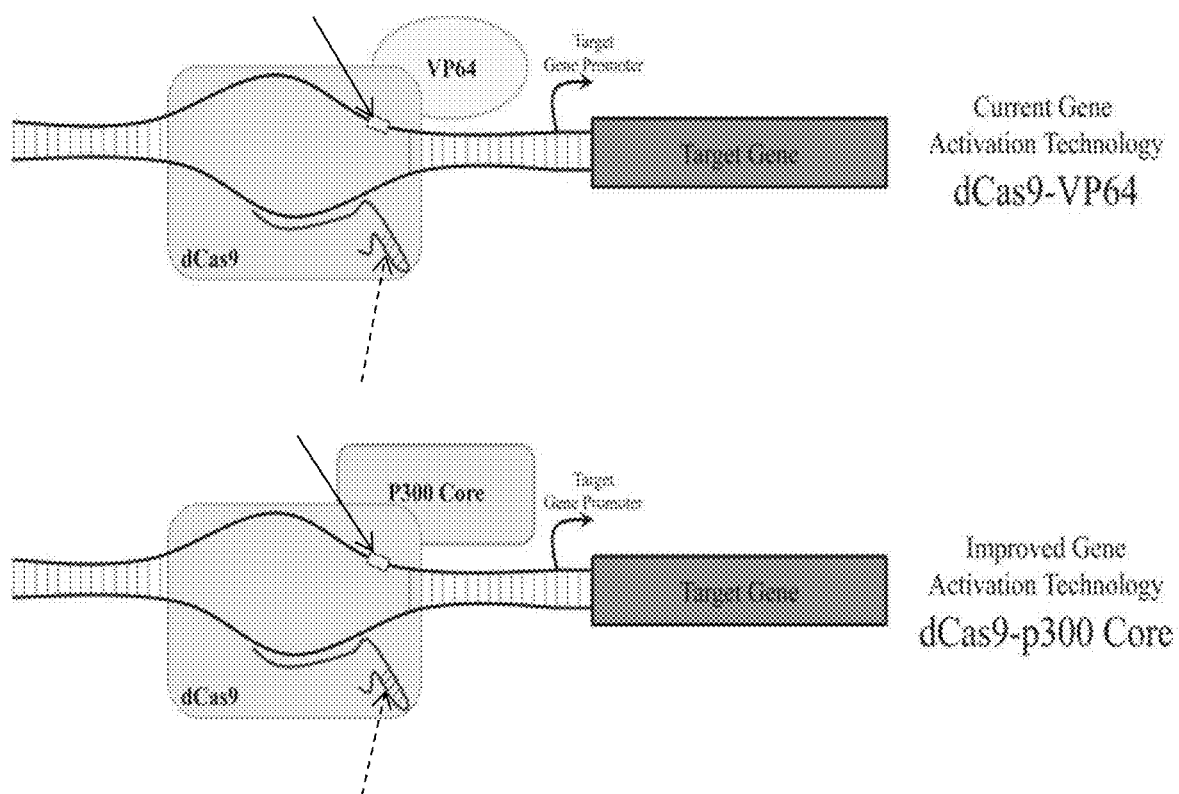


Fig. 59

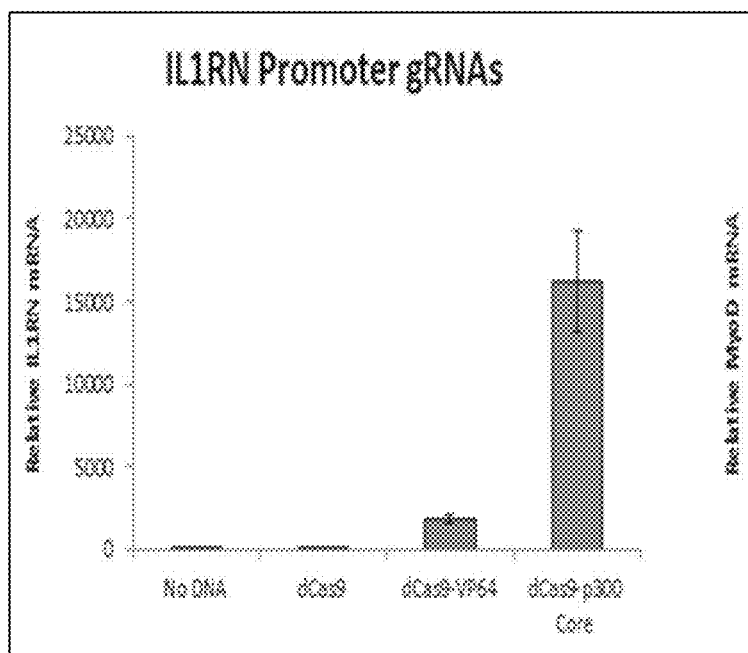


Fig. 60A

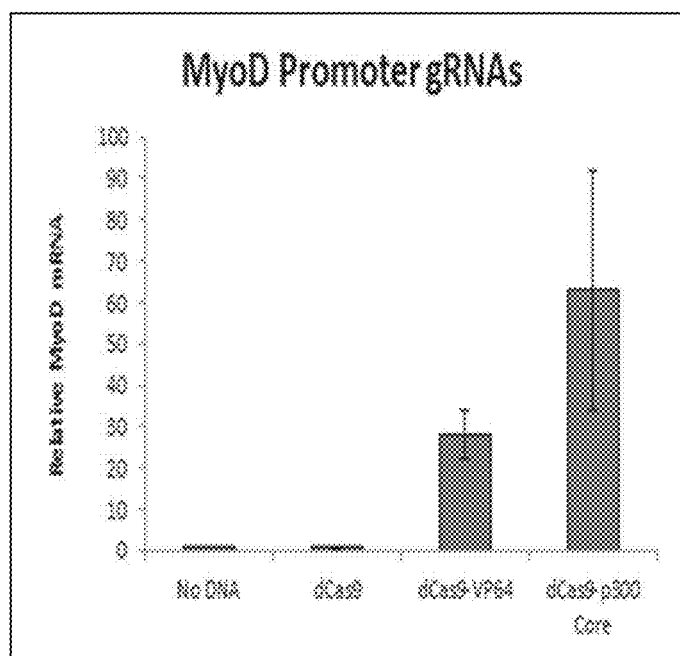


Fig. 60B

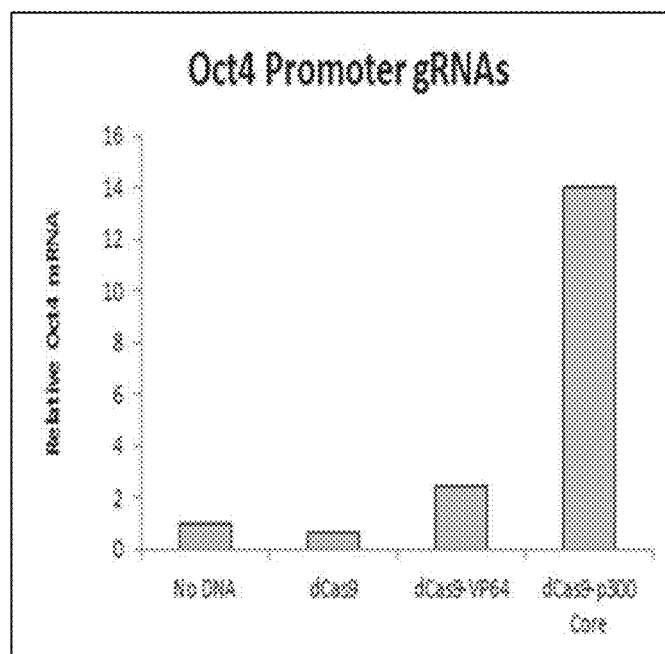


Fig. 60C

i. dCas9

MDYKDHDG DYKDHD IDYKDDDDMAPKKKKRKVGRGMDKKYSIGLAIGTNSVGNVAVITDEY
KVPSKKKFKVLGNTDRHSIKFNLI GALLFDSGETAEATRLKRTARRRYTRRRNRICYLQEI
FSNEMAKVDDSFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDS
TDKADLRLLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASG
VDAKAILSARLSKSRRLLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLO
LSKDTYDDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILKVNTETKAPLSASMIKRY
DEHHQDLTLKALVRQQLPEKYKEIPFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDG
TEELLVKINREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILT
FRIPYYVGPLARGNSRFAMTRKSEETITPWNFEVVDKGASAQSPIERMTNFDKNLPNE
KVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNKVTVRQLK
EDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTL
FEDREMIEERLRTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIADKQSGKTILDFLKS
DGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDLSLHEHIANLAGSPAIIKKGILQTVKVV
ELVKVMGRHKPENIVIEMARENQTTQGGQKNSRERMKAIEEGIKELGSQLKEHPVENTQ
LQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRG
KSDNVPSEEVVKMKKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKROLVETR
QITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAH
DAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIKSEQFEGKATAKYFFYSNIMNF
FKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKSKKLKSVKELLG
ITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKQNE
LALFSKYVNFYLYLASHYEKLKGSFEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADA
NLDKVL SAYNKH RDKPIRECAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDA
TLIHQSITGLYETRIDLSQLGGDPKKKKRKVG

Amino Acid Legend:

"Flag" Epitope

Nuclear Localization Sequence

Streptococcus pyogenes Cas9 (D10A, H840A)

VP64 Effector

p300 Core Effector

"HA" Epitope

Fig. 61A

ii. dCas9^{VP64}

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKKRKVGRGMDKKYSIGLAIGTNSVGNVAVITDEY
KVPSKKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKKTARRRYTERKNEICYLQEI
FSNEMAKYDDSEFFHRLEESFLVEEDKKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDS
TDKADLRLLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNOLFEENPINASC
VDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLO
LSKDTYDEDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDG
TEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILT
FRIPYYVGPGLARGNSRFAWMTRKSEETITPWNFEDEVVDGASAOSEFIERMTNFDKNLPNE
KVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLK
EDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTLTL
FEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIDKQSGKTIIDFLKS
DGFANRNFMLIHDDSLTFKEDIQKAQVSGGDSLHEHIANLAGSPAIKKGILQTVKVV
ELVKVMGRHKPENIVIEMARENQTTQGGQKNSRERMKRIEKGKELGSGQILKEHPVENTQ
LQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVAIVPQSFLKDDSIDNKVLTRSDNRG
KSDNVPSEEVVKMKKNYWROLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKROLVETR
QITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVRINNYHHAH
DAYLNAVVGITALIKKYFKLESEFVYGDYKVVYDVRKMIKSEQEIGKATAKYFFYSNIMNF
FKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKSKKKLKS VKELLG
ITIMERSSEFEKNPIDFLEAGYKEVKKDLIIKLPKYSLFELENGRKKPLASAGELQKGN
LALFSKYVNFLYLASHYEKLKGSFEDNEQKQLFVEQHKHYLDEII EQISEFSKRVILADA
NLDKVL SAYNKH RDKPIREQAENI IHLETLTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDA
TLIHQSITGLYETRIDLSQLGGDP IAGSKASPKKKRKVGRADALDDFDLDMLGSDALDDF
DLDMLGSDALDDFDLDMLGSDALDDFDLDMLINYPYDVFDYAS

Fig. 61B

iii. dCas9^{sp300} Core

MDYKDHDG DYKDHD IDYKDDDDMAPKKRKVKGRGMDKKYSIGLAI GTNSVGVAVITDEY
KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI
FSNEMAKVDDSFHRL EESFLVEEDKKHERHPI FGNIVDEVAYHEKYPTIYHLRKKLVDS
TDKADLR LIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASG
VDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIA LSLGLTFNPKSNFDLAEDAKLQ
LSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRYNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDG
TEELLVKLNREDLLRKQRTFDNGSIPHOIHLSELHAILRRQEDFYPLKDNSEKIEKILT
FRIFYYVGPLARGNSRFAMTRKSEETITPWNFEVVDKGASAQSFIERMTNFDKNLPNE
KVLFPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEOKKAIVDLLFKTNRKVTVKQLK
EDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTL
FEDREMI EERLKYAHLFDDKVMKQLKRRRYTGWGRLSEKLINGIRDKQSGKTILDFLKS
DGFANRNFMLIHDDSLTFKEDIQKAQVSGGSDSLHEHIANLAGSPA KKGILQTVRVVD
ELVKVMGRHKPENIVIEMARENQTTQRGQKNSRERMKRIE EGikelGSOILKEHPVENTO
LQNEKLYLYYLONGRDMYVDQELDINRLSDYDVAIVPOSFLKDDSIDNKVLTRSDENRG
KSDNVPSEEVVRKMKNYWRQLNAKLITQRKFDNLTKAERGGLSELDKAGFIKSOLVETR
QITKHVAQILDSRMNTKYDENDKLIREVKVITLKS KLVSDFRKDFQFYKVR EINNYHHAH
DAYLNAVVG TALIKKYPKLESEFVYG DYKVYDVRKMI AKSEQEIGKATAKYFFYSNIMNF
FKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNI VKKTEVQTGGF
SKESI LPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVA KVEKRSKKLKS VKELLG
ITIMERS SF EKNPIDFLEAGYKEVKKDLIIKLPKYSLFEL ENGRKRMLASAGELQK GNE
LALPSKYVNFY LASHYEKLKGS PEDNEQKQLFVEQHKHYLDEII EQISEFSKRVILADA
NLDKVL SAYNKHRDKPIRECAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDA
TLIHOSITGLYETRIDLSQLGGDP IAGSKASPKKKRKVGRAIFKPEELRQAIMPTLEALY
RQDPESLPFRQPVDFQLLGI PDYFDIVKSPMDLSTIKRKLDTGQYQEPWQYVDDIWL MFN
NAWLYNRKTSRVYKYCSKLSEVFEOEIDPVMQSLGYCCGRKLEFS POTLCCYGKOLCTIP
RDAYTYSYONRYHFCEKCFNEIOGESVSLGDDPSOPOTTINKEOFSKRKNDTLDPELVE
CTECGRKMHQICVLHHEI IWPAGFVCDGCLKXSARTKENKPSAKRLPSTR LGTFLENRV
NDFLRRQNHPESGEVTVRVVHASDKTVEVKPKMKARFVDSGEMAESFFYRTKALFAFEEI
DGVDLCFFGMHVQ EYGSDCPPPNORRVYISYLD SVHFFRPKCLRTAVYHEILIGYLEYVK
KLGYYTTGHIWACPPSEGDDYIFHCHPPDOKIPKPKRLOEWYKQMLDKAVSERIVHDYKDI
FKQATEDRLTSAKELPYFEGDFWPNVLEESI KELEQEEERKREENTSNESTDVTKGDSK
NAKKKNKKT SKNKSSL SRGNKKKPGMPNVSNL SOKLYATMEKHKEVFFVIRLIAGPAA
NSLPPIVDPDELIPCDLMDGRDAFILTLARDKHLEFSSLRRAQWSTMCM LVELHTOSODYF
YDVPDYAS

Fig. 61C

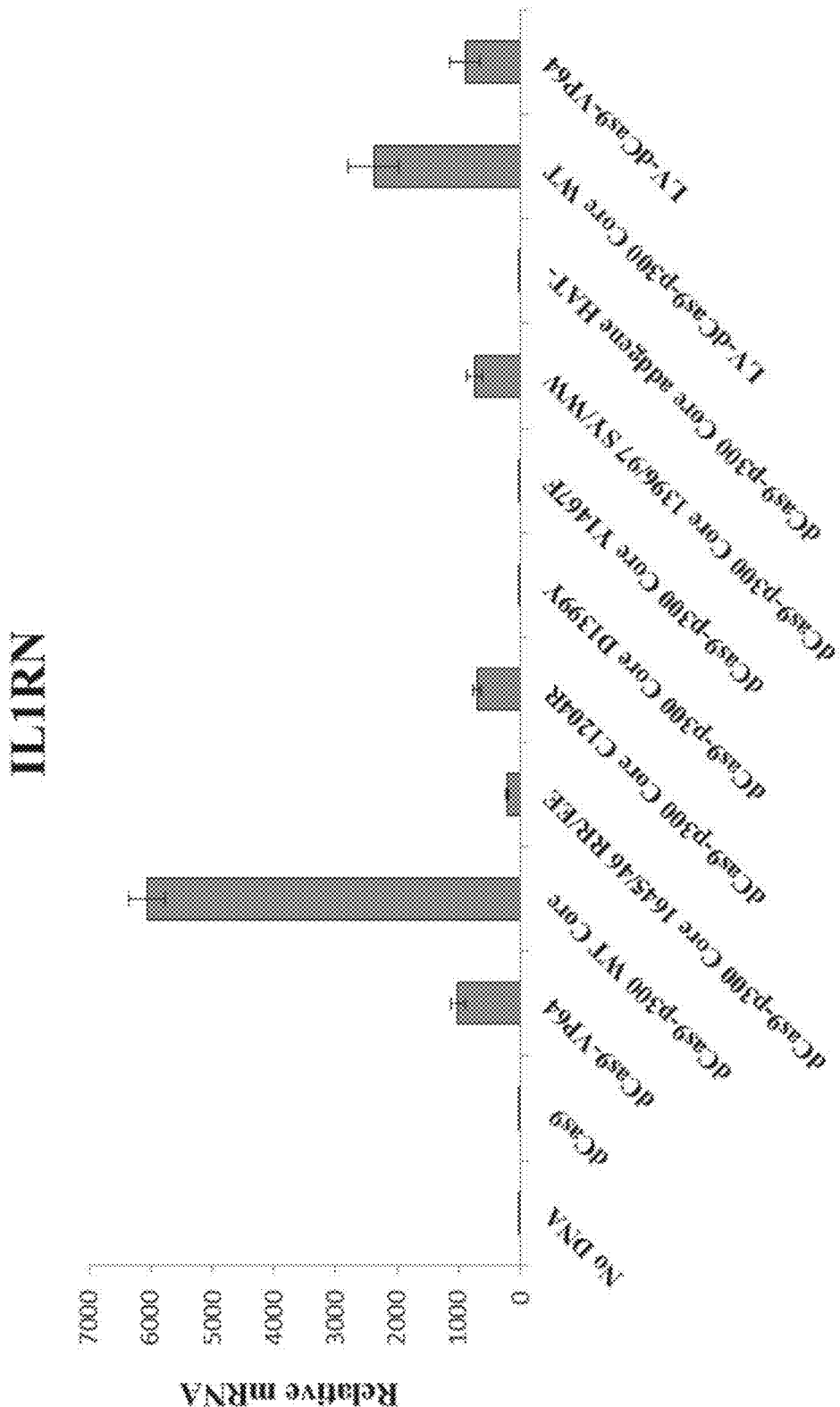


Fig. 62

Fig. 63A

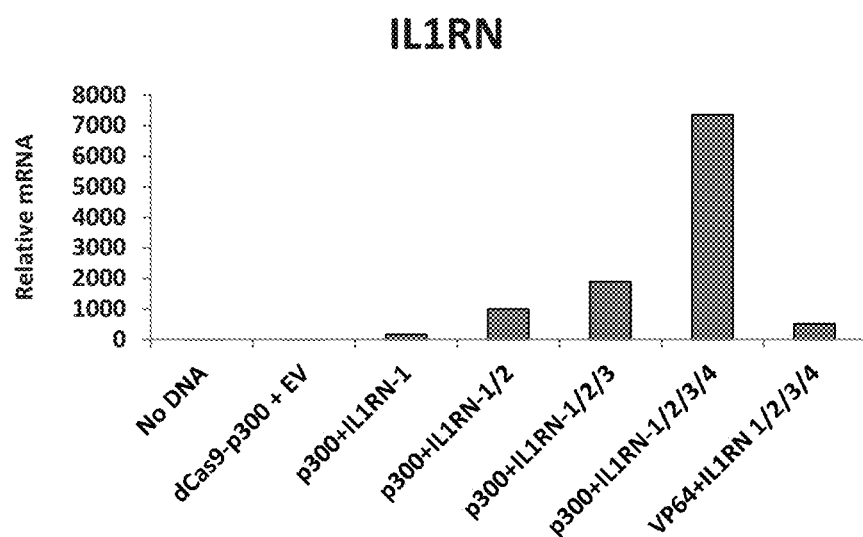


Fig. 63B

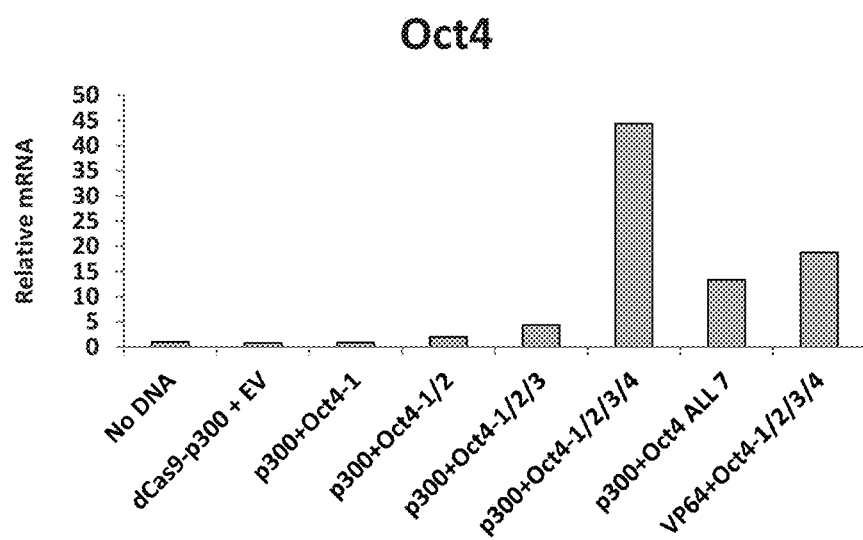


Fig. 64A

Genomic PCR to detect targeted integration

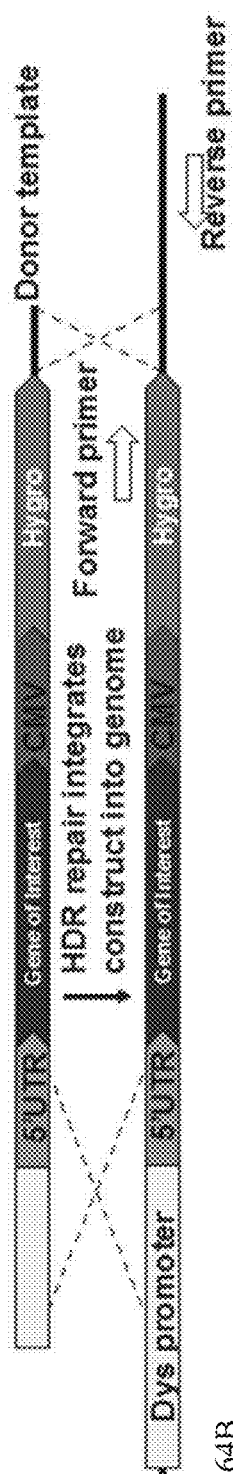


Fig. 64B

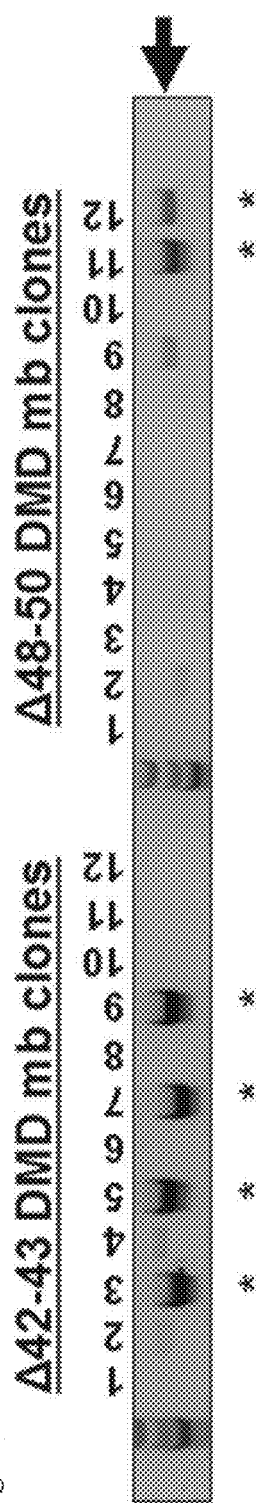
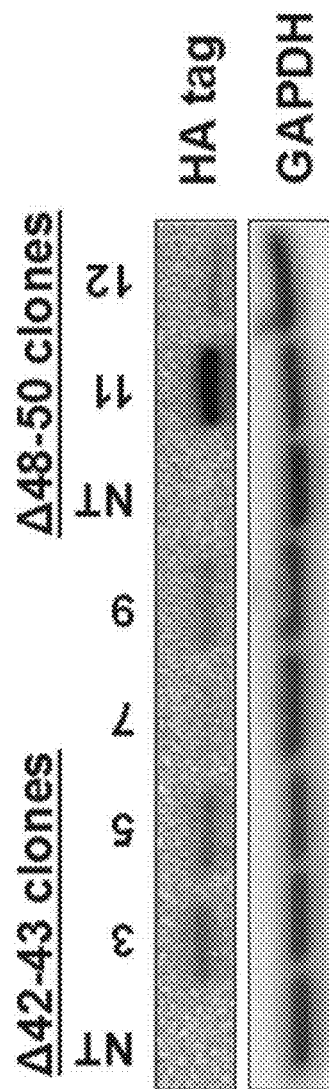


Fig. 64C



RNA-GUIDED GENE EDITING AND GENE REGULATION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 14/895,316, filed Dec. 2, 2015, which is the national stage filing under 35 U.S.C. 371 of International Patent Application No. PCT/US2014/041190, filed Jun. 5, 2014, which claims the benefit of priority to U.S. Provisional Application No. 61/831,481, filed Jun. 5, 2013, U.S. Provisional Application No. 61/839,127, filed Jun. 25, 2013, U.S. Provisional Application No. 61/904,911, filed Nov. 15, 2013, U.S. Provisional Application No. 61/967,466, filed Mar. 19, 2014, and U.S. Provisional Application No. 61/981,575, filed Apr. 18, 2014, the entire contents of each of which are hereby incorporated by reference.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with government support under federal grant numbers DP2-OD008586 and R01DA036865 awarded by NIH and CBET-1151035 awarded by the National Science Foundation. The U.S. Government has certain rights to this invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Feb. 27, 2018, is named 028193-9164-US03_Subt_Seq_List.txt and is 331,614 bytes in size.

TECHNICAL FIELD

[0004] The present disclosure relates to the field of gene expression alteration, genome engineering and genomic alteration of genes using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas) 9-based systems and viral delivery systems. The present disclosure also relates to the field of genome engineering and genomic alteration of genes in muscle, such as skeletal muscle and cardiac muscle.

BACKGROUND

[0005] Synthetic transcription factors have been engineered to control gene expression for many different medical and scientific applications in mammalian systems, including stimulating tissue regeneration, drug screening, compensating for genetic defects, activating silenced tumor suppressors, controlling stem cell differentiation, performing genetic screens, and creating synthetic gene circuits. These transcription factors can target promoters or enhancers of endogenous genes, or be purposefully designed to recognize sequences orthogonal to mammalian genomes for transgene regulation. The most common strategies for engineering novel transcription factors targeted to user-defined sequences have been based on the programmable DNA-binding domains of zinc finger proteins and transcription-activator like effectors (TALEs). Both of these approaches involve applying the principles of protein-DNA interactions of these domains to engineer new proteins with unique DNA-binding specificity. Although these methods have been widely successful for many applications, the protein engi-

neering necessary for manipulating protein-DNA interactions can be laborious and require specialized expertise.

[0006] Additionally, these new proteins are not always effective. The reasons for this are not yet known but may be related to the effects of epigenetic modifications and chromatin state on protein binding to the genomic target site. In addition, there are challenges in ensuring that these new proteins, as well as other components, are delivered to each cell. Existing methods for delivering these new proteins and their multiple components include delivery to cells on separate plasmids or vectors which leads to highly variable expression levels in each cell due to differences in copy number. Additionally, gene activation following transfection is transient due to dilution of plasmid DNA, and temporary gene expression may not be sufficient for inducing therapeutic effects. Furthermore, this approach is not amenable to cell types that are not easily transfected. Thus another limitation of these new proteins is the potency of transcriptional activation.

[0007] Site-specific nucleases can be used to introduce site-specific double strand breaks at targeted genomic loci. This DNA cleavage stimulates the natural DNA-repair machinery, leading to one of two possible repair pathways. In the absence of a donor template, the break will be repaired by non-homologous end joining (NHEJ), an error-prone repair pathway that leads to small insertions or deletions of DNA. This method can be used to intentionally disrupt, delete, or alter the reading frame of targeted gene sequences. However, if a donor template is provided along with the nucleases, then the cellular machinery will repair the break by homologous recombination, which is enhanced several orders of magnitude in the presence of DNA cleavage. This method can be used to introduce specific changes in the DNA sequence at target sites. Engineered nucleases have been used for gene editing in a variety of human stem cells and cell lines, and for gene editing in the mouse liver. However, the major hurdle for implementation of these technologies is delivery to particular tissues in vivo in a way that is effective, efficient, and facilitates successful genome modification.

[0008] Hereditary genetic diseases have devastating effects on children in the United States. These diseases currently have no cure and can only be managed by attempts to alleviate the symptoms. For decades, the field of gene therapy has promised a cure to these diseases. However technical hurdles regarding the safe and efficient delivery of therapeutic genes to cells and patients have limited this approach. Duchenne Muscular Dystrophy (DMD) is the most common hereditary monogenic disease and occurs in 1 in 3500 males. DMD is the result of inherited or spontaneous mutations in the dystrophin gene. Dystrophin is a key component of a protein complex that is responsible for regulating muscle cell integrity and function. DMD patients typically lose the ability to physically support themselves during childhood, become progressively weaker during the teenage years, and die in their twenties. Current experimental gene therapy strategies for DMD require repeated administration of transient gene delivery vehicles or rely on permanent integration of foreign genetic material into the genomic DNA. Both of these methods have serious safety concerns. Furthermore, these strategies have been limited by an inability to deliver the large and complex dystrophin gene sequence.

SUMMARY

[0009] The present invention is directed to a fusion protein comprising two heterologous polypeptide domains. The first polypeptide domain comprises a Clustered Regularly Interspaced Short Palindromic Repeats associated (Cas) protein and the second polypeptide domain has an activity selected from the group consisting of transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nuclease activity, nucleic acid association activity, methylase activity, and demethylase activity. The Cas protein may comprise Cas9. The Cas9 may comprise at least one amino acid mutation which knocks out nuclease activity of Cas9. The at least one amino acid mutation may be at least one of D10A and H840A. The Cas protein may comprise iCas9 (amino acids 36-1403 of SEQ ID NO: 1). The second polypeptide domain may have transcription activation activity. The second polypeptide domain may comprise at least one VP16 transcription activation domain repeat. The second polypeptide domain may comprise a VP16 tetramer ("VP64") or a p65 activation domain. The fusion protein may further comprise a linker connecting the first polypeptide domain to the second polypeptide domain. The fusion protein may comprise iCas9-VP64.

[0010] The present invention is directed to a DNA targeting system comprising said fusion protein and at least one guide RNA (gRNA). The at least one gRNA may comprise a 12-22 base pair complementary polynucleotide sequence of the target DNA sequence followed by a protospacer-adjacent motif. The at least one gRNA may target a promoter region of a gene, an enhancer region of a gene, or a transcribed region of a gene. The at least one gRNA may target an intron of a gene. The at least one gRNA may target an exon of a gene. The at least one gRNA may target a the promoter region of a gene selected from the group consisting of ASCL1, BRN2, MYT1L, NANOG, VEGFA, TERT, IL1B, IL1R2, IL1RN, HBG1, HBG2, and MYOD1. The at least one gRNA may comprise at least one of SEQ ID NOs: 5-40, 65-144, 492-515, 540-563, and 585-625.

[0011] The present invention is directed to a DNA targeting system that binds to a dystrophin gene comprising Cas9 and at least one guide RNA (gRNA). The at least one gRNA may target an intron of the dystrophin gene. The at least one gRNA may target an exon of the dystrophin gene. The at least one guide RNA may comprise at least one of SEQ ID NOs: 5-40, 65-144, 492-515, 540-563, and 585-625. The DNA targeting system may comprise between one and ten different gRNAs.

[0012] The present invention is directed to an isolated polynucleotide encoding said fusion protein or said DNA targeting system.

[0013] The present invention is directed to a vector comprising said isolated polynucleotide.

[0014] The present invention is directed to a cell comprising said isolated polynucleotide or said vector.

[0015] The present invention is directed to a method of modulating mammalian gene expression in a cell. The method comprises contacting the cell with said fusion protein, said DNA targeting system, said isolated polynucleotide, or said vector. The gene expression may be induced.

[0016] The present invention is directed to a method of transdifferentiating or inducing differentiation of a cell. The method comprises contacting the cell with said fusion protein, said DNA targeting system, said isolated polynucle-

otide, or said vector. The cell may be a fibroblast cell or an induced pluripotent stem cells. The fibroblast cell may be transdifferentiated into a neuronal cell or a myogenic cell. The DNA targeting system may be contacted with the cell and at least one gRNA targets a promoter region of at least one gene selected from the group consisting of ASCL1, BRN2, MYOD1, and MYT1L. The DNA targeting system may comprise at least one gRNA that targets the promoter region of the ASCL1 gene and at least one gRNA that targets the promoter region of the BRN2 gene. The DNA targeting system may comprise between one and twenty different gRNAs. The DNA targeting system may comprise 8 or 16 different gRNAs. The DNA targeting system may comprise dCas9-VP64. The DNA targeting system may be delivered to the cell virally or non-virally.

[0017] The present invention is directed to a method of correcting a mutant gene in a cell. The method comprises administering to a cell containing said DNA targeting system, said isolated polynucleotide, or said vector. The correction of the mutant gene may comprise homology-directed repair. The method may further comprise administering to the cell a donor DNA. The mutant gene may comprise a frameshift mutation which causes a premature stop codon and a truncated gene product. The correction of the mutant gene may comprise nuclease mediated non-homologous end joining. The correction of the mutant gene may comprise a deletion of a premature stop codon, a disruption of a splice acceptor site, a deletion of one or more exons, or disruption of a splice donor sequence. The deletion of one or more exons may result in the correction of the reading frame.

[0018] The present invention is directed to a method of treating a subject in need thereof having a mutant dystrophin gene. The method comprises administering to the subject said DNA targeting system, said isolated polynucleotide, or said vector. The subject may be suffering from Duchenne muscular dystrophy.

[0019] The present invention is directed to a method of correcting a mutant dystrophin gene in a cell. The method comprises administering to a cell containing a mutant dystrophin gene said DNA targeting system, said isolated polynucleotide, said vector, or said cell. The mutant dystrophin gene may comprise a premature stop codon, disrupted reading frame via gene deletion, an aberrant splice acceptor site, or an aberrant splice donor site, and wherein the target region is upstream or downstream of the premature stop codon, disrupted reading frame, aberrant splice acceptor site, or the aberrant splice donor site. The correction of the mutant dystrophin gene may comprise homology-directed repair. The method may further comprise administering to the cell a donor DNA. The mutant dystrophin gene may comprise a frameshift mutation which causes a premature stop codon and a truncated gene product. The correction of the mutant dystrophin gene may comprise nuclease mediated non-homologous end joining. The correction of the mutant dystrophin gene may comprise a deletion of a premature stop codon, correction of a disrupted reading frame, or modulation of splicing by disruption of a splice acceptor site or disruption of a splice donor sequence. The correction of the mutant dystrophin gene may comprise a deletion of exons 45-55 or exon 51.

[0020] The present invention is directed to a kit comprising said fusion protein, said DNA targeting system, said isolated polynucleotide, said vector, or said cell.

[0021] The present invention is directed to a method of modulating mammalian gene expression in a cell. The method comprises contacting the cell with a polynucleotide encoding a DNA targeting system. The DNA targeting system comprises said fusion protein and at least one guide RNA (gRNA). The DNA targeting system may comprise between one and ten different gRNAs. The different gRNAs may bind to different target regions within the target gene. The target regions may be separated by at least one nucleotide. The target regions may be separated by about 15 to about 700 base pairs. Each of the different gRNAs may bind to at least one different target genes. The different target genes may be located on same chromosome. The different target genes may be located on different chromosomes. The at least one target region may be within a non-open chromatin region, an open chromatin region, a promoter region of the target gene, an enhancer region of the target gene, a transcribed region of the target gene, or a region upstream of a transcription start site of the target gene. The at least one target region may be located between about 1 to about 1000 base pairs upstream of a transcription start site of a target gene. The at least one target region may be located between about 1 to about 600 base pairs upstream of a transcription start site of a target gene. The gene expression may be induced. The DNA targeting system may comprise two different gRNAs, three different gRNAs, four different gRNAs, five different gRNAs, six different gRNAs, seven different gRNAs, eight different gRNAs, nine different gRNAs, or ten different gRNAs. The at least one guide RNA may target a promoter region of a gene selected from the group consisting of ASCL1, BRN2, MYT1L, NANOG, VEGFA, TERT, IL1B, IL1R2, IL1RN, HBG1, HBG2, and MYOD1. The at least one guide RNA may comprise at least one of SEQ ID NOs: 5-40, 65-144, 492-515, 540-563, and 585-625. The at least one target region may be within an intron or an exon of a target gene.

[0022] The present invention is directed to a composition for inducing mammalian gene expression in a cell. The composition comprises said fusion protein and at least one guide RNA (gRNA).

[0023] The present invention is directed to a composition for inducing mammalian gene expression in a cell. The composition comprises an isolated polynucleotide sequence encoding said fusion protein and at least one guide RNA (gRNA). The at least one guide RNA may target a promoter region of a gene selected from the group consisting of ASCL1, BRN2, MYT1L, NANOG, VEGFA, TERT, IL1B, IL1R2, IL1RN, HBG1, HBG2, and MYOD1. The at least one guide RNA may comprise at least one of SEQ ID NOs 5-40, 65-144, 492-515, 540-563, and 585-625.

[0024] The present invention is directed to a cell comprising said composition for inducing mammalian gene expression in a cell.

[0025] The present invention is directed to a kit comprising said composition for inducing mammalian gene expression in a cell or said cell comprising said composition for inducing mammalian gene expression in a cell.

[0026] The present invention is directed to a kit for inducing mammalian gene expression in a cell. The kit comprises said composition for inducing mammalian gene expression in a cell or said cell comprising said composition for inducing mammalian gene expression in a cell.

[0027] The present invention is directed to a composition for genome editing in a muscle of a subject. The composition

comprises a modified adeno-associated virus (AAV) vector and a nucleotide sequence encoding a site-specific nuclease. The muscle is skeletal muscle or cardiac muscle. The modified AAV vector may have enhanced cardiac and skeletal muscle tissue tropism. The site-specific nuclease may comprise a zinc finger nuclease, a TAL effector nuclease, or a CRISPR/Cas9 system. The site-specific nuclease may bind a gene or locus in the cell of the muscle. The gene or locus may be dystrophin gene. The composition may further comprise a donor DNA or transgene.

[0028] The present invention is directed to a kit comprising said composition for genome editing in a muscle of a subject.

[0029] The present invention is directed to a method of genome editing in a muscle of a subject. The method comprises administering to the muscle said composition for genome editing in a muscle of a subject, wherein the muscle is skeletal muscle or cardiac muscle. The genome editing may comprise correcting a mutant gene or inserting a transgene. Correcting a mutant gene may comprise deleting, rearranging, or replacing the mutant gene. Correcting the mutant gene may comprise nuclease-mediated non-homologous end joining or homology-directed repair.

[0030] The present invention is directed to a method of treating a subject. The method comprises administering said composition for genome editing in a muscle of a subject to a muscle of the subject, wherein the muscle is skeletal muscle or cardiac muscle. The subject may be suffering from a skeletal muscle condition or a genetic disease. The subject may be suffering from Duchenne muscular dystrophy.

[0031] The present invention is directed to a method of correcting a mutant gene in a subject, the method comprises administering said composition for genome editing in a muscle of a subject. The muscle is skeletal muscle or cardiac muscle. The composition may be injected into the skeletal muscle of the subject. The composition may be injected systemically to the subject. The skeletal muscle may be tibialis anterior muscle.

[0032] The present invention is directed to a modified lentiviral vector for genome editing in a subject comprising a first polynucleotide sequence encoding said fusion protein and a second polynucleotide sequence encoding at least one sgRNA. The first polynucleotide sequence may be operably linked to a first promoter. The first promoter may be a constitutive promoter, an inducible promoter, a repressible promoter, or a regulatable promoter. The second polynucleotide sequence may encode between one and ten different sgRNAs. The second polynucleotide sequence may encode two different sgRNAs, three different sgRNAs, four different sgRNAs, five different sgRNAs, six different sgRNAs, seven different sgRNAs, eight different sgRNAs, nine different sgRNAs, or ten different sgRNAs. Each of the polynucleotide sequences encoding the different sgRNAs may be operably linked to a promoter. Each of the promoters operably linked to the different sgRNAs may be the same promoter. Each of the promoters operably linked to the different sgRNAs may be different promoters. The promoter may be a constitutive promoter, an inducible promoter, a repressible promoter, or a regulatable promoter. The sgRNA may bind to a target gene. Each of the sgRNA may bind to a different target region within one target loci. Each of the sgRNA may bind to a different target region within different gene loci. The fusion protein may comprise Cas9 protein or iCas9-VP64 protein. The fusion protein may comprise a

VP64 domain, a p300 domain, or a KRAB domain. The two or more endogenous genes may be transcriptionally activated. The two or more endogenous genes may be repressed.

[0033] The present invention is directed to a method of activating an endogenous gene in a cell. The method comprises contacting a cell with said modified lentiviral vector. The endogenous gene may be transiently activated. The endogenous gene may be stably activated. The endogenous gene may be transiently repressed. The endogenous gene may be stably repressed. The fusion protein may be expressed at similar levels to the sgRNAs. The fusion protein may be expressed at different levels to the sgRNAs. The cell may be a primary human cell.

[0034] The present invention is directed to a method of multiplex gene editing in a cell. The method comprises contacting a cell with said modified lentiviral vector. The multiplex gene editing may comprise correcting at least one mutant gene or inserting a transgene. Correcting a mutant gene may comprise deleting, rearranging, or replacing the at least one mutant gene. Correcting the at least one mutant gene may comprise nuclease-mediated non-homologous end joining or homology-directed repair. The multiplex gene editing may comprise deleting at least one gene, wherein the gene is an endogenous normal gene or a mutant gene. The multiplex gene editing may comprise deleting at least two genes. The multiplex gene editing may comprise deleting between two and ten genes.

[0035] The present invention is directed to a method of modulating gene expression of at least one target gene in a cell. The method comprises contacting a cell with said modified lentiviral vector. The gene expression of at least two genes may be modulated. The gene expression of between two genes and ten genes may be modulated. The gene expression of the at least one target gene may be modulated when gene expression levels of the at least one target gene are increased or decreased compared to normal gene expression levels for the at least one target gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIGS. 1A-1F show RNA-guided activation of the human IL1RN gene by iCas9-VP64. (FIG. 1A, FIG. 1B) An RNA-guided transcriptional activator was created by fusing the inactivated Cas9 (iCas9, D10A/H840A) to the VP64 transactivation domain. iCas9-VP64 recognizes genomic target sites through the hybridization of a guide RNA (gRNA) to a 20 bp target sequence. (FIG. 1C) Expression plasmids for four gRNAs or crRNA/tracrRNAs targeted to sequences in the IL1RN promoter were co-transfected with the iCas9-VP64 expression plasmid into HEK293T cells. Activation of IL1RN expression was assessed by qRT-PCR. (FIG. 1D) The four gRNA expression plasmids were co-transfected with iCas9-VP64 individually or in combination. Robust gene activation was observed by qRT-PCR only in response to the combination of gRNAs. (FIG. 1E) Activation of IL1RN expression was confirmed by assessing secretion of the IL-1ra gene product into the media by ELISA. IL-1ra was only detected in three of the six samples treated with the combination of gRNAs. For FIGS. 1C-1E, data are shown as the mean $\pm$ s.e.m. (n=3 independent experiments). Treatment with the combination of gRNAs was statistically different than all other treatments (*P $\leq$ 0.02) by Tukey's test. (FIG. 1F) RNA-seq was performed on samples treated with empty expression vector (n=2) or co-transfected with the expression plasmids for iCas9-VP64 and the four gRNAs targeting

IL1RN (n=2). The only statistically significant changes in gene expression between these treatments were an increase in the four IL1RN isoforms (false discovery rate $\leq$ 3 $\times$ 10 $^{-4}$) and a decrease in IL32 (false discovery rate=0.03).

[0037] FIGS. 2A-2H show RNA-guided activation of human genes relevant to cell and gene therapy, genetic reprogramming, and regenerative medicine. HEK293T cells were transfected with the iCas9-VP64 expression plasmid and four gRNAs individually or in combination. Target gene expression was measured by qRT-PCR and normalized to GAPDH mRNA levels. Data are shown as the mean $\pm$ s.e.m. (n=3 independent experiments). Treatment with the combination of gRNAs was statistically different than all other treatments (*P<0.05) by Tukey's test.

[0038] FIG. 3 shows expression of iCas9-VP64. Expression of iCas9-VP64 in transfected HEK293 cells was confirmed by western blot for the N-terminal Flag epitope tag. The wt Cas9 expression plasmid does not contain the epitope tag.

[0039] FIG. 4 shows positions of gRNA target sites and DNase hypersensitivity of human target genes. The four gRNA target sites for each locus are designated as custom tracks above each gene and DNase-seq data indicating DNase-hypersensitive open chromatin regions is shown below each gene. DNase-seq was performed in HEK293T cells to identify DNase hypersensitive regions, as previously described (Song et al., Cold Spring Harbor protocols 2010, pdb prot5384 (2010); Song et al. *Genome Res* 21, 1757-1767 (2011)). The results show that open chromatin was not a requirement for gene activation by combinations of gRNAs with iCas9-VP64.

[0040] FIG. 5 shows the absence of nuclease activity by iCas9-VP64. Wild-type Cas9 or inactivated (D10A, H840A) iCas9-VP64 expression plasmids were co-transfected with expression plasmids for four different guide RNAs targeting the IL1RN promoter. Nuclease activity was determined by the Surveyor assay (Guschin et al., *Methods Mol Biol* 649, 247-256 (2010)). The lower molecular weight bands indicative of nuclease activity and DNA repair by non-homologous end joining are only present following treatment with wild-type Cas9, supporting abrogation of nuclease activity by iCas9-VP64.

[0041] FIG. 6 shows RNA-seq for samples treated with gRNAs targeting HBG1 and HBG2. RNA-seq was performed on samples treated with a control empty expression vector (n=3) or cotransfected with the expression plasmids for iCas9-VP64 and the four gRNAs targeting HBG1 (n=2). Three of these gRNAs also target HBG2. Increases in both HBG1 and HBG2 relative to control were observed but were not statistically significant due to low expression levels. The only statistically significant changes in gene expression between these treatments were decreases in IL32 (false discovery rate=0.0007) and TNFRSF9 (false discovery rate=0.002).

[0042] FIG. 7 shows upregulation of Ascl1 and γ -globin by iCas9-VP64. HEK293T cells were transfected with iCas9-VP64 and four gRNAs targeting the ASCL1 or HBG1 promoter. Levels of corresponding Ascl1 and γ -globin protein production were assessed by western blot. Low levels of these proteins were detectable in HEK293T cells and increases in expression were detectable following iCas9-VP64 treatment in two independent experiments.

[0043] FIGS. 8A-8H show activation of downstream targets of Ascl1 in iCas9-VP64-treated murine embryonic

fibroblasts. Mouse embryonic fibroblasts (MEFs) were transfected with a control GFP expression plasmid or the iCas9-VP64 expression plasmid and a combination of four gRNA expression plasmids targeting ASCL1 at a ratio of 50:50 or 75:25. (FIG. 8A) The gRNA target sites in the human ASCL1 promoter (SEQ ID NO: 3) are conserved in the mouse ASCL1 promoter (SEQ ID NO: 4). Target sites are indicated by solid lines and the transcribed region is indicated by dashed line. (FIG. 8B) ASCL1 expression in MEFs increased at two days after iCas9-VP64/gRNA treatment as determined by qRT-PCR. (FIGS. 8C-8H) After 10 days in neural induction media, cells were stained for Ascl1 and Tuj1, an early marker of neuronal differentiation (FIGS. 8C-8D), or for Tuj1 and MAP2, a marker of more mature neuronal differentiation (FIGS. 8D-8F). Some Tuj1-positive cells adopted neuronal morphologies (FIGS. 8F-8G) and a single cell was found to be positive for Tuj1 and MAP2 (FIG. 8G). (FIG. 8H) Tuj1-positive cells were readily identified in the iCas9-VP64/gRNA-treated cultures (~0.05%) but were absent in controls. n=3 independent samples and data are represented as mean standard error of the mean. gRNA 75/25 is significantly different than gRNA 50/50 and control (*P<0.01, Tukey's test).

[0044] FIG. 9A shows the iCas9-VP64 protein sequence (SEQ ID NO: 1) and FIG. 9B shows the sequence of the gRNA expression cassette with U6 promoter (SEQ ID NO: 2).

[0045] FIG. 10 shows the standard curves for qRT-PCR. For each gene, the experimental sample with the highest expression level was diluted to create a standard curve that was assayed by qRT-PCR to ensure efficient amplification over an appropriate dynamic range. The efficiencies of all amplification reactions were within 90-115%.

[0046] FIGS. 11A-11C show the validation of RNA-guided repair. FIG. 11A shows the Surveyor assay results of genomic DNA harvested from HEK 293T cells two days after Cas9 was co-transfected into the cells with empty vector (negative control) or gRNA. FIG. 11B shows the location of the gRNA target. FIG. 11C shows the expected cleavage sizes for each gRNA.

[0047] FIG. 12 shows RNA-guided repair in DMD 8036 (del48-50) cells as shown by Surveyor assay.

[0048] FIG. 13 shows RNA-guided repair in DMD 8036 (del48-50) cells as shown by PCR across the entire locus. The PCR of a wild-type dystrophin gene generates a fragment of 1447 bp in size, whereas PCR of the mutant gene in the DMD 8036 cell line shows a deletion of approximately 817 bp. The deletion band after introduction of the CRISPR/Cas9-based system was approximately 630 bp.

[0049] FIG. 14 shows RNA-guided repair in DMD 8036 (del48-50) cells as shown by Western blot with MANDYS8 (anti-dystrophin antibody) and GAPDH antibody (positive control).

[0050] FIG. 15 shows ChIP sequencing data illustrating the specific binding of iCas9-VP64 targeting the IL1RN promoter. HEK 293T cells were transfected with iCas9-VP64 targeting the IL1RN promoter.

[0051] FIGS. 16A-16D CRISPR/Cas9 targeting of the dystrophin gene. (FIG. 16A) sgRNA sequences were designed to bind sequences in the exon 45-55 mutational hotspot region of the dystrophin gene, such that gene editing could restore dystrophin expression from a wide variety of patient-specific mutations. Arrows within introns indicate sgRNA targets designed to delete entire exons from the

genome. Arrows within exons indicate sgRNA targets designed to create targeted frameshifts in the dystrophin gene. (FIG. 16B) Example of frame correction following introduction of small insertions or deletions by NHEJ DNA repair in exon 51 using the CR3 sgRNA. FIG. 16B discloses SEQ ID NOS 633-636, respectively, in order of appearance. (FIG. 16C) Schematic of multiplex sgRNA targets designed to delete exon 51 and restore the dystrophin reading frame in a patient mutation with the deletion of exons 48-50. (FIG. 16D) Schematic of multiplex sgRNA targets designed to delete the entire exon 45-55 region to address a variety of DMD patient mutations.

[0052] FIG. 17 shows images of TBE-PAGE gels used to quantify Surveyor assay results to measure day 3 gene modification in Table 7. Asterisks mark expected sizes of bands indicative of nuclease activity.

[0053] FIG. 18 shows images of TBE-PAGE gels used to quantify Surveyor assay results to measure day 10 gene modification in Table 7. Asterisks mark expected sizes of bands indicative of nuclease activity.

[0054] FIGS. 19A-19D show fluorescence-activated flow sorting to enrich genetically modified DMD myoblasts. (FIG. 19A) A plasmid expressing a human-codon optimized SpCas9 protein linked to a GFP marker using a T2A ribosomal skipping peptide sequence was co-electroporated into human DMD myoblasts with one or two plasmids carrying sgRNA expression cassettes. (FIG. 19B) The indicated sgRNA expression cassettes were independently co-transfected into HEK293 Ts with a separate plasmid expressing SpCas9 with (bottom) or without (top) a GFP marker linked to SpCas9 by a T2A ribosomal skipping peptide sequence. Gene modification frequencies were assessed at 3 days post-transfection by the Surveyor assay. (FIG. 19C) DMD myoblasts with deletions of exons 48-50 in the dystrophin gene were treated with sgRNAs that correct the dystrophin reading frame in these patient cells. Gene modification was assessed at 20 days post-electroporation in unsorted (bulk) or GFP+ sorted cells. (FIG. 19D) GFP expression in DMD myoblasts 3 days after electroporation with indicated expression plasmids. Transfection efficiencies and sorted cell populations are indicated by the gated region.

[0055] FIGS. 20A-20D show targeted frameshifts to restore the dystrophin reading frame using CRISPR/Cas9. (FIG. 20A) The 5' region of exon 51 was targeted using a sgRNA (SEQ ID NO: 637), CR3, that binds immediately upstream of the first out-of-frame stop codon. PAM: proto-spacer-adjacent motif. (FIG. 20B) The exon 51 locus was PCR amplified from HEK293T cells treated with SpCas9 and CR3 expression cassettes. Sequences of individual clones were determined by Sanger sequencing. The top sequence (bolded, exon in red) is the native, unmodified sequence. The number of clones for each sequence is indicated in parentheses. (FIG. 20C) Summary of total gene editing efficiency and reading frame conversions resulting from gene modification shown in FIG. 20B. (FIG. 20D) Western blot for dystrophin expression in human DMD myoblasts treated with SpCas9 and the CR3 sgRNA expression cassette (FIG. 19C) to create targeted frameshifts to restore the dystrophin reading frame. Dystrophin expression was probed using an antibody against the rod-domain of the dystrophin protein after 6 days of differentiation.

[0056] FIGS. 21A-21D show deletion of exon 51 from the human genome using multiplex CRISPR/Cas9 gene editing.

(FIG. 21A) End-point genomic PCR across the exon 51 locus in human DMD myoblasts with a deletion of exons 48-50. The top arrow indicates the expected position of full-length PCR amplicons and the two lower arrows indicate the expected position of PCR amplicons with deletions caused by the indicated sgRNA combinations. (FIG. 21B) PCR products from FIG. 21A were cloned and individual clones were sequenced to determine insertions and deletions present at the targeted locus (SEQ ID NOS 424, 638, 425-428, 639 and 429-431, respectively, in order of appearance). The top row shows the wild-type unmodified sequence and the triangles indicate SpCas9 cleavage sites. At the right are representative chromatograms showing the sequences of the expected deletion junctions (SEQ ID NOS 640-642, respectively, in order of appearance). (FIG. 21C) End-point RT-PCR analysis of dystrophin mRNA transcripts in CRISPR/Cas9-modified human Δ 48-50 DMD myoblasts treated with the indicated sgRNAs. A representative chromatogram of the expected deletion PCR product is shown at the right. Asterisk: band resulting from hybridization of the deletion product strand to the unmodified strand. (FIG. 21D) Rescue of dystrophin protein expression by CRISPR/Cas9 genome editing was assessed by western blot for the dystrophin protein with GAPDH as a loading control. The arrow indicates the expected restored dystrophin protein band.

[0057] FIGS. 22A-22D show deletion of the entire exon 45-55 region in human DMD myoblasts by multiplex CRISPR/Cas9 gene editing. (FIG. 22A) End-point genomic PCR of genomic DNA to detect deletion of the region between intron 44 and intron 55 after treating HEK293 Ts or DMD myoblasts with the indicated sgRNAs. (FIG. 22B) Individual clones of PCR products (SEQ ID NOS 432, 643 and 433, respectively, in order of appearance) of the expected size for the deletions from DMD myoblasts in FIG. 22A were analyzed by Sanger sequencing to determine the sequences of genomic deletions present at the targeted locus. Below is a representative chromatograms showing the sequence of the expected deletion junctions (SEQ ID NO: 644). (FIG. 22C) End-point RT-PCR analysis of dystrophin mRNA transcripts in CRISPR/Cas9-modified human Δ 48-50 DMD myoblasts treated with the indicated sgRNAs. A representative chromatogram of the expected deletion PCR product is shown at the right (SEQ ID NO: 645). (FIG. 22D) Analysis of restored dystrophin protein expression by western blot following electroporation of DMD myoblasts with sgRNAs targeted to intron 44 and/or intron 55.

[0058] FIG. 23 shows verification of flow cytometry-based enrichment of gene-modified DMD myoblasts used for in vivo cell transplantation experiment. DMD myoblasts were treated with Cas9 with or without sgRNA expression vectors for CR1 and CR5 and sorted for GFP+ cells by flow cytometry. Deletions at the exon 51 locus were detected by end-point PCR using primers flanking the locus. Neg ctrl: DMD myoblasts treated with Cas9 only and sorted for GFP+ cells.

[0059] FIG. 24 shows expression of restored human dystrophin in vivo following transplantation of CRISPR/Cas9-treated human DMD myoblasts into immunodeficient mice. Human Δ 48-50 DMD myoblasts were treated with SpCas9, CR1, and CR5 to delete exon 51 and sorted for GFP expression as shown in FIGS. 19A-19D. These sorted cells and untreated control cells were injected into the hind limbs of immunodeficient mice and assessed for human-specific protein expression in muscle fibers after 4 weeks post-

transplantation. Cryosections were stained with anti-human spectrin, which is expressed by both uncorrected and corrected myoblasts that have fused into mouse myofibers, or anti-human dystrophin antibodies as indicated. White arrows indicate muscle fibers positive for human dystrophin.

[0060] FIGS. 25A-25F show additional immunofluorescence images probing human dystrophin expression. Serial sections from regions stained with anti-human spectrin are shown inset in top left. (FIG. 25A-FIG. 25C) Sections from muscles injected with untreated human DMD myoblasts. (FIG. 25D-FIG. 25F) Sections from muscles injected with CR1/5 treated human DMD myoblasts enriched by flow cytometry. White arrows indicate dystrophin positive fibers.

[0061] FIGS. 26A-26D shows evaluation of CRISPR/Cas9 toxicity and off-target effects for CR1/CR5-mediated deletion of exon 51 in human cells. (FIG. 26A) Results of a cytotoxicity assay in HEK293T cells treated with human-optimized SpCas9 and the indicated sgRNA constructs. Cytotoxicity is based on survival of GFP-positive cells that are co-transfected with the indicated nuclease. I-SceI is a well-characterized non-toxic meganuclease and GZF3 is a known toxic zinc finger nuclease. (FIG. 26B) Surveyor analysis at off-target sites in sorted hDMD cells treated with expression cassettes encoding Cas9 the indicated sgRNAs. These three off-target sites tested in hDMD cells were identified from a panel of 50 predicted sites tested in HEK293T cells (FIG. 27 and Table 4). TGT: on-target locus for indicated sgRNA. OT: off-target locus. (FIG. 26C, FIG. 26D) End-point nested PCR to detect chromosomal translocations in (FIG. 26C) HEK293T cells treated with Cas9 and CR1 or (FIG. 26D) sorted hDMD cells treated with Cas9, CR1, and CR5. The schematic depicts the relative location of nested primer pairs customized for each translocation event. The expected size of each band was estimated based on the primer size and the location of the predicted sgRNA cut site at each locus. Asterisks indicate bands detected at the expected size. The identities of the bands in FIG. 26C were verified by Sanger sequencing from each end (FIG. 30). A representative chromatogram for the P2/P5 translocation in HEK293T cells is shown (SEQ ID NO: 646).

[0062] FIG. 27 shows images of TBE-PAGE gels used to quantify Surveyor assay results to measure on-target and off-target gene modification in Table 4. Asterisks mark expected sizes of bands indicative of nuclease activity.

[0063] FIGS. 28A-28C show end-point nested PCR to detect chromosomal translocations caused by CRISPR/Cas9 off-target activity for CR3 and CR6/CR36 in human cells. Nested end-point PCR analysis was used to detect translocations in (FIG. 28A) HEK293T or sorted hDMD cells treated with Cas9 and CR3 as indicated, (FIG. 28B) HEK293T cells treated with Cas9 and CR36 alone, or (FIG. 28C) sorted hDMD cells treated with Cas9, CR6, and CR36 expression cassettes. The second nested PCR reaction for translocation was amplified using custom primers for each predicted translocation locus to maximize specificity (See Table 4). The schematic depicts the relative location of nested primer pairs used to probe for the presence of translocations. Each possible translocation event was first amplified from genomic DNA isolated from cells treated with or without the indicated sgRNA(s). A second nested PCR reaction was performed using primers within the predicted PCR amplicons that would result from translocations. Expected size was estimated based on the indicated

primer binding site and the predicted sgRNA cut site at each locus. *indicates bands detected at the expected size and verified by Sanger sequencing from each end. # indicates amplicons in which Sanger sequencing showed sequences other than the predicted translocation, likely a result of mispriming during the nested PCR.

[0064] FIG. 29 shows Sanger sequencing chromatograms (SEQ ID NOS 647-654, respectively, in order of appearance) for bands detected in FIGS. 28A-28C resulting from translocations between CR3 and CR3-OT1, on chromosomes X and 1, respectively, in HEK293T cells treated with Cas9 and CR3 gene cassettes. Arrows show regions of homology to the indicated chromosome nearby the expected break points caused by the appropriate sgRNAs. Note that sequencing reads become out of phase near the break point due to the error-prone nature of DNA repair by non-homologous end-joining.

[0065] FIG. 30 shows Sanger sequencing chromatograms (SEQ ID NOS 655-660, respectively, in order of appearance) for bands detected in FIG. 26C resulting from translocations between CR1 and CR1-OT1, on chromosomes X and 16, respectively, in HEK293T cells treated with Cas9 and CR1 gene cassettes. Arrows show regions of homology to the indicated chromosome nearby the expected break points caused by the appropriate sgRNAs. Note that sequencing reads become out of phase near the break point due to the error-prone nature of DNA repair by non-homologous end-joining.

[0066] FIG. 31 shows an overview of in vivo AAV injections and tissue harvest.

[0067] FIGS. 32A-32C show Surveyor analysis of Rosa26 ZFN activities in skeletal muscle in vitro and in vivo following delivery of AAV-SASTG-ROSA. Arrows indicate expected bands resulting from Surveyor cleavage. n.d.: not detected. (FIG. 32A) Proliferating C2C12s were transduced with the indicated amount of virus and harvested at 4 days post-infection. Arrows indicate expected bands sizes resulting from Surveyor cleavage. (FIG. 32B) C2C12s were incubated in differentiation medium for 5 days and then transduced with the indicated amount of AAV-SASTG-ROSA virus in 24 well plates. Samples were collected at 10 days post-transduction. (FIG. 32C) The indicated amount of AAV-SASTG-ROSA was injected directly into the tibialis anterior of C57BL/6J mice and muscles were harvested 4 weeks post-infection. The harvested TA muscles were partitioned into 8 separate pieces for genomic DNA analysis, each shown in a separate lane.

[0068] FIG. 33 shows Rosa T2A opt DNA sequence (SEQ ID NO: 434) and Rosa T2A opt protein sequence (SEQ ID NO: 435).

[0069] FIG. 34 shows SASTG capsid DNA sequence (SEQ ID NO: 436) and SASTG capsid peptide sequence (SEQ ID NO: 437).

[0070] FIG. 35 shows DZF16 ZFN target site sequence (SEQ ID NO: 442), DZF16-L6 left full amino acid sequence (SEQ ID NO: 443) and DZF16-R6 right full amino acid sequence (SEQ ID NO: 444).

[0071] FIG. 36 shows E51C3 ZFN target site sequence (SEQ ID NO: 445), E51C3-3L left full amino acid sequence (SEQ ID NO: 446) and E51C3-3R right full amino acid sequence (SEQ ID NO: 447).

[0072] FIG. 37 shows DZF15 ZFN target site sequence (SEQ ID NO: 448), DZF15-L6 left full amino acid sequence (SEQ ID NO: 449), DZF15-R6 right full amino acid

sequence (SEQ ID NO: 450), DZF15-L5 left full amino acid sequence (SEQ ID NO: 451), DZF15-R5 right full amino acid sequence (SEQ ID NO: 452).

[0073] FIG. 38 shows E51C4 ZFN target site sequence (SEQ ID NO: 453), E51C4-4L left full amino acid sequence (SEQ ID NO: 454) and E51C4-4R right full amino acid sequence (SEQ ID NO: 455).

[0074] FIG. 39 shows schematic diagrams of a “Single vector, multiplex CRISPR system,” Dual vector, multiplex CRISPR system,” and “Single vector, single gRNA system.”

[0075] FIG. 40 shows the nucleotide sequences of SaCas9-NLS (with the NLS underlined) (SEQ ID NO: 459) and SaCas9 gRNA (SEQ ID NO: 460).

[0076] FIG. 41 shows the nucleotide sequences of NmCas9 (with the NLS 1 underlined, the NLS 2 underlined and bolded, and the HA tag bolded), NmCas9 short hairpin from Thomson *PNAS* 2013 (SEQ ID NO: 462), and NmCas9 long hairpin from Church Nature Biotech 2013 (SEQ ID NO: 463).

[0077] FIGS. 42A-42C show validation of sgRNA and lentiviral Cas9 expression constructs. (FIG. 42A) Constructs encoding unique Pol III promoters expressing sgRNAs targeting the AAVS1 locus or a construct containing the hU6 promoter immediately followed by poly-thymidine to terminate expression (“PolyT”) were transfected into HEK293T cells. End-point RT-PCR was used to probe for expression of each indicated promoter/sgRNA construct two days post-transfection. -RT: no reverse transcriptase control. (FIG. 42B) HEK293 Ts were transfected with expression vectors encoding the AAVS1 zinc-finger nuclease or Cas9-T2A-GFP and the indicated promoter/sgRNA expression cassettes and assessed for gene modification levels 3 days post-transfection using the Surveyor assay. (FIG. 42C) HEK293T cells were transduced with lentiviral constructs encoding the indicated Cas9-T2A-GFP constructs without sgRNAs and assessed for Cas9 expression by western blot 7 days post-transduction by probing for a FLAG epitope tag on the N-terminus of the Cas9 protein.

[0078] FIG. 43 shows Golden Gate assembly of single lentiviral CRISPR/Cas9 expression cassettes. FIG. 43 discloses SEQ ID NOS 661-672, respectively, in order of appearance.

[0079] FIGS. 44A-44B show single lentiviral delivery of a multiplex CRISPR/Cas9 system. (FIG. 44A) Four sgRNAs targeting distinct genomic loci were cloned into a lentiviral vector expressing the active Cas9 nuclease. (FIG. 44B) HEK293 Ts and primary human dermal fibroblasts were transduced with lentivirus expressing the indicated sgRNAs and assayed for cleavage events using the Surveyor assay. HEK293 Ts were assayed 7 days post transduction. The human fibroblasts were assayed 10 days post transduction.

[0080] FIGS. 45A-45D show transient gene activation in HEK293 Ts stably expressing dCas9-VP64. HEK293 Ts were transduced with lentivirus to stably express dCas9-VP64 and were subsequently transfected with plasmid expressing the indicated sgRNA combinations. By varying the number of sgRNAs delivered, tunable endogenous gene activation of the endogenous IL1RN (FIG. 45A) and HBG1 (FIG. 45B) loci was achieved 3 days post transfection. Peak levels of endogenous IL1RN (FIG. 45C) and HBG1 (FIG. 45D) were observed 3-6 days post transfection and the level of activation returned to background levels between days 15-20. Importantly, the cell lines were able to reactive

following a second transfection on day 20 albeit at a lower level than previously observed.

[0081] FIGS. 46A-46D show stable gene activation in HEK293 Ts using a single lentiviral multiplex dCas9-VP64 vector. HEK293 Ts were transduced with lentivirus to stably express dCas9-VP64 and the indicated combinations of gRNAs. By varying the number of sgRNAs delivered, tunable endogenous gene activation of the endogenous IL1RN (FIG. 46A) and HBG1 (FIG. 46B) loci was achieved 7 days post transduction. Peak levels of endogenous IL1RN (FIG. 46C) and HBG1 (FIG. 46D) were observed 6 days post transduction and the level of activation was sustained out to day 21.

[0082] FIG. 47 shows IL1RN mRNA expression levels.

[0083] FIG. 48 shows a schematic representing the direct conversion of fibroblasts to neurons through ectopic expression of the BAM neuronal transcription factors.

[0084] FIGS. 49A-49C show (FIG. 49A) Schematic of the dCas9-VP64 construct. dCas9-VP64 is a catalytically inactive form of the Cas9 protein fused to a tetramer of the VP16 transcriptional activation domain. (FIG. 49B) Schematic showing the mechanism of RNA-guided recruitment of dCas9-VP64 to a genomic target. (FIG. 49C) Schematic of the experimental protocol to generate iNs with CRISPR/Cas9 transcription factors.

[0085] FIGS. 50A-50B show endogenous ASCL1 expression at day 3 determined by (FIG. 50A) qRT-PCR or total ASCL1 protein detected by (FIG. 50B) immunofluorescence in MEFs transduced with dCas9-VP64 and transfected with either gRNAs targeted to the ASCL1 promoter, ASCL1 cDNA, or luciferase. Asterisk (*) indicates significant ($p < 0.05$) increase in ASCL1 expression with the co-delivery of 8 gRNAs compared to 4 gRNAs. Ectopic expression of ASCL1 produced more protein than induced by dCas9-VP64 and 8 gRNAs targeted to the Ascl1 promoter, but did not activate the endogenous locus by day 3 in culture.

[0086] FIG. 51A shows TUJ1 and MAP2-positive cells generated by ectopic BAM factors or by dCas9-VP64 and gRNAs targeted to the BRN2 and ASCL1 promoters. FIG. 51B shows cells with neuronal morphology expressing a hSyn-RFP reporter at day 11 in N3 medium.

[0087] FIG. 52A shows a cell with neuronal morphology positive for the GCaMP5 calcium indicator in the presence (bottom) or absence (top) of KCl in the culture medium. FIG. 52B shows a trace of normalized fluorescent intensity over time showing depolarization of the cell in response to KCl addition.

[0088] FIGS. 53A-53C show the CRISPR/Cas9 platform for control of mammalian gene regulation. (FIG. 53A) Cas9-based effectors bind genomic sequences in the presence of a chimeric gRNA molecule consisting of a constant region that complexes with Cas9 preceded by an exchangeable 20 bp protospacer that confers target site specificity. FIG. 53A discloses SEQ ID NO: 673. (FIG. 53B) Cas9-based synthetic transcription factors repress transcription of a target gene by interfering with RNA polymerase activity or by binding within the promoter and blocking the binding sites of endogenous transcription factors. (FIG. 53C) Targeting regulatory elements such as enhancers could also potentially block the expression of multiple distal genes.

[0089] FIG. 54 shows targeting the HS2 enhancer using CRISPR/dCas9-KRAB. The HS2 region is a potent enhancer that distally regulates the expression of globin

genes >10 kb downstream. A panel of single gRNAs was designed to target sites along the enhancer region.

[0090] FIGS. 55A-55E show that single gRNAs targeting the HS2 enhancer effect potent transcriptional repression of globin genes. (FIG. 55A) dCas9 and dCas9-KRAB repressors were delivered on a lentiviral vector. Single gRNAs were transiently transfected for screening. When assayed by quantitative RT-PCR at 3 days post-transfection, K562s expressing dCas9-KRAB achieve up to 80% repression of (FIG. 55B) γ -globin, (FIG. 55C) ϵ -globin, and (FIG. 55D) β -globin genes, as compared to control cells that received no gRNA treatment. (FIG. 55E) Protein expression in cells expressing dCas9 or dCas9-KRAB and treated with Cr4 or Cr8 show mild repression of γ -globin expression at day 3, compared to β -actin controls.

[0091] FIGS. 56A-56C show expression of globin locus genes with varying doses of gRNA plasmid delivered to cells treated with (FIG. 56A) no lentivirus, (FIG. 56B) dCas9 lentivirus, or (FIG. 56C) dCas9-KRAB lentivirus. Increasing the dose of Cr4 gRNA plasmid delivered enhanced repression in dCas9-KRAB treated cells, indicating that both the dCas9-KRAB effector and targeted gRNA play a role in achieving repression.

[0092] FIGS. 57A-57D show that stably delivering single gRNAs with dCas9-KRAB silences expression of the globin genes. (FIG. 57A) dCas9 and dCas9-KRAB repressors were co-expressed on a lentiviral vector with single gRNAs. When assayed by quantitative RT-PCR at 7 days post-transduction, K562s expressing dCas9-KRAB achieve up to 95% repression of (FIG. 57B) γ -globin, (FIG. 57C) ϵ -globin, and (FIG. 57D) β -globin genes, as compared to control cells that received no lentiviral treatment.

[0093] FIG. 58 shows that isolating the p300 HAT "Core" for targeted epigenetic modification of histones only via dCas9 fusion.

[0094] FIG. 59 shows a simplified schematic of *S. pyogenes* dCas9-VP64 fusion (top) and dCas9-p300 core fusion (bottom). The Protospacer Adjacent Motifs (PAM) are shown with arrows at target gene loci and synthetic guide RNA (gRNA) is shown with hatched arrows.

[0095] FIGS. 60A-60C show representative data at three human loci demonstrating the efficacy of activation using dCas9-p300 in relation to dCas9-VP64 and dCas9 without any fused effector domain in the human 293T cell culture line.

[0096] FIGS. 61A-61C show the amino acid sequences of the dCas9 constructs. The legend for all FIGS. 61A-61C is shown in FIG. 61A. FIGS. 61A-61C disclose SEQ ID NOS 674-676, respectively, in order of appearance.

[0097] FIG. 62 shows that HAT-dCase9-p300 fusion proteins fail to activate gene expression.

[0098] FIGS. 63A-63B show that gRNA's also act synergistically with dCas9-p300 Core.

[0099] FIGS. 64A-64C show TALEN mediated integration of minidystrophin at the 5'UTR of the Dp427m skeletal muscle isoform of dystrophin in skeletal myoblast cell lines derived from human DMD patients carrying different deletions in the dystrophin gene. DMD patient cells were electroporated with constructs encoding a TALEN pair active at the 5'UTR locus and a donor template carrying the minidystrophin gene. (FIG. 64A) Schematic showing how minidystrophin is integrated into the 5'UTR. (FIG. 64B) Hygromycin-resistant clonal cell lines were isolated and screened by PCR for successful site-specific integrations at the 5'UTR

using the primers shown in FIG. 64A. Asterisks indicate clones selected for further analysis in FIG. 64C. (FIG. 64C) Clonally isolated DMD myoblasts with detected integration events were differentiated for 6 days and assessed for expression of an HA tag fused to the C terminus of minidystrophin.

DETAILED DESCRIPTION

[0100] As described herein, certain methods and engineered CRISPR/CRISPR-associated (Cas) 9-based system compositions have been discovered to be useful for altering the expression of genes, genome engineering, and correcting or reducing the effects of mutations in genes involved in genetic diseases. The CRISPR/Cas9-based system involves a Cas9 protein and at least one guide RNA, which provide the DNA targeting specificity for the system. In particular, the present disclosure describes a Cas9 fusion protein that combines the DNA sequence targeting function of the CRISPR/Cas9-based system with an additional activity, thus allowing changes in gene expression and/or epigenetic status. The system may also be used in genome engineering and correcting or reducing the effects of gene mutations.

[0101] The present disclosure also provides certain compositions and methods for delivering CRISPR/CRISPR-associated (Cas) 9-based system and multiple gRNAs to target one or more endogenous genes. Co-transfection of multiple sgRNAs targeted to a single promoter allow for synergistic activation, however, co-transfection of multiple plasmids leads to variable expression levels in each cell due to differences in copy number. Additionally, gene activation following transfection is transient due to dilution of plasmid DNA over time. Moreover, many cell types are not easily transfected and transient gene expression may not be sufficient for inducing a therapeutic effect. To address these limitations, a single lentiviral system was developed to express Cas9 and up to four sgRNAs from independent promoters. A platform is disclosed that expresses Cas9 or dCas9 fusion proteins and up to four gRNAs from a single lentiviral vector. The lentiviral vector expresses a constitutive or inducible Cas9 or dCas9-VP64 in addition to one, two, three, or four gRNAs expressed from independent promoters. This system enables control of both the magnitude and timing of CRISPR/Cas9-based gene regulation. Furthermore, the lentiviral platform provides the potent and sustained levels of gene expression that will facilitate therapeutic applications of the CRISPR/Cas9 system in primary cells. Finally, this system may be used for editing multiple genes simultaneously, such as the concurrent knockout of several oncogenes.

[0102] The present disclosure also provides certain compositions and methods for delivering site-specific nucleases to skeletal muscle and cardiac muscle using modified adeno-associated virus (AAV) vectors. The site-specific nucleases, which may be engineered, are useful for altering the expression of genes, genome engineering, correcting or reducing the effects of mutations in genes involved in genetic diseases, or manipulating genes involved in other conditions affecting skeletal muscle or cardiac muscle or muscle regeneration. The engineered site-specific nucleases may include a zinc finger nuclease (ZFN), a TAL effector nuclease (TALEN), and/or a CRISPR/Cas9 system for genome editing. As described herein, genes in skeletal muscle tissue were successfully edited in vivo using this unique delivery system. The disclosed invention provides a means to rewrite

the human genome for therapeutic applications and target model species for basic science applications.

[0103] Gene editing is highly dependent on cell cycle and complex DNA repair pathways that vary from tissue to tissue. Skeletal muscle is a very complex environment, consisting of large myofibers with more than 100 nuclei per cell. Gene therapy and biologics in general have been limited for decades by in vivo delivery hurdles. These challenges include stability of the carrier in vivo, targeting the right tissue, getting sufficient gene expression and active gene product, and avoiding toxicity that might overcome activity, which is common with gene editing tools. Other delivery vehicles, such as direct injection of plasmid DNA, work to express genes in skeletal muscle and cardiac muscle in other contexts, but do not work well with these site-specific nucleases for achieving detectable levels of genome editing.

[0104] While many gene sequences are unstable in AAV vectors and therefore undeliverable, these site-specific nucleases are surprisingly stable in the AAV vectors. When these site-specific nucleases are delivered and expressed, they remained active in the skeletal muscle tissue. The protein stability and activity of the site-specific nucleases are highly tissue type- and cell type-dependent. These active and stable nucleases are able to modify gene sequences in the complex environment of skeletal muscle. The current invention describes a way to deliver active forms of this class of therapeutics to skeletal muscle or cardiac muscle that is effective, efficient and facilitates successful genome modification.

[0105] The present disclosure also provides certain fusion epigenetic effector molecules, a dCas9-p300 fusion protein, which provides a robust and potentially more widely applicable tool for synthetic transcriptional modulation compared to the dCas9-VP64 fusion. The activated target genes to a substantially greater extent than the dCas9-VP64 fusion protein at all loci tested. In addition, the p300 has intrinsic endogenous activity at enhancers within the human genome. The dCas9-p300 fusion protein may be able to activate endogenous target gene promoters and enhancer regions.

[0106] The dCas9-p300 fusion protein can be used in human tissue culture cell lines to activate gene expression. This fusion protein may be used to direct the epigenetic state of target loci within human cells with precision and predictability in order to control differentiation, modulate cellular regulation, and apply innovative potential therapies. Current technologies are limited in the strength of activation and the extent and sustainability of epigenetic modulation; obstacles which may be obviated via utilization of this new fusion protein.

[0107] Section headings as used in this section and the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

1. DEFINITIONS

[0108] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are

incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0109] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[0110] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0111] “Adeno-associated virus” or “AAV” as used interchangeably herein refers to a small virus belonging to the genus Dependovirus of the Parvoviridae family that infects humans and some other primate species. AAV is not currently known to cause disease and consequently the virus causes a very mild immune response.

[0112] “Binding region” as used herein refers to the region within a nuclease target region that is recognized and bound by the nuclease.

[0113] “Cardiac muscle” or “heart muscle” as used interchangeably herein means a type of involuntary striated muscle found in the walls and histological foundation of the heart, the myocardium. Cardiac muscle is made of cardiomyocytes or myocardiocytes. Myocardiocytes show striations similar to those on skeletal muscle cells but contain only one, unique nucleus, unlike the multinucleated skeletal cells.

[0114] “Cardiac muscle condition” as used herein refers to a condition related to the cardiac muscle, such as cardiomyopathy, heart failure, arrhythmia, and inflammatory heart disease.

[0115] “Coding sequence” or “encoding nucleic acid” as used herein means the nucleic acids (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence can further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to which the nucleic acid is administered. The coding sequence may be codon optimize.

[0116] “Complement” or “complementary” as used herein means a nucleic acid can mean Watson-Crick (e.g., A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules. “Complementarity” refers to a property shared between two nucleic acid sequences, such that when they are aligned antiparallel to each other, the nucleotide bases at each position will be complementary.

[0117] “Correcting,” “genome editing” and “restoring” as used herein refers to changing a mutant gene that encodes a truncated protein or no protein at all, such that a full-length functional or partially full-length functional protein expression is obtained. Correcting or restoring a mutant gene may include replacing the region of the gene that has the mutation

or replacing the entire mutant gene with a copy of the gene that does not have the mutation with a repair mechanism such as homology-directed repair (HDR). Correcting or restoring a mutant gene may also include repairing a frameshift mutation that causes a premature stop codon, an aberrant splice acceptor site or an aberrant splice donor site, by generating a double stranded break in the gene that is then repaired using non-homologous end joining (NHEJ). NHEJ may add or delete at least one base pair during repair which may restore the proper reading frame and eliminate the premature stop codon. Correcting or restoring a mutant gene may also include disrupting an aberrant splice acceptor site or splice donor sequence. Correcting or restoring a mutant gene may also include deleting a non-essential gene segment by the simultaneous action of two nucleases on the same DNA strand in order to restore the proper reading frame by removing the DNA between the two nuclease target sites and repairing the DNA break by NHEJ.

[0118] “Donor DNA”, “donor template” and “repair template” as used interchangeably herein refers to a double-stranded DNA fragment or molecule that includes at least a portion of the gene of interest. The donor DNA may encode a full-functional protein or a partially-functional protein.

[0119] “Duchenne Muscular Dystrophy” or “DMD” as used interchangeably herein refers to a recessive, fatal, X-linked disorder that results in muscle degeneration and eventual death. DMD is a common hereditary monogenic disease and occurs in 1 in 3500 males. DMD is the result of inherited or spontaneous mutations that cause nonsense or frame shift mutations in the dystrophin gene. The majority of dystrophin mutations that cause DMD are deletions of exons that disrupt the reading frame and cause premature translation termination in the dystrophin gene. DMD patients typically lose the ability to physically support themselves during childhood, become progressively weaker during the teenage years, and die in their twenties.

[0120] “Dystrophin” as used herein refers to a rod-shaped cytoplasmic protein which is a part of a protein complex that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix through the cell membrane. Dystrophin provides structural stability to the dystroglycan complex of the cell membrane that is responsible for regulating muscle cell integrity and function. The dystrophin gene or “DMD gene” as used interchangeably herein is 2.2 megabases at locus Xp21. The primary transcription measures about 2,400 kb with the mature mRNA being about 14 kb. 79 exons code for the protein which is over 3500 amino acids.

[0121] “Exon 51” as used herein refers to the 51st exon of the dystrophin gene. Exon 51 is frequently adjacent to frame-disrupting deletions in DMD patients and has been targeted in clinical trials for oligonucleotide-based exon skipping. A clinical trial for the exon 51 skipping compound eteplirsen recently reported a significant functional benefit across 48 weeks, with an average of 47% dystrophin positive fibers compared to baseline. Mutations in exon 51 are ideally suited for permanent correction by NHEJ-based genome editing.

[0122] “Frameshift” or “frameshift mutation” as used interchangeably herein refers to a type of gene mutation wherein the addition or deletion of one or more nucleotides causes a shift in the reading frame of the codons in the mRNA. The shift in reading frame may lead to the alteration

in the amino acid sequence at protein translation, such as a missense mutation or a premature stop codon.

[0123] “Functional” and “full-functional” as used herein describes protein that has biological activity. A “functional gene” refers to a gene transcribed to mRNA, which is translated to a functional protein.

[0124] “Fusion protein” as used herein refers to a chimeric protein created through the joining of two or more genes that originally coded for separate proteins. The translation of the fusion gene results in a single polypeptide with functional properties derived from each of the original proteins.

[0125] “Genetic construct” as used herein refers to the DNA or RNA molecules that comprise a nucleotide sequence that encodes a protein. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term “expressible form” refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

[0126] “Genetic disease” as used herein refers to a disease, partially or completely, directly or indirectly, caused by one or more abnormalities in the genome, especially a condition that is present from birth. The abnormality may be a mutation, an insertion or a deletion. The abnormality may affect the coding sequence of the gene or its regulatory sequence. The genetic disease may be, but not limited to DMD, hemophilia, cystic fibrosis, Huntington’s chorea, familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson’s disease, congenital hepatic porphyria, inherited disorders of hepatic metabolism, Lesch Nyhan syndrome, sickle cell anemia, thalassemias, xeroderma pigmentosum, Fanconi’s anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom’s syndrome, retinoblastoma, and Tay-Sachs disease.

[0127] “Homology-directed repair” or “HDR” as used interchangeably herein refers to a mechanism in cells to repair double strand DNA lesions when a homologous piece of DNA is present in the nucleus, mostly in G2 and S phase of the cell cycle. HDR uses a donor DNA template to guide repair and may be used to create specific sequence changes to the genome, including the targeted addition of whole genes. If a donor template is provided along with the site specific nuclease, such as with a CRISPR/Cas9-based systems, then the cellular machinery will repair the break by homologous recombination, which is enhanced several orders of magnitude in the presence of DNA cleavage. When the homologous DNA piece is absent, non-homologous end joining may take place instead.

[0128] “Genome editing” as used herein refers to changing a gene. Genome editing may include correcting or restoring a mutant gene. Genome editing may include knocking out a gene, such as a mutant gene or a normal gene. Genome editing may be used to treat disease or enhance muscle repair by changing the gene of interest.

[0129] “Identical” or “identity” as used herein in the context of two or more nucleic acids or polypeptide sequences means that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the

specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

[0130] “Mutant gene” or “mutated gene” as used interchangeably herein refers to a gene that has undergone a detectable mutation. A mutant gene has undergone a change, such as the loss, gain, or exchange of genetic material, which affects the normal transmission and expression of the gene. A “disrupted gene” as used herein refers to a mutant gene that has a mutation that causes a premature stop codon. The disrupted gene product is truncated relative to a full-length undisrupted gene product.

[0131] “Non-homologous end joining (NHEJ) pathway” as used herein refers to a pathway that repairs double-strand breaks in DNA by directly ligating the break ends without the need for a homologous template. The template-independent re-ligation of DNA ends by NHEJ is a stochastic, error-prone repair process that introduces random micro-insertions and micro-deletions (indels) at the DNA break-point. This method may be used to intentionally disrupt, delete, or alter the reading frame of targeted gene sequences. NHEJ typically uses short homologous DNA sequences called microhomologies to guide repair. These microhomologies are often present in single-stranded overhangs on the end of double-strand breaks. When the overhangs are perfectly compatible, NHEJ usually repairs the break accurately, yet imprecise repair leading to loss of nucleotides may also occur, but is much more common when the overhangs are not compatible.

[0132] “Normal gene” as used herein refers to a gene that has not undergone a change, such as a loss, gain, or exchange of genetic material. The normal gene undergoes normal gene transmission and gene expression.

[0133] “Nuclease mediated NHEJ” as used herein refers to NHEJ that is initiated after a nuclease, such as a cas9, cuts double stranded DNA.

[0134] “Nucleic acid” or “oligonucleotide” or “polynucleotide” as used herein means at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

[0135] Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be

DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

[0136] “Operably linked” as used herein means that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

[0137] “Partially-functional” as used herein describes a protein that is encoded by a mutant gene and has less biological activity than a functional protein but more than a non-functional protein.

[0138] “Premature stop codon” or “out-of-frame stop codon” as used interchangeably herein refers to nonsense mutation in a sequence of DNA, which results in a stop codon at location not normally found in the wild-type gene. A premature stop codon may cause a protein to be truncated or shorter compared to the full-length version of the protein.

[0139] “Promoter” as used herein means a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which may be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

[0140] “Repeat variable diresidue” or “RVD” as used interchangeably herein refers to a pair of adjacent amino acid residues within a DNA recognition motif (also known as “RVD module”), which includes 33-35 amino acids, of a TALE DNA-binding domain. The RVD determines the nucleotide specificity of the RVD module. RVD modules may be combined to produce an RVD array. The “RVD array length” as used herein refers to the number of RVD modules that corresponds to the length of the nucleotide sequence within the TALEN target region that is recognized by a TALEN, i.e., the binding region.

[0141] “Site-specific nuclease” as used herein refers to an enzyme capable of specifically recognizing and cleaving DNA sequences. The site-specific nuclease may be engi-

neered. Examples of engineered site-specific nucleases include zinc finger nucleases (ZFNs), TAL effector nucleases (TALENs), and CRISPR/Cas9-based systems.

[0142] “Skeletal muscle” as used herein refers to a type of striated muscle, which is under the control of the somatic nervous system and attached to bones by bundles of collagen fibers known as tendons. Skeletal muscle is made up of individual components known as myocytes, or “muscle cells”, sometimes colloquially called “muscle fibers.” Myocytes are formed from the fusion of developmental myoblasts (a type of embryonic progenitor cell that gives rise to a muscle cell) in a process known as myogenesis. These long, cylindrical, multinucleated cells are also called myofibers.

[0143] “Skeletal muscle condition” as used herein refers to a condition related to the skeletal muscle, such as muscular dystrophies, aging, muscle degeneration, wound healing, and muscle weakness or atrophy.

[0144] “Spacers” and “spacer region” as used interchangeably herein refers to the region within a TALEN or ZFN target region that is between, but not a part of, the binding regions for two TALENs or ZFNs.

[0145] “Subject” and “patient” as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (for example, a monkey, such as a cynomolgous or rhesus monkey, chimpanzee, etc.) and a human). In some embodiments, the subject may be a human or a non-human. The subject or patient may be undergoing other forms of treatment.

[0146] “Target gene” as used herein refers to any nucleotide sequence encoding a known or putative gene product. The target gene may be a mutated gene involved in a genetic disease.

[0147] “Target region” as used herein refers to the region of the target gene to which the site-specific nuclease is designed to bind and cleave.

[0148] “Transcription activator-like effector” or “TALE” as used herein refers to a protein structure that recognizes and binds to a particular DNA sequence. The “TALE DNA-binding domain” refers to a DNA-binding domain that includes an array of tandem 33-35 amino acid repeats, also known as RVD modules, each of which specifically recognizes a single base pair of DNA. RVD modules may be arranged in any order to assemble an array that recognizes a defined sequence.

[0149] A binding specificity of a TALE DNA-binding domain is determined by the RVD array followed by a single truncated repeat of 20 amino acids. A TALE DNA-binding domain may have 12 to 27 RVD modules, each of which contains an RVD and recognizes a single base pair of DNA. Specific RVDs have been identified that recognize each of the four possible DNA nucleotides (A, T, C, and G). Because the TALE DNA-binding domains are modular, repeats that recognize the four different DNA nucleotides may be linked together to recognize any particular DNA sequence. These targeted DNA-binding domains may then be combined with catalytic domains to create functional enzymes, including artificial transcription factors, methyltransferases, integrases, nucleases, and recombinases.

[0150] “Transcription activator-like effector nucleases” or “TALENs” as used interchangeably herein refers to engineered fusion proteins of the catalytic domain of a nuclease,

such as endonuclease FokI, and a designed TALE DNA-binding domain that may be targeted to a custom DNA sequence. A “TALEN monomer” refers to an engineered fusion protein with a catalytic nuclease domain and a designed TALE DNA-binding domain. Two TALEN monomers may be designed to target and cleave a TALEN target region.

[0151] “Transgene” as used herein refers to a gene or genetic material containing a gene sequence that has been isolated from one organism and is introduced into a different organism. This non-native segment of DNA may retain the ability to produce RNA or protein in the transgenic organism, or it may alter the normal function of the transgenic organism’s genetic code. The introduction of a transgene has the potential to change the phenotype of an organism.

[0152] “Variant” used herein with respect to a nucleic acid means (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

[0153] “Variant” with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes may be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., *J. Mol. Biol.* 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes may be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ± 2 are substituted. The hydrophilicity of amino acids may also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide. Substitutions may be performed with amino acids having hydrophilicity values within ± 2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

[0154] “Vector” as used herein means a nucleic acid sequence containing an origin of replication. A vector may be a viral vector, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be a self-replicating extrachromosomal vector, and preferably, is a DNA plasmid.

For example, the vector may encode an iCas9-VP64 fusion protein comprising the amino acid sequence of SEQ ID NO: 1 or at least one gRNA nucleotide sequence of any one of SEQ ID NOs: 5-40, 65-144, 492-515, 540-563, and 585-625. Alternatively, the vector may encode Cas9 and at least one gRNA nucleotide sequence of any one of SEQ ID NOs: 5-40, 65-144, 492-515, 540-563, and 585-625.

[0155] “Zinc finger” as used herein refers to a protein structure that recognizes and binds to DNA sequences. The zinc finger domain is the most common DNA-binding motif in the human proteome. A single zinc finger contains approximately 30 amino acids and the domain typically functions by binding 3 consecutive base pairs of DNA via interactions of a single amino acid side chain per base pair.

[0156] “Zinc finger nuclease” or “ZFN” as used interchangeably herein refers to a chimeric protein molecule comprising at least one zinc finger DNA binding domain effectively linked to at least one nuclease or part of a nuclease capable of cleaving DNA when fully assembled.

[0157] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. For example, any nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those that are well known and commonly used in the art. The meaning and scope of the terms should be clear; in the event however of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

2. COMPOSITIONS FOR GENOME EDITING

[0158] The present invention is directed to compositions for genome editing, genomic alteration or altering gene expression of a target gene. The compositions may include a may include viral vector and fusion protein such as a site-specific nuclease or CRISPR/Cas9-system with at least one gRNA.

[0159] a. Compositions for Genome Editing in Muscle

[0160] The present invention is directed to a composition for genome editing a target gene in skeletal muscle or cardiac muscle of a subject. The composition includes a modified AAV vector and a nucleotide sequence encoding a site-specific nuclease. The composition delivers active forms of site-specific nucleases to skeletal muscle or cardiac muscle. The composition may further comprise a donor DNA or a transgene. These compositions may be used in genome editing, genome engineering, and correcting or reducing the effects of mutations in genes involved in genetic diseases and/or other skeletal or cardiac muscle conditions.

[0161] The target gene may be involved in differentiation of a cell or any other process in which activation, repression, or disruption of a gene may be desired, or may have a mutation such as a deletion, frameshift mutation, or a nonsense mutation. If the target gene has a mutation that causes a premature stop codon, an aberrant splice acceptor site or an aberrant splice donor site, the site-specific nucleases may be designed to recognize and bind a nucleotide sequence upstream or downstream from the premature stop

codon, the aberrant splice acceptor site or the aberrant splice donor site. The site-specific nucleases may also be used to disrupt normal gene splicing by targeting splice acceptors and donors to induce skipping of premature stop codons or restore a disrupted reading frame. The site-specific nucleases may or may not mediate off-target changes to protein-coding regions of the genome.

3. CRISPR SYSTEM

[0162] “Clustered Regularly Interspaced Short Palindromic Repeats” and “CRISPRs”, as used interchangeably herein refers to loci containing multiple short direct repeats that are found in the genomes of approximately 40% of sequenced bacteria and 90% of sequenced archaea. The CRISPR system is a microbial nuclease system involved in defense against invading phages and plasmids that provides a form of acquired immunity. The CRISPR loci in microbial hosts contain a combination of CRISPR-associated (Cas) genes as well as non-coding RNA elements capable of programming the specificity of the CRISPR-mediated nucleic acid cleavage. Short segments of foreign DNA, called spacers, are incorporated into the genome between CRISPR repeats, and serve as a ‘memory’ of past exposures. Cas9 forms a complex with the 3' end of the sgRNA, and the protein-RNA pair recognizes its genomic target by complementary base pairing between the 5' end of the sgRNA sequence and a predefined 20 bp DNA sequence, known as the protospacer. This complex is directed to homologous loci of pathogen DNA via regions encoded within the crRNA, i.e., the protospacers, and protospacer-adjacent motifs (PAMs) within the pathogen genome. The non-coding CRISPR array is transcribed and cleaved within direct repeats into short crRNAs containing individual spacer sequences, which direct Cas nucleases to the target site (protospacer). By simply exchanging the 20 bp recognition sequence of the expressed sgRNA, the Cas9 nuclease can be directed to new genomic targets. CRISPR spacers are used to recognize and silence exogenous genetic elements in a manner analogous to RNAi in eukaryotic organisms.

[0163] Three classes of CRISPR systems (Types I, II and III effector systems) are known. The Type II effector system carries out targeted DNA double-strand break in four sequential steps, using a single effector enzyme, Cas9, to cleave dsDNA. Compared to the Type I and Type III effector systems, which require multiple distinct effectors acting as a complex, the Type II effector system may function in alternative contexts such as eukaryotic cells. The Type II effector system consists of a long pre-crRNA, which is transcribed from the spacer-containing CRISPR locus, the Cas9 protein, and a tracrRNA, which is involved in pre-crRNA processing. The tracrRNAs hybridize to the repeat regions separating the spacers of the pre-crRNA, thus initiating dsRNA cleavage by endogenous RNase III. This cleavage is followed by a second cleavage event within each spacer by Cas9, producing mature crRNAs that remain associated with the tracrRNA and Cas9, forming a Cas9: crRNA-tracrRNA complex.

[0164] The Cas9:crRNA-tracrRNA complex unwinds the DNA duplex and searches for sequences matching the crRNA to cleave. Target recognition occurs upon detection of complementarity between a “protospacer” sequence in the target DNA and the remaining spacer sequence in the crRNA. Cas9 mediates cleavage of target DNA if a correct protospacer-adjacent motif (PAM) is also present at the 3'

end of the protospacer. For protospacer targeting, the sequence must be immediately followed by the protospacer-adjacent motif (PAM), a short sequence recognized by the Cas9 nuclease that is required for DNA cleavage. Different Type II systems have differing PAM requirements. The *S. pyogenes* CRISPR system may have the PAM sequence for this Cas9 (SpCas9) as 5'-NRG-3', where R is either A or G, and characterized the specificity of this system in human cells. A unique capability of the CRISPR/Cas9 system is the straightforward ability to simultaneously target multiple distinct genomic loci by co-expressing a single Cas9 protein with two or more sgRNAs. For example, the *Streptococcus pyogenes* Type II system naturally prefers to use an “NGG” sequence, where “N” can be any nucleotide, but also accepts other PAM sequences, such as “NAG” in engineered systems (Hsu et al., *Nature Biotechnology* (2013) doi:10.1038/nbt.2647). Similarly, the Cas9 derived from *Neisseria meningitidis* (NmCas9) normally has a native PAM of NNNNGATT, but has activity across a variety of PAMs, including a highly degenerate NNNNGNNN PAM (Esvelt et al. *Nature Methods* (2013) doi:10.1038/nmeth.2681).

4. CRISPR/CAS9-BASED SYSTEM

[0165] An engineered form of the Type II effector system of *Streptococcus pyogenes* was shown to function in human cells for genome engineering. In this system, the Cas9 protein was directed to genomic target sites by a synthetically reconstituted “guide RNA” (“gRNA”, also used interchangeably herein as a chimeric single guide RNA (“sgRNA”)), which is a crRNA-tracrRNA fusion that obviates the need for RNase III and crRNA processing in general (see FIG. 53A). Provided herein are CRISPR/Cas9-based engineered systems for use in genome editing and treating genetic diseases. The CRISPR/Cas9-based engineered systems may be designed to target any gene, including genes involved in a genetic disease, aging, tissue regeneration, or wound healing. The CRISPR/Cas9-based systems may include a Cas9 protein or Cas9 fusion protein and at least one gRNA. The Cas9 fusion protein may, for example, include a domain that has a different activity than what is endogenous to Cas9, such as a transactivation domain.

[0166] The target gene may be involved in differentiation of a cell or any other process in which activation of a gene may be desired, or may have a mutation such as a frameshift mutation or a nonsense mutation. If the target gene has a mutation that causes a premature stop codon, an aberrant splice acceptor site or an aberrant splice donor site, the CRISPR/Cas9-based system may be designed to recognize and bind a nucleotide sequence upstream or downstream from the premature stop codon, the aberrant splice acceptor site or the aberrant splice donor site. The CRISPR-Cas9-based system may also be used to disrupt normal gene splicing by targeting splice acceptors and donors to induce skipping of premature stop codons or restore a disrupted reading frame. The CRISPR/Cas9-based system may or may not mediate off-target changes to protein-coding regions of the genome.

[0167] a. Cas9

[0168] The CRISPR/Cas9-based system may include a Cas9 protein or a Cas9 fusion protein. Cas9 protein is an endonuclease that cleaves nucleic acid and is encoded by the CRISPR loci and is involved in the Type II CRISPR system. The Cas9 protein may be from any bacterial or archaea species, such as *Streptococcus pyogenes*. The Cas9 protein

may be mutated so that the nuclease activity is inactivated. An inactivated Cas9 protein from *Streptococcus pyogenes* (iCas9, also referred to as “dCas9”) with no endonuclease activity has been recently targeted to genes in bacteria, yeast, and human cells by gRNAs to silence gene expression through steric hindrance. As used herein, “iCas9” and “dCas9” both refer to a Cas9 protein that has the amino acid substitutions D10A and H840A and has its nuclease activity inactivated. For example, the CRISPR/Cas9-based system may include a Cas9 of SEQ ID NO: 459 or 461.

[0169] b. Cas9 Fusion Protein

[0170] The CRISPR/Cas9-based system may include a fusion protein. The fusion protein may comprise two heterologous polypeptide domains, wherein the first polypeptide domain comprises a Cas protein and the second polypeptide domain has an activity such as transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nuclease activity, nucleic acid association activity, methylase activity, or demethylase activity. The fusion protein may include a Cas9 protein or a mutated Cas9 protein, as described above, fused to a second polypeptide domain that has an activity such as transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nuclease activity, nucleic acid association activity, methylase activity, or demethylase activity.

[0171] (1) Transcription Activation Activity

[0172] The second polypeptide domain may have transcription activation activity, i.e., a transactivation domain. For example, gene expression of endogenous mammalian genes, such as human genes, may be achieved by targeting a fusion protein of iCas9 and a transactivation domain to mammalian promoters via combinations of gRNAs. The transactivation domain may include a VP16 protein, multiple VP16 proteins, such as a VP48 domain or VP64 domain, or p65 domain of NF kappa B transcription activator activity. For example, the fusion protein may be iCas9-VP64.

[0173] (2) Transcription Repression Activity

[0174] The second polypeptide domain may have transcription repression activity. The second polypeptide domain may have a Kruppel associated box activity, such as a KRAB domain, ERF repressor domain activity, Mx1 repressor domain activity, SID4X repressor domain activity, Mad-SID repressor domain activity or TATA box binding protein activity. For example, the fusion protein may be dCas9-KRAB.

[0175] (3) Transcription Release Factor Activity

[0176] The second polypeptide domain may have transcription release factor activity. The second polypeptide domain may have eukaryotic release factor 1 (ERF1) activity or eukaryotic release factor 3 (ERF3) activity.

[0177] (4) Histone Modification Activity

[0178] The second polypeptide domain may have histone modification activity. The second polypeptide domain may have histone deacetylase, histone acetyltransferase, histone demethylase, or histone methyltransferase activity. The histone acetyltransferase may be p300 or CREB-binding protein (CBP) protein, or fragments thereof. For example, the fusion protein may be dCas9-p300.

[0179] (5) Nuclease Activity

[0180] The second polypeptide domain may have nuclease activity that is different from the nuclease activity of the

Cas9 protein. A nuclease, or a protein having nuclease activity, is an enzyme capable of cleaving the phosphodiester bonds between the nucleotide subunits of nucleic acids. Nucleases are usually further divided into endonucleases and exonucleases, although some of the enzymes may fall in both categories. Well known nucleases are deoxyribonuclease and ribonuclease.

[0181] (6) Nucleic Acid Association Activity

[0182] The second polypeptide domain may have nucleic acid association activity or nucleic acid binding protein-DNA-binding domain (DBD) is an independently folded protein domain that contains at least one motif that recognizes double- or single-stranded DNA. A DBD can recognize a specific DNA sequence (a recognition sequence) or have a general affinity to DNA. nucleic acid association region selected from the group consisting of helix-turn-helix region, leucine zipper region, winged helix region, winged helix-turn-helix region, helix-loop-helix region, immunoglobulin fold, B3 domain, Zinc finger, HMG-box, Wor3 domain, TAL effector DNA-binding domain.

[0183] (7) Methylase Activity

[0184] The second polypeptide domain may have methylase activity, which involves transferring a methyl group to DNA, RNA, protein, small molecule, cytosine or adenine. The second polypeptide domain may include a DNA methyltransferase.

[0185] (8) Demethylase Activity

[0186] The second polypeptide domain may have demethylase activity. The second polypeptide domain may include an enzyme that remove methyl (CH₃-) groups from nucleic acids, proteins (in particular histones), and other molecules. Alternatively, the second polypeptide may convert the methyl group to hydroxymethylcytosine in a mechanism for demethylating DNA. The second polypeptide may catalyze this reaction. For example, the second polypeptide that catalyzes this reaction may be Tet1.

[0187] c. gRNA

[0188] The gRNA provides the targeting of the CRISPR/Cas9-based system. The gRNA is a fusion of two noncoding RNAs: a crRNA and a tracrRNA. The sgRNA may target any desired DNA sequence by exchanging the sequence encoding a 20 bp protospacer which confers targeting specificity through complementary base pairing with the desired DNA target. gRNA mimics the naturally occurring crRNA: tracrRNA duplex involved in the Type II Effector system. This duplex, which may include, for example, a 42-nucleotide crRNA and a 75-nucleotide tracrRNA, acts as a guide for the Cas9 to cleave the target nucleic acid. The “target region”, “target sequence” or “protospacer” as used interchangeably herein refers to the region of the target gene to which the CRISPR/Cas9-based system targets. The CRISPR/Cas9-based system may include at least one gRNA, wherein the gRNAs target different DNA sequences. The target DNA sequences may be overlapping. The target sequence or protospacer is followed by a PAM sequence at the 3' end of the protospacer. Different Type II systems have differing PAM requirements. For example, the *Streptococcus pyogenes* Type II system uses an “NGG” sequence, where “N” can be any nucleotide.

[0189] The number of gRNA administered to the cell may be at least 1 gRNA, at least 2 different gRNA, at least 3 different gRNA at least 4 different gRNA, at least 5 different gRNA, at least 6 different gRNA, at least 7 different gRNA, at least 8 different gRNA, at least 9 different gRNA, at least

10 different gRNAs, at least 11 different gRNAs, at least 12 different gRNAs, at least 13 different gRNAs, at least 14 different gRNAs, at least 15 different gRNAs, at least 16 different gRNAs, at least 17 different gRNAs, at least 18 different gRNAs, at least 18 different gRNAs, at least 20 different gRNAs, at least 25 different gRNAs, at least 30 different gRNAs, at least 35 different gRNAs, at least 40 different gRNAs, at least 45 different gRNAs, or at least 50 different gRNAs. The number of gRNA administered to the cell may be between at least 1 gRNA to at least 50 different gRNAs, at least 1 gRNA to at least 45 different gRNAs, at least 1 gRNA to at least 40 different gRNAs, at least 1 gRNA to at least 35 different gRNAs, at least 1 gRNA to at least 30 different gRNAs, at least 1 gRNA to at least 25 different gRNAs, at least 1 gRNA to at least 20 different gRNAs, at least 1 gRNA to at least 16 different gRNAs, at least 1 gRNA to at least 12 different gRNAs, at least 1 gRNA to at least 8 different gRNAs, at least 1 gRNA to at least 4 different gRNAs, at least 4 gRNAs to at least 50 different gRNAs, at least 4 different gRNAs to at least 45 different gRNAs, at least 4 different gRNAs to at least 40 different gRNAs, at least 4 different gRNAs to at least 35 different gRNAs, at least 4 different gRNAs to at least 30 different gRNAs, at least 4 different gRNAs to at least 25 different gRNAs, at least 4 different gRNAs to at least 20 different gRNAs, at least 4 different gRNAs to at least 16 different gRNAs, at least 4 different gRNAs to at least 12 different gRNAs, at least 4 different gRNAs to at least 8 different gRNAs, at least 8 different gRNAs to at least 50 different gRNAs, at least 8 different gRNAs to at least 45 different gRNAs, at least 8 different gRNAs to at least 40 different gRNAs, at least 8 different gRNAs to at least 35 different gRNAs, 8 different gRNAs to at least 30 different gRNAs, at least 8 different gRNAs to at least 25 different gRNAs, 8 different gRNAs to at least 20 different gRNAs, at least 8 different gRNAs to at least 16 different gRNAs, or 8 different gRNAs to at least 12 different gRNAs.

[0190] The gRNA may comprise a complementary polynucleotide sequence of the target DNA sequence followed by a PAM sequence. The gRNA may comprise a “G” at the 5' end of the complementary polynucleotide sequence. The gRNA may comprise at least a 10 base pair, at least a 11 base pair, at least a 12 base pair, at least a 13 base pair, at least a 14 base pair, at least a 15 base pair, at least a 16 base pair, at least a 17 base pair, at least a 18 base pair, at least a 19 base pair, at least a 20 base pair, at least a 21 base pair, at least a 22 base pair, at least a 23 base pair, at least a 24 base pair, at least a 25 base pair, at least a 30 base pair, or at least a 35 base pair complementary polynucleotide sequence of the target DNA sequence followed by a PAM sequence. The PAM sequence may be “NGG”, where “N” can be any nucleotide. The gRNA may target at least one of the promoter region, the enhancer region or the transcribed region of the target gene. The gRNA may include a nucleic acid sequence of at least one of SEQ ID NOs: 5-40, 65-144, 492-515, 540-563, 585-625, 462 (FIG. 40), 464 (FIG. 41), and 465 (FIG. 41).

[0191] The gRNA may target any nucleic acid sequence. The nucleic acid sequence target may be DNA. The DNA may be any gene. For example, the gRNA may target a gene, such as BRN2, MYT1L, ASCL1, NANOG, VEGFA, TERT, IL1B, IL1R2, IL1RN, HBG1, HBG2, MYOD1, OCT4, and DMD.

[0192] (1) Dystrophin

[0193] Dystrophin is a rod-shaped cytoplasmic protein which is a part of a protein complex that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix through the cell membrane. Dystrophin provides structural stability to the dystroglycan complex of the cell membrane. The dystrophin gene is 2.2 megabases at locus Xp21. The primary transcription measures about 2,400 kb with the mature mRNA being about 14 kb. 79 exons code for the protein which is over 3500 amino acids. Normal skeleton muscle tissue contains only small amounts of dystrophin but its absence or abnormal expression leads to the development of severe and incurable symptoms. Some mutations in the dystrophin gene lead to the production of defective dystrophin and severe dystrophic phenotype in affected patients. Some mutations in the dystrophin gene lead to partially-functional dystrophin protein and a much milder dystrophic phenotype in affected patients.

[0194] DMD is the result of inherited or spontaneous mutations that cause nonsense or frame shift mutations in the dystrophin gene. Naturally occurring mutations and their consequences are relatively well understood for DMD. It is known that in-frame deletions that occur in the exon 45-55 region contained within the rod domain can produce highly functional dystrophin proteins, and many carriers are asymptomatic or display mild symptoms. Furthermore, more than 60% of patients may theoretically be treated by targeting exons in this region of the dystrophin gene. Efforts have been made to restore the disrupted dystrophin reading frame in DMD patients by skipping non-essential exons during mRNA splicing to produce internally deleted but functional dystrophin proteins. The deletion of internal dystrophin exons retain the proper reading frame but cause the less severe Becker muscular dystrophy.

[0195] (2) CRISPR/Cas9-Based System for Targeting Dystrophin

[0196] A CRISPR/Cas9-based system specific for dystrophin gene are disclosed herein. The CRISPR/Cas9-based system may include Cas9 and at least one gRNA to target the dystrophin gene. The CRISPR/Cas9-based system may bind and recognize a target region. The target regions may be chosen immediately upstream of possible out-of-frame stop codons such that insertions or deletions during the repair process restore the dystrophin reading frame by frame conversion. Target regions may also be splice acceptor sites or splice donor sites, such that insertions or deletions during the repair process disrupt splicing and restore the dystrophin reading frame by splice site disruption and exon exclusion. Target regions may also be aberrant stop codons such that insertions or deletions during the repair process restore the dystrophin reading frame by eliminating or disrupting the stop codon.

[0197] Single or multiplexed sgRNAs may be designed to restore the dystrophin reading frame by targeting the mutational hotspot at exons 45-55 and introducing either intronic small insertions and deletions, or large deletions of one or more exons. Following treatment with Cas9 and one or more sgRNAs, dystrophin expression may be restored in Duchenne patient muscle cells in vitro. Human dystrophin was detected in vivo following transplantation of genetically corrected patient cells into immunodeficient mice. Significantly, the unique multiplex gene editing capabilities of the CRISPR/Cas9 system enable efficiently generating large deletions of this mutational hotspot region that can correct

up to 62% of patient mutations by universal or patient-specific gene editing approaches.

[0198] The CRISPR/Cas9-based system may use gRNA of varying sequences and lengths. Examples of gRNAs may be found in Table 6. The CRISPR/Cas9-based system may target a nucleic acid sequence of SEQ ID NOs: 65-144, or a complement thereof. The gRNA may include a nucleotide sequence selected from the group consisting of SEQ ID NO: 65-144, or a complement thereof. For example, the disclosed CRISPR/Cas9-based systems were engineered to mediate highly efficient gene editing at exon 51 of the dystrophin gene. These CRISPR/Cas9-based systems restored dystrophin protein expression in cells from DMD patients.

[0199] (a) Exons 51 and 45-55

[0200] Exon 51 is frequently adjacent to frame-disrupting deletions in DMD. Elimination of exon 51 from the dystrophin transcript by exon skipping can be used to treat approximately 15% of all DMD patients. This class of DMD mutations is ideally suited for permanent correction by NIEJ-based genome editing and HDR. The CRISPR/Cas9-based systems described herein have been developed for targeted modification of exon 51 in the human dystrophin gene. These CRISPR/Cas9-based systems were transfected into human DMD cells and mediated efficient gene modification and conversion to the correct reading frame. Protein restoration was concomitant with frame restoration and detected in a bulk population of CRISPR/Cas9-based system-treated cells. Similarly, the elimination of exons 45-55 of the dystrophin transcript can be used to treat approximately 62% of all DMD patients.

[0201] (3) AAV/CRISPR Constructs

[0202] AAV may be used to deliver CRISPRs using various construct configurations (see FIG. 39). For example, AAV may deliver Cas9 and gRNA expression cassettes on separate vectors. Alternatively, if the small Cas9 proteins, derived from species such as *Staphylococcus aureus* or *Neisseria meningitidis*, are used then both the Cas9 and up to two gRNA expression cassettes may be combined in a single AAV vector within the 4.7 kb packaging limit (see FIG. 39).

5. MULTIPLEX CRISPR/CAS9-BASED SYSTEM

[0203] The present disclosure is directed to a multiplex CRISPR/Cas9-Based System which includes a CRISPR/CRISPR-associated (Cas) 9-based system, such as Cas9 or dCas9, and multiple gRNAs to target one or more endogenous genes. This platform utilizes a convenient Golden Gate cloning method to rapidly incorporate up to four independent sgRNA expression cassettes into a single lentiviral vector. Each sgRNA was efficiently expressed and could mediate multiplex gene editing at diverse loci in immortalized and primary human cell lines. Transient transcriptional activation in cell lines stably expressing dCas9-VP64 was demonstrated to be tunable by synergistic activation with one to four sgRNAs. Furthermore, the single lentiviral vector can induce sustained and long-term endogenous gene expression in immortalized and primary human cells. This system allows for rapid assembly of a single lentiviral vector that enables efficient multiplex gene editing or activation in model and primary cell lines.

[0204] The multiplex CRISPR/Cas9-Based System provides potency of transcriptional activation and tunable induction of transcriptional activation. Readily generated by Golden Gate assembly, the final vector expresses a consti-

tutive Cas9 or dCas9-VP64 in addition to one, two, three, or four sgRNAs expressed from independent promoters. Each promoter is capable of efficiently expressing sgRNAs that direct similar levels of Cas9 nuclease activity. Furthermore, lentiviral delivery of a single vector expressing Cas9 and four sgRNAs targeting independent loci resulted in simultaneous multiplex gene editing of all four loci. Tunable transcriptional activation at two endogenous genes in both transient and stable contexts was achieved using lentiviral delivery of Cas9 with or without sgRNAs. Highly efficient and long-term gene activation in primary human cells is accomplished. This system is therefore an attractive and efficient method to generate multiplex gene editing and long-term transcriptional activation in human cells.

[0205] The multiplex CRISPR/Cas9-Based System allows efficient multiplex gene editing for simultaneously inactivating multiple genes. The CRISPR/Cas9 system can simultaneously target multiple distinct genomic loci by co-expressing a single Cas9 protein with two or more sgRNAs, making this system uniquely suited for multiplex gene editing or synergistic activation applications. The CRISPR/Cas9 system greatly expedites the process of molecular targeting to new sites by simply modifying the expressed sgRNA molecule. The single lentiviral vector may be combined with methods for achieving inducible control of these components, either by chemical or optogenetic regulation, to facilitate investigation of the dynamics of gene regulation in both time and space.

[0206] The multiplex CRISPR/Cas9-based systems may transcriptionally activate two or more endogenous genes. The multiplex CRISPR/Cas9-based systems may transcriptionally repress two or more endogenous genes. For example, at least two endogenous genes, at least three endogenous genes, at least four endogenous genes, at least five endogenous genes, or at least ten endogenous genes may be activated or repressed by the multiplex CRISPR/Cas9-based system. Between two and fifteen genes, between two and ten genes, between two and five genes, between five and fifteen genes, or between five and ten genes may be activated or repressed by the multiplex CRISPR/Cas9-based system.

[0207] (1) Modified Lentiviral Vector

[0208] The multiplex CRISPR/Cas9-based system includes a modified lentiviral vector. The modified lentiviral vector includes a first polynucleotide sequence encoding a fusion protein and a second polynucleotide sequence encoding at least one sgRNA. The fusion protein may be the fusion protein of the CRISPR/Cas9-based system, as described above. The first polynucleotide sequence may be operably linked to a promoter. The promoter may be a constitutive promoter, an inducible promoter, a repressible promoter, or a regulatable promoter.

[0209] The second polynucleotide sequence encodes at least 1 sgRNA. For example, the second polynucleotide sequence may encode at least 1 sgRNA, at least 2 sgRNAs, at least 3 sgRNAs, at least 4 sgRNAs, at least 5 sgRNAs, at least 6 sgRNAs, at least 7 sgRNAs, at least 8 sgRNAs, at least 9 sgRNAs, at least 10 sgRNAs, at least 11 sgRNA, at least 12 sgRNAs, at least 13 sgRNAs, at least 14 sgRNAs, at least 15 sgRNAs, at least 16 sgRNAs, at least 17 sgRNAs, at least 18 sgRNAs, at least 19 sgRNAs, at least 20 sgRNAs, at least 25 sgRNA, at least 30 sgRNAs, at least 35 sgRNAs, at least 40 sgRNAs, at least 45 sgRNAs, or at least 50 sgRNAs. The second polynucleotide sequence may encode between 1 sgRNA and 50 sgRNAs, between 1 sgRNA and

45 sgRNAs, between 1 sgRNA and 40 sgRNAs, between 1 sgRNA and 35 sgRNAs, between 1 sgRNA and 30 sgRNAs, between 1 sgRNA and 25 different sgRNAs, between 1 sgRNA and 20 sgRNAs, between 1 sgRNA and 16 sgRNAs, between 1 sgRNA and 8 different sgRNAs, between 4 different sgRNAs and 50 different sgRNAs, between 4 different sgRNAs and 45 different sgRNAs, between 4 different sgRNAs and 40 different sgRNAs, between 4 different sgRNAs and 35 different sgRNAs, between 4 different sgRNAs and 30 different sgRNAs, between 4 different sgRNAs and 25 different sgRNAs, between 4 different sgRNAs and 20 different sgRNAs, between 4 different sgRNAs and 16 different sgRNAs, between 4 different sgRNAs and 8 different sgRNAs, between 8 different sgRNAs and 45 different sgRNAs, between 8 different sgRNAs and 40 different sgRNAs, between 8 different sgRNAs and 35 different sgRNAs, between 8 different sgRNAs and 30 different sgRNAs, between 8 different sgRNAs and 25 different sgRNAs, between 8 different sgRNAs and 20 different sgRNAs, between 8 different sgRNAs and 16 different sgRNAs, between 8 different sgRNAs and 50 different sgRNAs, between 16 different sgRNAs and 45 different sgRNAs, between 16 different sgRNAs and 40 different sgRNAs, between 16 different sgRNAs and 35 different sgRNAs, between 16 different sgRNAs and 30 different sgRNAs, between 16 different sgRNAs and 25 different sgRNAs, or between 16 different sgRNAs and 20 different sgRNAs. Each of the polynucleotide sequences encoding the different sgRNAs may be operably linked to a promoter. The promoters that are operably linked to the different sgRNAs may be the same promoter. The promoters that are operably linked to the different sgRNAs may be different promoters. The promoter may be a constitutive promoter, an inducible promoter, a repressible promoter, or a regulatable promoter.

[0210] The at least one sgRNA may bind to a target gene or loci. If more than one sgRNA is included, each of the sgRNAs binds to a different target region within one target loci or each of the sgRNA binds to a different target region within different gene loci. The fusion protein may include Cas9 protein or iCas9-VP64 protein. The fusion protein may include a VP64 domain, a p300 domain, or a KRAB domain.

6. SITE-SPECIFIC NUCLEASES

[0211] The composition, as described above, includes a nucleotide sequence encoding a site-specific nuclease that binds and cleaves a target region. The site-specific nuclease may be engineered. For example, an engineered site-specific nuclease may be a CRISPR/Cas9-based system, a ZFN, or a TALEN. The site-specific nuclease may bind and cleave a gene or locus in the genome of a cell in the skeletal muscle or cardiac muscle. For example, the gene or locus may be the Rosa26 locus or the dystrophin gene.

[0212] a. CRISPR/Cas9-based system

[0213] The CRISPR/Cas9-based system, as described above, may be used to introduce site-specific double strand breaks at targeted genomic loci.

[0214] b. Zinc Finger Nucleases (ZFN)

[0215] The site-specific nuclease may be a ZFN. A single zinc finger contains approximately 30 amino acids and the domain functions by binding 3 consecutive base pairs of DNA via interactions of a single amino acid side chain per base pair. The modular structure of the zinc finger motif

permits the conjunction of several domains in series, allowing for the recognition and targeting of extended sequences in multiples of 3 nucleotides. These targeted DNA-binding domains can be combined with a nuclease domain, such as FokI, to generate a site-specific nuclease, called a “zinc finger nuclease” (ZFNs) that can be used to introduce site-specific double strand breaks at targeted genomic loci. This DNA cleavage stimulates the natural DNA-repair machinery, leading to one of two possible repair pathways, NTEJ and HFIDR. For example, the ZFN may target the Rosa26 locus (Perez-Pinera et al. *Nucleic Acids Research* (2012) 40:3741-3752) or a dystrophin gene. Examples of ZFNs are shown in Table 1 and FIGS. 35-38. In Table 1, the DNA recognition helices are underlined and “Fok ELD-S” and “Fok KKR-S” refers to the FokI nuclease domain that is fused to the zinc finger protein DNA-binding domains. In FIGS. 35-38, the target DNA sequence in the target sites (i.e., in SEQ ID NOs: 442, 445, 448, and 453) and the DNA recognition helices in the ZFN amino acid sequences (i.e., in SEQ ID NOs: 443, 444, 446, 447, 449-452, 454, and 455) are underlined, respectively.

TABLE 1

Full amino acid sequences of identified ZFNs.
ZFN B left Fok ELD-S full amino acid sequence (SEQ ID NO: 438)
MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKVGRLPEGKPYKCECGK
SFSRKDALRGHQRTHTGKPYKCECGKSFSHRTTLTNHQRTHTGKPY
KCPECGKSFSQNALAGHQRTHTGKPYKCECGKSFSHKNALQNHQRT
HTGKPYKCECGKSFSDPGHLVRHQRTHTGKPYKCECGKSFSSTSGN
LVRHQRTHTGAAARALVKSELEKKSELRHKLKYPVPHYIELIETARNP
TQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYTVGSPIDYGVIVD
TKAYSGGYNLPIGQADEMERYVEENQTRDKHLNPNEWKVPYSSVTEFK
FLFVSGHFGKGYKAQLTRLNHNITCNGAVLSVEELLIGGEMIKAGTTLT
EEVRRKFNNGEINF*
ZFN B right Fok KKR-S full amino acid sequence (SEQ ID NO: 439)
MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKVGRLPEGKPYKCECGK
SFSQQRSLVGHQRTHTGKPYKCECGKSFSDDKDLTRHQRTHTGKPY
KCPECGKSFSSTSGHLVRHQRTHTGKPYKCECGKSFSQRAHLERHQRT
HTGKPYKCECGKSFSSTSGSLVRHQRTHTGKPYKCECGKSFSSTSGN
LVRHQRTHTGAAARALVKSELEKKSELRHKLKYPVPHYIELIETARNP
TQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYTVGSPIDYGVIVD
TKAYSGGYNLPIGQADEMQRYVKNQTRNKHINPNEWKVPYSSVTEFK
FLFVSGHFGKGYKAQLTRLNRKTNCGAVLSVEELLIGGEMIKAGTTLT
EEVRRKFNNGEINF*
ZFN J left Fok KKR-S full amino acid sequence (SEQ ID NO: 440)
MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKVGRLPEGKPYKCECGK
NFSSKQALAVHTRHTGKPYKCECGKQCRICMRNFSQSTTLKRHLRHTGKPY
QCRICMRNFSRSDHLSLHLKTHLRGSQLVKSELEKKSELRHKLKYPVPH

TABLE 1-continued

Full amino acid sequences of identified ZFNs.
EYIELIEIARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYT
VGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVKENQTRNKHINPNEW
WKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNRKTNCNGAVLSVEELLI
GGEMIKAGTLTLEEVRRKFNNGEINF*
ZFN J right FokI ELD-S full amino acid sequence (SEQ ID NO: 441)
MDYKDHGDYDHDIDYKDDDDKMAPKKRKVGRLEPGERPEQCRCIMR
NESRAHLQNHTRTHTGKEPQCRICMRNESQSTTLKRHLRTHTGKPE
QCRCIMRNESDGGHLTRHLKTHLRGSQLVKSELEKKSELRHKLKYVPH
EYIELIETARNPTQDRILEMKVMEFFMKVYGYRGEHLGGSRKPDGAIYT
VGSPIDYGVIVDTKAYSGGYNLPIGQADEMERYVEENQTRDKHLNPNEW
WKVYPSSVTEFKFLFVSGHEKGNYKAQLTRLNHTNCNGAVLSVEELLI
GGEMIKAGTLTLEEVRRKFNNGEINF*

[0216] c. TAL Effector Nucleases (TALENs)

[0217] TALENs may be used to introduce site-specific double strand breaks at targeted genomic loci. Site-specific double-strand breaks are created when two independent TALENs bind to nearby DNA sequences, thereby permitting dimerization of FokI and cleavage of the target DNA. TALENs have advanced genome editing due to their high rate of successful and efficient genetic modification. This DNA cleavage may stimulate the natural DNA-repair machinery, leading to one of two possible repair pathways: homology-directed repair (HDR) or the non-homologous end joining (NHEJ) pathway. The TALENs may be designed to target any gene involved in a genetic disease.

[0218] The TALENs may include a nuclease and a TALE DNA-binding domain that binds to the target gene in a TALEN target region. The target gene may have a mutation such as a frameshift mutation or a nonsense mutation. If the target gene has a mutation that causes a premature stop codon, the TALEN may be designed to recognize and bind a nucleotide sequence upstream or downstream from the premature stop codon. A "TALEN target region" includes the binding regions for two TALENs and the spacer region, which occurs between the binding regions. The two TALENs bind to different binding regions within the TALEN target region, after which the TALEN target region is cleaved. Examples of TALENs are described in International Patent Application No. PCT/US2013/038536, which is incorporated by reference in its entirety.

7. TRANSCRIPTIONAL ACTIVATORS

[0219] The composition, as described above, includes a nucleotide sequence encoding a transcriptional activator that activates a target gene. The transcriptional activator may be engineered. For example, an engineered transcriptional activator may be a CRISPR/Cas9-based system, a zinc finger fusion protein, or a TALE fusion protein.

[0220] a. CRISPR/Cas9-Based System

[0221] The CRISPR/Cas9-based system, as described above, may be used to activate transcription of a target gene with RNA. The CRISPR/Cas9-based system may include a

fusion protein, as described above, wherein the second polypeptide domain has transcription activation activity or histone modification activity. For example, the second polypeptide domain may include VP64 or p300.

[0222] b. Zinc Finger Fusion Proteins

[0223] The transcriptional activator may be a zinc finger fusion protein. The zinc finger targeted DNA-binding domains, as described above, can be combined with a domain that has transcription activation activity or histone modification activity. For example, the domain may include VP64 or p300.

[0224] c. TALE Fusion Proteins

[0225] TALE fusion proteins may be used to activate transcription of a target gene. The TALE fusion protein may include a TALE DNA-binding domain and a domain that has transcription activation activity or histone modification activity. For example, the domain may include VP64 or p300.

8. COMPOSITIONS

[0226] The present invention is directed to a composition for altering gene expression and engineering or altering genomic DNA in a cell or subject. The composition may also include a viral delivery system.

[0227] a. Compositions for Genome Editing in Muscle

[0228] The present invention is directed to a composition for genome editing a target gene in skeletal muscle or cardiac muscle of a subject. The composition includes a modified AAV vector and a nucleotide sequence encoding a site-specific nuclease. The composition delivers active forms of site-specific nucleases to skeletal muscle or cardiac muscle. The composition may further comprise a donor DNA or a transgene. These compositions may be used in genome editing, genome engineering, and correcting or reducing the effects of mutations in genes involved in genetic diseases and/or other skeletal or cardiac muscle conditions.

[0229] The target gene may be involved in differentiation of a cell or any other process in which activation, repression, or disruption of a gene may be desired, or may have a mutation such as a deletion, frameshift mutation, or a nonsense mutation. If the target gene has a mutation that causes a premature stop codon, an aberrant splice acceptor site or an aberrant splice donor site, the site-specific nucleases may be designed to recognize and bind a nucleotide sequence upstream or downstream from the premature stop codon, the aberrant splice acceptor site or the aberrant splice donor site. The site-specific nucleases may also be used to disrupt normal gene splicing by targeting splice acceptors and donors to induce skipping of premature stop codons or restore a disrupted reading frame. The site-specific nucleases may or may not mediate off-target changes to protein-coding regions of the genome.

[0230] b. Adeno-Associated Virus Vectors

[0231] The composition, as described above, includes a modified adeno-associated virus (AAV) vector. The modified AAV vector may have enhanced cardiac and skeletal muscle tissue tropism. The modified AAV vector may be capable of delivering and expressing the site-specific nuclease in the cell of a mammal. For example, the modified AAV vector may be an AAV-SASTG vector (Piacentini et al. (2012) Human Gene Therapy 23:635-646). The modified AAV vector may deliver nucleases to skeletal and cardiac muscle in vivo. The modified AAV vector may be based on

one or more of several capsid types, including AAV1, AAV2, AAV5, AAV6, AAV8, and AAV9. The modified AAV vector may be based on AAV2 pseudotype with alternative muscle-tropic AAV capsids, such as AAV2/1, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV2.5 and AAV/SASTG vectors that efficiently transduce skeletal muscle or cardiac muscle by systemic and local delivery (Seto et al. *Current Gene Therapy* (2012) 12:139-151).

[0232] c. CRISPR/Cas9-Based System

[0233] The present disclosure also provides DNA targeting systems or compositions of at least one CRISPR/Cas9-based system, as described above. These compositions may be used in genome editing, genome engineering, and correcting or reducing the effects of mutations in genes involved in genetic diseases. The composition includes a CRISPR/Cas9-based system that includes a Cas9 protein or Cas9 fusion protein, as described above. The CRISPR/Cas9-based system may also include at least one gRNA, as described above.

[0234] d. Multiplex CRISPR/Cas9-Based System

[0235] The present disclosure also provides multiplex CRISPR/Cas9-based systems, as described above. These compositions may be used in genome editing, genome engineering, and correcting or reducing the effects of mutations in genes involved in genetic diseases. These compositions may be used to target more than one gene. The composition includes a modified lentiviral vector comprising a CRISPR/Cas9-based system that includes a Cas9 protein or Cas9 fusion protein, as described above and more than one gRNA, as described above.

9. METHODS OF USES

[0236] Potential applications of the compositions are diverse across many areas of science and biotechnology. The disclosed compositions may be used to repair genetic mutations that cause disease. The disclosed compositions may be used to disrupt genes such that gene disruption leads to increases in muscle regeneration or muscle strength, or decreases in muscle aging. The disclosed compositions may be used to introduce therapeutic genes to be expressed systemically from skeletal muscle or cardiac muscle, such as clotting factors or monoclonal antibodies. The disclosed compositions may be used to modulate mammalian gene expression. The disclosed compositions may be used to transdifferentiate or induce the differentiation of a cell or correct a mutant gene in a cell. Examples of activation of genes related to cell and gene therapy, genetic reprogramming, and regenerative medicine are provided. RNA-guided transcriptional activators may be used to reprogram cell lineage specification. Activation of endogenous genes encoding the key regulators of cell fate, rather than forced overexpression of these factors, may potentially lead to more rapid, efficient, stable, or specific methods for genetic reprogramming, transdifferentiation, and/or induced differentiation.

10. METHODS OF GENOME EDITING IN MUSCLE

[0237] The present disclosure is directed to a method of genome editing in a skeletal muscle or cardiac muscle of a subject. The method comprises administering to the skeletal muscle or cardiac muscle of the subject the composition for genome editing in skeletal muscle or cardiac muscle, as

described above. The genome editing may include correcting a mutant gene or inserting a transgene. Correcting the mutant gene may include deleting, rearranging, or replacing the mutant gene. Correcting the mutant gene may include nuclease-mediated NHEJ or HDR.

11. METHODS OF USING CRISPR/CAS9-BASED SYSTEM

[0238] Potential applications of the CRISPR/Cas9-based system are diverse across many areas of science and biotechnology. The disclosed CRISPR/Cas9-based systems may be used to modulate mammalian gene expression. The disclosed CRISPR/Cas9-based systems may be used to transdifferentiate or induce differentiation of a cell or correct a mutant gene in a cell. Examples of activation of genes related to cell and gene therapy, genetic reprogramming, and regenerative medicine are provided. RNA-guided transcriptional activators may be used to reprogram cell lineage specification. Although reprogramming was incomplete and inefficient in these experiments, there are many ways by which this method could be improved, including repeated transfections of iCas9-VP64/gRNA combinations, stable expression of these factors, and targeting multiple genes, such as Brn2 and Myt1l in addition to Ascl1 for transdifferentiation into a neuronal phenotype. Activation of endogenous genes encoding the key regulators of cell fate, rather than forced overexpression of these factors, may potentially lead to more rapid, efficient, stable, or specific methods for genetic reprogramming and transdifferentiation or induced differentiation of a cell. Finally, Cas9 fusions to other domains, including repressive and epigenetic-modifying domains, could provide a greater diversity of RNA-guided transcriptional regulators to complement other RNA-based tools for mammalian cell engineering.

[0239] a. Methods of Activating Gene Expression

[0240] The present disclosure provides a mechanism for activating the expression of endogenous genes, such as mammalian genes, based on targeting a transcriptional activator to promoters via RNA using a CRISPR/Cas9 based system, as described above. This is fundamentally different from previously described methods based on engineering sequence-specific DNA-binding proteins and may provide opportunities for targeted gene regulation. Because the generation of gRNA expression plasmids simply involves synthesizing two short custom oligonucleotides and one cloning step, it is possible to generate many new gene activators quickly and economically. The gRNAs can also be transfected directly to cells following in vitro transcription. Multiple gRNAs targeted to single promoters were shown, but simultaneous targeting of multiple promoters could also be possible. Recognition of genomic target sites with RNAs, rather than proteins, may also circumvent limitations of targeting epigenetically modified sites, such as methylated DNA.

[0241] In contrast to current methods based on engineering DNA-binding proteins, Cas9 fused to a transcriptional activation domain can be targeted by combinations of guide RNA molecules to induce the expression of endogenous human genes. This straightforward and versatile approach for targeted gene activation circumvents the need for engineering new proteins and allows for widespread synthetic gene regulation.

[0242] The method may include administering to a cell or subject a CRISPR/Cas9-based system, a polynucleotide or

vector encoding said CRISPR/Cas9-based system, or DNA targeting systems or compositions of at least one CRISPR/Cas9-based system, as described above. The method may include administering a CRISPR/Cas9-based system, such as administering a Cas9 fusion protein containing transcription activation domain or a nucleotide sequence encoding said Cas9 fusion protein. The Cas9 fusion protein may include a transcription activation domain such as aVP16 protein or a transcription co-activator such as a p300 protein.

[0243] (1) dCas9-VP16

[0244] The Cas9 fusion protein may include a transcription activation domain such as aVP16 protein. The transcription activation domain may contain at least 1 VP16 protein, at least 2 VP16 proteins, at least 3 VP16 proteins, at least 4 VP16 proteins (i.e., a VP64 activator domain), at least 5 VP16 proteins, at least 6 VP16 proteins, at least 6 VP16 proteins, or at least 10 VP16 proteins. The Cas9 protein may be a Cas9 protein in which the nuclease activity is inactivated. For example, the Cas9 protein in the fusion protein may be iCas9 (amino acids 36-1403 of SEQ ID NO: 1), which includes the amino acid substitutions of D10A and H840A. The Cas9 fusion protein may be iCas9-VP64.

[0245] (2) dCas9-p300

[0246] The Cas9 fusion protein may include a transcription co-activation domain such as a p300 protein. The p300 protein (also known as EP300 or E1A binding protein p300) is encoded by the EP300 gene and regulates the activity of many genes in tissues throughout the body. The p300 protein plays a role in regulating cell growth and division, prompting cells to mature and assume specialized functions (differentiate) and preventing the growth of cancerous tumors. The p300 protein activates transcription by connecting transcription factors with a complex of proteins that carry out transcription in the cell's nucleus. The p300 interaction with transcription factors is managed by one or more of p300 domains: the nuclear receptor interaction domain (RID), the CREB and MYB interaction domain (KIX), the cysteine/histidine regions (TAZ1/CH and TAZ2/CH3) and the interferon response binding domain (IBiD). The last four domains, KIX, TAZ1, TAZ2 and IBiD of p300, each bind tightly to a sequence spanning both transactivation domains 9aaTADs of transcription factor p53. The protein functions as histone acetyltransferase that regulates transcription via chromatin remodeling, and is important in the processes of cell proliferation and differentiation. It mediates cAMP-gene regulation by binding specifically to phosphorylated CREB protein.

[0247] The p300 protein may activate Mothers against decapentaplegic homolog 7, MAF, TSG101, Peroxisome proliferator-activated receptor alpha, NPAS2, PAX6, DDX5, MYBL2, Mothers against decapentaplegic homolog 1, Mothers against decapentaplegic homolog 2, Lymphoid enhancer-binding factor 1, SNIP1, TRERF1, STAT3, EID1, RAR-related orphan receptor alpha, ELK1, HIF1A, ING5, Peroxisome proliferator-activated receptor gamma, SS18, TCF3, Zif268, Estrogen receptor alpha, GPS2, MyoD, YY1, ING4, PROX1, CITED1, HNF1A, MEF2C, MEF2D, MAML1, Twist transcription factor, PTMA, IRF2, DTX1, Flap structure-specific endonuclease 1, Myocyte-specific enhancer factor 2A, CDX2, BRCA1, HNRPU, STAT6, CITED2, RELA, TGS1, CEBPB, Mdm2, NCOA6, NFATC2, Thyroid hormone receptor alpha, BCL3, TFAP2A, PCNA, P53 and TAL1.

[0248] The transcription co-activation domain may include a human p300 protein or a fragment thereof. The transcription co-activation domain may include a wild-type human p300 protein or a mutant human p300 protein, or fragments thereof. The transcription co-activation domain may include the core lysine-acetyltransferase domain of the human p300 protein, i.e., the p300 HAT Core (also known as "p300 WT Core"; see FIG. 58). The Cas9 protein may be a Cas9 protein in which the nuclease activity is inactivated. For example, the Cas9 protein in the fusion protein may be iCas9 (amino acids 36-1403 of SEQ ID NO: 1), which includes the amino acid substitutions of D10A and H840A. The Cas9 fusion protein may be iCas9-p300 WT Core.

[0249] (3) gRNA

[0250] The method may also include administering to a cell or subject a CRISPR/Cas9-based system at least one gRNA, wherein the gRNAs target different DNA sequences. The target DNA sequences may be overlapping. The number of gRNA administered to the cell may be at least 1 gRNA, at least 2 different gRNAs, at least 3 different gRNAs at least 4 different gRNAs, at least 5 different gRNAs, at least 6 different gRNAs, at least 7 different gRNAs, at least 8 different gRNAs, at least 9 different gRNAs, at least 10 different gRNAs, at least 11 different gRNAs, at least 12 different gRNAs, at least 13 different gRNAs, at least 14 different gRNAs, at least 15 different gRNAs, at least 16 different gRNAs, at least 17 different gRNAs, at least 18 different gRNAs, at least 18 different gRNAs, at least 20 different gRNAs, at least 25 different gRNAs, at least 30 different gRNAs, at least 35 different gRNAs, at least 40 different gRNAs, at least 45 different gRNAs, or at least 50 different gRNAs. The number of gRNA administered to the cell may be between at least 1 gRNA to at least 50 different gRNAs, at least 1 gRNA to at least 45 different gRNAs, at least 1 gRNA to at least 40 different gRNAs, at least 1 gRNA to at least 35 different gRNAs, at least 1 gRNA to at least 30 different gRNAs, at least 1 gRNA to at least 25 different gRNAs, at least 1 gRNA to at least 20 different gRNAs, at least 1 gRNA to at least 16 different gRNAs, at least 1 gRNA to at least 12 different gRNAs, at least 1 gRNA to at least 8 different gRNAs, at least 1 gRNA to at least 4 different gRNAs, at least 4 gRNAs to at least 50 different gRNAs, at least 4 different gRNAs to at least 45 different gRNAs, at least 4 different gRNAs to at least 40 different gRNAs, at least 4 different gRNAs to at least 35 different gRNAs, at least 4 different gRNAs to at least 30 different gRNAs, at least 4 different gRNAs to at least 25 different gRNAs, at least 4 different gRNAs to at least 20 different gRNAs, at least 4 different gRNAs to at least 16 different gRNAs, at least 4 different gRNAs to at least 12 different gRNAs, at least 4 different gRNAs to at least 8 different gRNAs, at least 8 different gRNAs to at least 50 different gRNAs, at least 8 different gRNAs to at least 45 different gRNAs, at least 8 different gRNAs to at least 40 different gRNAs, at least 8 different gRNAs to at least 35 different gRNAs, 8 different gRNAs to at least 30 different gRNAs, at least 8 different gRNAs to at least 25 different gRNAs, 8 different gRNAs to at least 20 different gRNAs, at least 8 different gRNAs to at least 16 different gRNAs, at least 8 different gRNAs to at least 12 different gRNAs, at least 8 different gRNAs to at least 8 different gRNAs,

[0251] The gRNA may comprise a complementary polynucleotide sequence of the target DNA sequence followed by NGG. The gRNA may comprise a "G" at the 5' end of the

complementary polynucleotide sequence. The gRNA may comprise at least a 10 base pair, at least a 11 base pair, at least a 12 base pair, at least a 13 base pair, at least a 14 base pair, at least a 15 base pair, at least a 16 base pair, at least a 17 base pair, at least a 18 base pair, at least a 19 base pair, at least a 20 base pair, at least a 21 base pair, at least a 22 base pair, at least a 23 base pair, at least a 24 base pair, at least a 25 base pair, at least a 30 base pair, or at least a 35 base pair complementary polynucleotide sequence of the target DNA sequence followed by NGG. The gRNA may target at least one of the promoter region, the enhancer region or the transcribed region of the target gene. The gRNA may include a nucleic acid sequence of at least one of SEQ ID NOs: 5-40, 65-144, 492-515, 540-563, and 585-625.

[0252] b. Methods of Repressing Gene Expression

[0253] The present disclosure provides a mechanism for repressing the expression of endogenous genes, such as mammalian genes, based on targeting genomic regulatory elements, such as distal enhancers, via RNA using a CRISPR/Cas9 based system, as described above. The Cas9 fusion protein may include a transcriptional repressor, such as the KRAB repressor. The Cas9 fusion protein may be dCas9-KRAB. The dCas9-KRAB may additionally affect epigenetic gene regulation by recruiting heterochromatin-forming factors to the targeted locus. The CRISPR/dCas9-KRAB system may be used to repress the transcription of genes, but can also be used to target genomic regulatory elements which were previously inaccessible by traditional repression methods such as RNA interference (FIG. 53B). Delivering dCas9-KRAB with gRNAs targeted to a distal enhancer may disrupt expression of multiple genes regulated by the targeted enhancer (see FIG. 53C). The targeted enhancer may be any enhancer for a gene such as the HS2 enhancer.

[0254] a. Methods of Transdifferentiation or Induced Differentiation

[0255] The present disclosure provides a mechanism for transdifferentiating or inducing differentiation of cells by activating endogenous genes via RNA using a CRISPR/Cas9-based system, as described above.

[0256] (1) Transdifferentiation

[0257] The CRISPR/Cas9-based system may be used to transdifferentiate cells. Transdifferentiation, also known as lineage reprogramming or direct conversion, is a process where cells convert from one differentiated cell type to another without undergoing an intermediate pluripotent state or progenitor cell type. It is a type of metaplasia, which includes all cell fate switches, including the interconversion of stem cells. Transdifferentiation of cells has potential uses for disease modeling, drug discovery, gene therapy and regenerative medicine. Activation of endogenous genes, such as BRN2, MYT1L, ASCL1, NANOG, and/or MYOD1, using the CRISPR/Cas9 based system described above may lead to transdifferentiation of several cell types, such as fibroblasts, cardiomyocytes, hepatocytes, chondrocytes, mesenchymal progenitor cells, hematopoietic stem cells, or smooth muscle cells, into neuronal and myogenic phenotypes, respectively.

[0258] (2) Inducing Differentiation

[0259] The CRISPR/Cas9-based system may be used to induce differentiation of cells, such as stem cells, cardiomyocytes, hepatocytes, chondrocytes, mesenchymal progenitor cells, hematopoietic stem cells, or smooth muscle

cells. For example, stem cells, such as embryonic stem cells or pluripotent stem cells, may be induced to differentiate into muscle cells or vascular endothelial cell, i.e., induce neuronal or myogenic differentiation.

12. USES OF MULTIPLEX CRISPR/CAS9-BASED SYSTEM

[0260] The multiplex CRISPR/Cas9-Based System takes advantage of the simplicity and low cost of sgRNA design and may be helpful in exploiting advances in high-throughput genomic research using CRISPR/Cas9 technology. For example, the single lentiviral vectors described here are useful in expressing Cas9 and numerous sgRNAs in difficult cell lines, such as primary fibroblasts described here (FIG. 47). The multiplex CRISPR/Cas9-Based System may be used in the same ways as the CRISPR/Cas9-Based System described above.

[0261] In addition to the described transcriptional activation and nuclease functionality, this system will be useful for expressing other novel Cas9-based effectors that control epigenetic modifications for diverse purposes, including interrogation of genome architecture and pathways of endogenous gene regulation. As endogenous gene regulation is a delicate balance between multiple enzymes, multiplexing Cas9 systems with different functionalities will allow for examining the complex interplay among different regulatory signals. The vector described here should be compatible with aptamer-modified sgRNAs and orthogonal Cas9s to enable independent genetic manipulations using a single set of sgRNAs.

[0262] The multiplex CRISPR/Cas9-Based System may be used to activate at least one endogenous gene in a cell. The method includes contacting a cell with the modified lentiviral vector. The endogenous gene may be transiently activated or stably activated. The endogenous gene may be transiently repressed or stably repressed. The fusion protein may be expressed at similar levels to the sgRNAs. The fusion protein may be expressed at different levels compared to the sgRNAs. The cell may be a primary human cell.

[0263] The multiplex CRISPR/Cas9-Based System may be used in a method of multiplex gene editing in a cell. The method includes contacting a cell with the modified lentiviral vector. The multiplex gene editing may include correcting a mutant gene or inserting a transgene. Correcting a mutant gene may include deleting, rearranging or replacing the mutant gene. Correcting the mutant gene may include nuclease-mediated non-homologous end joining or homology-directed repair. The multiplex gene editing may include deleting or correcting at least one gene, wherein the gene is an endogenous normal gene or a mutant gene. The multiplex gene editing may include deleting or correcting at least two genes. For example, at least two genes, at least three genes, at least four genes, at least five genes, at least six genes, at least seven genes, at least eight genes, at least nine genes, or at least ten genes may be deleted or corrected.

[0264] The multiplex CRISPR/Cas9-Based System may be used in a method of multiplex modulation of gene expression in a cell. The method includes contacting a cell with the modified lentiviral vector. The method may include modulating the gene expression levels of at least one gene. The gene expression of the at least one target gene is modulated when gene expression levels of the at least one target gene are increased or decreased compared to normal

gene expression levels for the at least one target gene. The gene expression levels may be RNA or protein levels.

13. METHODS OF CORRECTING A MUTANT GENE AND TREATING A SUBJECT

[0265] The present disclosure is also directed to a method of correcting a mutant gene in a subject. The method comprises administering to the skeletal muscle or cardiac muscle of the subject the composition for genome editing in skeletal muscle or cardiac muscle, as described above. Use of the composition to deliver the site-specific nuclease to the skeletal muscle or cardiac muscle may restore the expression of a full-functional or partially-functional protein with a repair template or donor DNA, which can replace the entire gene or the region containing the mutation. The site-specific nuclease may be used to introduce site-specific double strand breaks at targeted genomic loci. Site-specific double-strand breaks are created when the site-specific nuclease binds to a target DNA sequences, thereby permitting cleavage of the target DNA. This DNA cleavage may stimulate the natural DNA-repair machinery, leading to one of two possible repair pathways: homology-directed repair (HDR) or the non-homologous end joining (NHEJ) pathway.

[0266] The present disclosure is directed to genome editing with a site-specific nuclease without a repair template, which can efficiently correct the reading frame and restore the expression of a functional protein involved in a genetic disease. The disclosed site-specific nucleases may involve using homology-directed repair or nuclease-mediated non-homologous end joining (NHEJ)-based correction approaches, which enable efficient correction in proliferation-limited primary cell lines that may not be amenable to homologous recombination or selection-based gene correction. This strategy integrates the rapid and robust assembly of active site-specific nucleases with an efficient gene editing method for the treatment of genetic diseases caused by mutations in nonessential coding regions that cause frameshifts, premature stop codons, aberrant splice donor sites or aberrant splice acceptor sites.

[0267] a. Nuclease Mediated Non-Homologous End Joining

[0268] Restoration of protein expression from an endogenous mutated gene may be through template-free NHEJ-mediated DNA repair. In contrast to a transient method targeting the target gene RNA, the correction of the target gene reading frame in the genome by a transiently expressed site-specific nuclease may lead to permanently restored target gene expression by each modified cell and all of its progeny.

[0269] Nuclease mediated NHEJ gene correction may correct the mutated target gene and offers several potential advantages over the HDR pathway. For example, NHEJ does not require a donor template, which may cause non-specific insertional mutagenesis. In contrast to HDR, NHEJ operates efficiently in all stages of the cell cycle and therefore may be effectively exploited in both cycling and post-mitotic cells, such as muscle fibers. This provides a robust, permanent gene restoration alternative to oligonucleotide-based exon skipping or pharmacologic forced read-through of stop codons and could theoretically require as few as one drug treatment. NHEJ-based gene correction using a CRISPR/Cas9-based system, as well as other engineered nucleases including meganucleases and zinc finger nucleases, may be combined with other existing ex vivo and in

vivo platforms for cell- and gene-based therapies, in addition to the plasmid electroporation approach described here. For example, delivery of a CRISPR/Cas9-based system by mRNA-based gene transfer or as purified cell permeable proteins could enable a DNA-free genome editing approach that would circumvent any possibility of insertional mutagenesis.

[0270] b. Homology-Directed Repair

[0271] Restoration of protein expression from an endogenous mutated gene may involve homology-directed repair. The method as described above further includes administering a donor template to the cell. The donor template may include a nucleotide sequence encoding a full-functional protein or a partially-functional protein. For example, the donor template may include a miniaturized dystrophin construct, termed minidystrophin (“minidys”), a full-functional dystrophin construct for restoring a mutant dystrophin gene, or a fragment of the dystrophin gene that after homology-directed repair leads to restoration of the mutant dystrophin gene.

[0272] c. Methods of Correcting a Mutant Gene and Treating a Subject Using CRISPR/Cas9

[0273] The present disclosure is also directed to genome editing with the CRISPR/Cas9-based system to restore the expression of a full-functional or partially-functional protein with a repair template or donor DNA, which can replace the entire gene or the region containing the mutation. The CRISPR/Cas9-based system may be used to introduce site-specific double strand breaks at targeted genomic loci. Site-specific double-strand breaks are created when the CRISPR/Cas9-based system binds to a target DNA sequences using the gRNA, thereby permitting cleavage of the target DNA. The CRISPR/Cas9-based system has the advantage of advanced genome editing due to their high rate of successful and efficient genetic modification. This DNA cleavage may stimulate the natural DNA-repair machinery, leading to one of two possible repair pathways: homology-directed repair (HDR) or the non-homologous end joining (NHEJ) pathway. For example, a CRISPR/Cas9-based system directed towards the dystrophin gene may include a gRNA having a nucleic acid sequence of any one of SEQ ID NOs: 65-115.

[0274] The present disclosure is directed to genome editing with CRISPR/Cas9-based system without a repair template, which can efficiently correct the reading frame and restore the expression of a functional protein involved in a genetic disease. The disclosed CRISPR/Cas9-based system and methods may involve using homology-directed repair or nuclease-mediated non-homologous end joining (NHEJ)-based correction approaches, which enable efficient correction in proliferation-limited primary cell lines that may not be amenable to homologous recombination or selection-based gene correction. This strategy integrates the rapid and robust assembly of active CRISPR/Cas9-based system with an efficient gene editing method for the treatment of genetic diseases caused by mutations in nonessential coding regions that cause frameshifts, premature stop codons, aberrant splice donor sites or aberrant splice acceptor sites.

[0275] The present disclosure provides methods of correcting a mutant gene in a cell and treating a subject suffering from a genetic disease, such as DMD. The method may include administering to a cell or subject a CRISPR/Cas9-based system, a polynucleotide or vector encoding said CRISPR/Cas9-based system, or composition of said

CRISPR/Cas9-based system as described above. The method may include administering a CRISPR/Cas9-based system, such as administering a Cas9 protein or Cas9 fusion protein containing a second domain having nuclease activity, a nucleotide sequence encoding said Cas9 protein or Cas9 fusion protein, and/or at least one gRNA, wherein the gRNAs target different DNA sequences. The target DNA sequences may be overlapping. The number of gRNA administered to the cell may be at least 1 gRNA, at least 2 different gRNA, at least 3 different gRNA at least 4 different gRNA, at least 5 different gRNA, at least 6 different gRNA, at least 7 different gRNA, at least 8 different gRNA, at least 9 different gRNA, at least 10 different gRNA, at least 15 different gRNA, at least 20 different gRNA, at least 30 different gRNA, or at least 50 different gRNA, as described above. The gRNA may include a nucleic acid sequence of at least one of SEQ ID NOs: 65-115. The method may involve homology-directed repair or non-homologous end joining.

14. METHODS OF TREATING A DISEASE

[0276] The present disclosure is directed to a method of treating a subject in need thereof. The method comprises administering to a tissue of a subject the composition for altering gene expression and engineering or altering genomic DNA in a cell or subject genome editing, as described above. The method may comprise administering to the skeletal muscle or cardiac muscle of the subject the composition for genome editing in skeletal muscle or cardiac muscle, as described above. The subject may be suffering from a skeletal muscle or cardiac muscle condition causing degeneration or weakness or a genetic disease. For example, the subject may be suffering from Duchenne muscular dystrophy, as described above.

[0277] a. Duchenne Muscular Dystrophy

[0278] The method, as described above, may be used for correcting the dystrophin gene and recovering full-functional or partially-functional protein expression of said mutated dystrophin gene. In some aspects and embodiments the disclosure provides a method for reducing the effects (e.g., clinical symptoms/indications) of DMD in a patient. In some aspects and embodiments the disclosure provides a method for treating DMD in a patient. In some aspects and embodiments the disclosure provides a method for preventing DMD in a patient. In some aspects and embodiments the disclosure provides a method for preventing further progression of DMD in a patient.

15. CONSTRUCTS AND PLASMIDS

[0279] The compositions, as described above, may comprise genetic constructs that encodes the CRISPR/Cas9-based system, as disclosed herein. The genetic construct, such as a plasmid, may comprise a nucleic acid that encodes the CRISPR/Cas9-based system, such as the Cas9 protein and Cas9 fusion proteins and/or at least one of the gRNAs. The compositions, as described above, may comprise genetic constructs that encodes the modified AAV vector and a nucleic acid sequence that encodes the site-specific nuclease, as disclosed herein. The genetic construct, such as a plasmid, may comprise a nucleic acid that encodes the site-specific nuclease. The compositions, as described above, may comprise genetic constructs that encodes the modified lentiviral vector, as disclosed herein. The genetic construct, such as a plasmid, may comprise a nucleic acid

that encodes the Cas9-fusion protein and at least one sgRNA. The genetic construct may be present in the cell as a functioning extrachromosomal molecule. The genetic construct may be a linear minichromosome including centromere, telomeres or plasmids or cosmid.

[0280] The genetic construct may also be part of a genome of a recombinant viral vector, including recombinant lentivirus, recombinant adenovirus, and recombinant adenovirus associated virus. The genetic construct may be part of the genetic material in attenuated live microorganisms or recombinant microbial vectors which live in cells. The genetic constructs may comprise regulatory elements for gene expression of the coding sequences of the nucleic acid. The regulatory elements may be a promoter, an enhancer, an initiation codon, a stop codon, or a polyadenylation signal.

[0281] The nucleic acid sequences may make up a genetic construct that may be a vector. The vector may be capable of expressing the fusion protein, such as the Cas9-fusion protein or site-specific nuclease, in the cell of a mammal. The vector may be recombinant. The vector may comprise heterologous nucleic acid encoding the fusion protein, such as the Cas9-fusion protein or site-specific nuclease. The vector may be a plasmid. The vector may be useful for transfecting cells with nucleic acid encoding the Cas9-fusion protein or site-specific nuclease, which the transformed host cell is cultured and maintained under conditions wherein expression of the Cas9-fusion protein or the site-specific nuclease system takes place.

[0282] Coding sequences may be optimized for stability and high levels of expression. In some instances, codons are selected to reduce secondary structure formation of the RNA such as that formed due to intramolecular bonding.

[0283] The vector may comprise heterologous nucleic acid encoding the CRISPR/Cas9-based system or the site-specific nuclease and may further comprise an initiation codon, which may be upstream of the CRISPR/Cas9-based system or the site-specific nuclease coding sequence, and a stop codon, which may be downstream of the CRISPR/Cas9-based system or the site-specific nuclease coding sequence. The initiation and termination codon may be in frame with the CRISPR/Cas9-based system or the site-specific nuclease coding sequence. The vector may also comprise a promoter that is operably linked to the CRISPR/Cas9-based system or the site-specific nuclease coding sequence. The promoter operably linked to the CRISPR/Cas9-based system or the site-specific nuclease coding sequence may be a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter may also be a promoter from a human gene such as human ubiquitin C (hUbC), human actin, human myosin, human hemoglobin, human muscle creatine, or human metallothionein. The promoter may also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US Patent Application Publication No. US20040175727, the contents of which are incorporated herein in its entirety.

[0284] The vector may also comprise a polyadenylation signal, which may be downstream of the CRISPR/Cas9-based system or the site-specific nuclease. The polyadenylation signal may be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β -globin polyadenylation signal. The SV40 polyadenylation signal may be a polyadenylation signal from a pCEP4 vector (Invitrogen, San Diego, Calif.).

[0285] The vector may also comprise an enhancer upstream of the CRISPR/Cas9-based system, i.e., the Cas9 protein or Cas9 fusion protein coding sequence or sgRNAs, or the site-specific nuclease. The enhancer may be necessary for DNA expression. The enhancer may be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, HA, RSV or EBV. Polynucleotide function enhancers are described in U.S. Pat. Nos. 5,593,972, 5,962,428, and WO94/016737, the contents of each are fully incorporated by reference. The vector may also comprise a mammalian origin of replication in order to maintain the vector extrachromosomally and produce multiple copies of the vector in a cell. The vector may also comprise a regulatory sequence, which may be well suited for gene expression in a mammalian or human cell into which the vector is administered. The vector may also comprise a reporter gene, such as green fluorescent protein ("GFP") and/or a selectable marker, such as hygromycin ("Hygro").

[0286] The vector may be expression vectors or systems to produce protein by routine techniques and readily available starting materials including Sambrook et al., Molecular Cloning and Laboratory Manual, Second Ed., Cold Spring Harbor (1989), which is incorporated fully by reference. In some embodiments the vector may comprise the nucleic acid sequence encoding the CRISPR/Cas9-based system, including the nucleic acid sequence encoding the Cas9 protein or Cas9 fusion protein and the nucleic acid sequence encoding the at least one gRNA comprising the nucleic acid sequence of at least one of SEQ ID NOs: 5-40, 65-144, 492-515, 540-563, and 585-625.

16. PHARMACEUTICAL COMPOSITIONS

[0287] The composition may be in a pharmaceutical composition. The pharmaceutical composition may comprise about 1 ng to about 10 mg of DNA encoding the CRISPR/Cas9-based system or CRISPR/Cas9-based system protein component, i.e., the Cas9 protein or Cas9 fusion protein. The pharmaceutical composition may comprise about 1 ng to about 10 mg of the DNA of the modified AAV vector and nucleotide sequence encoding the site-specific nuclease. The pharmaceutical composition may comprise about 1 ng to about 10 mg of the DNA of the modified lentiviral vector. The pharmaceutical compositions according to the present invention are formulated according to the mode of administration to be used. In cases where pharmaceutical compositions are injectable pharmaceutical compositions, they are sterile, pyrogen free and particulate free. An isotonic formulation is preferably used. Generally, additives for isotonicity may include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include gelatin and albumin. In some embodiments, a vasoconstriction agent is added to the formulation.

[0288] The composition may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient may be functional molecules as vehicles, adjuvants, carriers, or diluents. The pharmaceutically acceptable excipient may be a transfection facilitating agent, which may include surface active agents, such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

[0289] The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and more preferably, the poly-L-glutamate is present in the composition for genome editing in skeletal muscle or cardiac muscle at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the genetic construct. In some embodiments, the DNA vector encoding the composition may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example WO9324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. Preferably, the transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid.

17. METHODS OF DELIVERY

[0290] Provided herein is a method for delivering the pharmaceutical formulations, preferably compositions described above, for providing genetic constructs. The delivery of the compositions may be the transfection or electroporation of the composition as a nucleic acid molecule that is expressed in the cell and delivered to the surface of the cell. The nucleic acid molecules may be electroporated using BioRad Gene Pulser Xcell or Amaxa Nucleofector IIb devices. Several different buffers may be used, including BioRad electroporation solution, Sigma phosphate-buffered saline product #D8537 (PBS), Invitrogen OptiMM I (OM), or Amaxa Nucleofector solution V (N.V.). Transfections may include a transfection reagent, such as Lipofectamine 2000.

[0291] Upon delivery of the composition to the tissue, and thereupon the vector into the cells of the mammal, the transfected cells will express the fusion protein, such as a CRISPR/Cas9-based system and/or a site-specific nuclease. The composition may be administered to a mammal to alter gene expression or to re-engineer or alter the genome. For example, the composition may be administered to a mammal to correct the dystrophin gene in a mammal. The mammal may be human, non-human primate, cow, pig, sheep, goat, antelope, bison, water buffalo, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, or chicken, and preferably human, cow, pig, or chicken.

[0292] a. CRISPR/Cas9-Based System

[0293] The vector encoding a CRISPR/Cas9-based system protein component, i.e., the Cas9 protein or Cas9 fusion protein, may be delivered to the mammal by DNA injection (also referred to as DNA vaccination) with and without in vivo electroporation, liposome mediated, nanoparticle facilitated, and/or recombinant vectors. The recombinant vector may be delivered by any viral mode. The viral mode may be recombinant lentivirus, recombinant adenovirus, and/or recombinant adeno-associated virus.

[0294] The nucleotide encoding a CRISPR/Cas9-based system protein component, i.e., the Cas9 protein or Cas9 fusion protein, may be introduced into a cell to genetically correct the target gene or alter gene expression of a gene, such as activate or repress endogenous genes. For example, a nucleotide encoding a CRISPR/Cas9-based system protein component, i.e., the Cas9 protein or Cas9 fusion protein, directed towards a mutant dystrophin gene by the gRNA may be introduced into a myoblast cell from a DMD patient. Alternatively, they may be introduced into a fibroblast cell from a DMD patient, and the genetically corrected fibroblast cell may be treated with MyoD to induce differentiation into myoblasts, which may be implanted into subjects, such as the damaged muscles of a subject to verify that the corrected dystrophin protein was functional and/or to treat the subject. The modified cells may also be stem cells, such as induced pluripotent stem cells, bone marrow-derived progenitors, skeletal muscle progenitors, human skeletal myoblasts from DMD patients, CD133+ cells, mesoangioblasts, and MyoD- or Pax7-transduced cells, or other myogenic progenitor cells. For example, the CRISPR/Cas9-based system may cause neuronal or myogenic differentiation of an induced pluripotent stem cell.

18. ROUTES OF ADMINISTRATION

[0295] The compositions may be administered to a subject by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian may readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. The compositions may be administered by traditional syringes, needleless injection devices, “microprojectile bombardment gone guns”, or other physical methods such as electroporation (“EP”), “hydrodynamic method”, or ultrasound.

[0296] The composition may be delivered to the mammal by several technologies including DNA injection (also referred to as DNA vaccination) with and without in vivo electroporation, liposome mediated, nanoparticle facilitated, recombinant vectors such as recombinant lentivirus, recombinant adenovirus, and recombinant adenovirus associated virus. The composition may be injected into the skeletal muscle or cardiac muscle. For example, the composition may be injected into the tibialis anterior muscle.

19. CELL TYPES

[0297] Any of these delivery methods and/or routes of administration could be utilized with a myriad of cell types,

for example, those cell types currently under investigation for cell-based therapies. Cell types may be fibroblasts, pluripotent stem cells, cardiomyocytes, hepatocytes, chondrocytes, mesenchymal progenitor cells, hematopoietic stem cells, smooth muscle cells, or K562 human erythroid leukemia cell line.

[0298] a. DMD

[0299] Cell types currently under investigation for cell-based therapies of DMD include immortalized myoblast cells, such as wild-type and DMD patient derived lines, for example $\Delta 48-50$ DMD, DMD 8036 (del48-50), C25C14 and DMD-7796 cell lines, primal DMD dermal fibroblasts, induced pluripotent stem cells, bone marrow-derived progenitors, skeletal muscle progenitors, human skeletal myoblasts from DMD patients, CD133+ cells, mesoangioblasts, cardiomyocytes, hepatocytes, chondrocytes, mesenchymal progenitor cells, hematopoietic stem cells, smooth muscle cells, and MyoD- or Pax7-transduced cells, or other myogenic progenitor cells. Immortalization of human myogenic cells may be used for clonal derivation of genetically corrected myogenic cells. Cells may be modified ex vivo to isolate and expand clonal populations of immortalized DMD myoblasts that contain a genetically corrected dystrophin gene and are free of other nuclease-introduced mutations in protein coding regions of the genome. Alternatively, transient in vivo delivery of nucleases by non-viral or non-integrating viral gene transfer, or by direct delivery of purified proteins and gRNAs containing cell-penetrating motifs may enable highly specific correction in situ with minimal or no risk of exogenous DNA integration.

20. KITS

[0300] Provided herein is a kit, which may be used to edit a genome in skeletal muscle or cardiac muscle, such as correcting a mutant gene. The kit comprises a composition for genome editing in skeletal muscle or cardiac muscle, as described above, and instructions for using said composition. Instructions included in kits may be affixed to packaging material or may be included as a package insert. While the instructions are typically written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term “instructions” may include the address of an internet site that provides the instructions.

[0301] The composition for genome editing in skeletal muscle or cardiac muscle may include a modified AAV vector and a nucleotide sequence encoding a site-specific nuclease, as described above. The site-specific nuclease may include a ZFN, a TALEN, or CRISPR/Cas9-based system, as described above, that specifically binds and cleaves a mutated gene. The site-specific nuclease, as described above, may be included in the kit to specifically bind and target a particular region in the mutated gene. The site-specific nuclease may be specific for a mutated dystrophin gene, as described above. The kit may further include donor DNA, a gRNA, or a transgene, as described above.

[0302] a. CRISPR/Cas9-based system

[0303] Provided herein is a kit, which may be used to correct a mutated gene. The kit comprises at least one component for correcting a mutated gene and instructions for using the CRISPR/Cas9-based system. Instructions

included in kits may be affixed to packaging material or may be included as a package insert. While the instructions are typically written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term “instructions” may include the address of an internet site that provides the instructions.

[0304] At least one component may include at least one CRISPR/Cas9-based system, as described above, which specifically targets a gene. The kit may include a Cas9 protein or Cas9 fusion protein, a nucleotide sequence encoding said Cas9 protein or Cas9 fusion protein, and/or at least one gRNA. The CRISPR/Cas9-based system, as described above, may be included in the kit to specifically bind and target a particular target region upstream, within or downstream of the coding region of the target gene. For example, a CRISPR/Cas9-based system may be specific for a promoter region of a target gene or a CRISPR/Cas9-based system may be specific for a mutated gene, for example a mutated dystrophin gene, as described above. The kit may include donor DNA, as described above.

21. EXAMPLES

[0305] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the present disclosure described herein are readily applicable and appreciable, and may be made using suitable equivalents without departing from the scope of the present disclosure or the aspects and embodiments disclosed herein. Having now described the present disclosure in detail, the same will be more clearly understood by reference to the following examples, which are merely intended only to illustrate some aspects and embodiments of the disclosure, and should not be viewed as limiting to the scope of the disclosure. The disclosures of all journal references, U.S. patents, and publications referred to herein are hereby incorporated by reference in their entireties.

[0306] The present invention has multiple aspects, illustrated by the following non-limiting examples.

Example 1

Materials and Methods

[0307] Cell culture and transfection. HEK293T cells were obtained from the American Tissue Collection Center (ATCC) through the Duke University Cancer Center Facilities and were maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37° C. with 5% CO₂. HEK293T cells were transfected with Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Transfection efficiencies were routinely higher than 80% as determined by fluorescence microscopy following delivery of a control eGFP expression plasmid. Cas9 expression plasmid was transfected at a mass ratio of 3:1 to either the individual gRNA expression plasmids or the identical amount of gRNA expression plasmid consisting of a mixture of equal amounts of the four gRNAs.

[0308] Primary mouse embryonic fibroblasts (PMEF-HL, Millipore, Billerica, Mass.) were seeded (75,000 per well) in 24-well TCPS plates (BD, Franklin Lakes, N.J.) and main-

tained at 37° C. and 5% CO₂ in complete MEF medium consisting of high glucose DMEM supplemented with 10% Premium Select FBS (Atlanta Biologicals, Lawrenceville, Ga.), 25 µg mL⁻¹ gentamicin (Invitrogen), 1× GutaMAX, non-essential amino acids, sodium pyruvate, and β-mercaptoethanol (Invitrogen). MEF transfections were performed with a single 1 µg cm⁻² dose of total plasmid DNA, delivered as cationic nanocomplexes following electrostatic condensation with poly(CBA-ABOL) in serum- and antibiotic-free OptiMEM, as described previously (Adler et al. *Molecular therapy. Nucleic acids* 1, e32 (2012)). OptiMEM was replaced with complete MEF medium 4 hrs after transfection. 48 hrs after transfection, MEFs were processed for qRT-PCR, or the complete MEF medium was replaced with N3 neural induction medium containing: DMEM/F-12 (Invitrogen), 1× N-2 Supplement (Invitrogen), 10 ng mL⁻¹ human bFGF2 (Stemgent, Cambridge, Mass.), and 25 µg mL⁻¹ gentamicin (Invitrogen). A GFP reporter vector (pmax-GFP, 3486 bp, Amaxa, Cologne, Germany) was used to optimize transfection conditions. Cas9 expression plasmid was transfected at a mass ratio of 3:1 or 1:1 to an equal mixture of four gRNA expression plasmids.

[0309] Plasmids. The plasmids encoding wild-type and H840A Cas9 were obtained from Addgene (Plasmid #39312 and Plasmid #39316; Jinek, et al. *Science* 337, 816-821 (2012)). H840A Cas9 was cloned into the vector pcDNA3.1 in frame with a FLAG epitope tag and a nuclear localization sequence (NLS) at the N-terminus with a primer pair that introduced the D10A mutation. The VP64 domain, an NLS, and an HA epitope tag were cloned in frame with the Cas9 ORF at the C-terminus (FIG. 1A, FIG. 9A). The tracrRNA and crRNA expression cassettes (Cong et al. *Science* 339, 819-823 (2013)) were ordered as gBlocks (Integrated DNA Technologies (IDT)) and cloned into a pZDonor plasmid (Sigma) with KpnI and SacII sites. A chimeric guide RNA expression cassette (Mali et al. *Science* 339, 823-826 (2013)) was also ordered as gBlocks with modifications to include a BbsI restriction site to facilitate rapid cloning of new guide RNA spacer sequences (FIG. 9B). The oligonucleotides containing the target sequences were obtained from IDT, hybridized, phosphorylated, and cloned in the appropriate plasmids using BbsI sites. The target sequences are provided in Table 2.

TABLE 2

Target sequences of gRNAs			
Target	Name	Sequence	SEQ ID NO
ASCL1	CR1	GCTGGGTGTCCCATGAAA	5
	CR2	CAGCCGCTCGCTGCAGCAG	6
	CR3	TGGAGAGTTTGCAAGGAGC	7
	CR4	GTTTATTCAGCCGGGAGTC	8
NANOG	CR1	CGCCAGGAGGGGTGGGTCTA	9
	CR2	CCTTGGTGAGACTGGTAGA	10
	CR3	GTCTTCAGGTTCTGTGCT	11
	CR4	ATATTCCTGATTAAAGT	12
VEGFA	CR1	TTAAAGTCGGCTGGTAGC	13
	CR2	CGGGCCGGGGCGGGGTCC	14
	CR3	GCCCGAGCCGCGTGTGGAA	15
	CR4	CCTTCATTGCGGCGGCTG	16
TERT	CR1	CCGACCCCTCCCGGTCCC	17
	CR2	CAGGACCGCGCTCCACG	18

TABLE 2-continued

Target sequences of gRNAs			
Target	Name	Sequence	SEQ ID NO
	CR3	TGCACCTTGGGAGCGCGAG	19
	CR4	CCGCACGCACCTGTTCCTCA	20
IL1B	CR1	AAAACAGCGAGGGAGAAAC	21
	CR2	TTAACTTGATTGTGAAATC	22
	CR3	AAAACAATGCATATTGCA	23
	CR4	AAAATCCAGTATTTTAATG	24
IL1R2	CR1	ACCCAGCACTGCAGCCTGG	25
	CR2	AACCTTATGCGCGCTTTCCT	26
	CR3	TCACCTTTAAACACCTCT	27
	CR4	GCATCTTTTCTCTTTAAT	28
IL1RN	CR1	TGTACTCTCTGAGGTGCTC	29
	CR2	ACGCAGATAAGAACCAGTT	30
	CR3	CATCAAGTCAGCCATCAGC	31
	CR4	GAGTCACCTCTCTGGAAAC	32
HBG1/2	CR1	GCTAGGGATGAAGAATAAA	33
	CR2	TTGACCAATAGCCTTGACA	34
	CR3	TGCAAAATATCTGTCTGAAA	35
	CR4	AAATTAGCAGTATCCTCTT	36
MYOD1	CR1	CCTGGGCTCCGGGGCGTTT	37
	CR2	GGCCCTGCGGCCACCCCG	38
	CR3	CTCCCTCCCTGCCCCGTAG	39
	CR4	AGGTTTGAAAGGGCGTGC	40

[0310] Western blot. Cells were lysed in 50 mM Tris-Cl (pH 7.4), 150 mM NaCl, 0.50 Triton X-100, and 0.1% SDS. Lysates were mixed with loading buffer, boiled for 5 min, and equal volumes of protein were run in NuPAGE® Novex 0-2% or 10% Bis-Tris Gel polyacrylamide gels and transferred to nitrocellulose membranes. Non-specific antibody binding was blocked with 50 mM Tris/150 mM NaCl/0.1% Tween-20 (TBS-T) with 5% nonfat milk for 30 min. The membranes were incubated with primary antibodies (HRP-conjugated anti-Flag (Cell Signaling, Cat #2044) in 5% BSA in TBS-T diluted 1:1000 overnight; anti-GAPDH (Cell Signaling, clone 14C10) in 5% milk in TBS-T diluted 1:5000 for 30 min; anti-ASCL1 (Santa Cruz, clone sc-48449) in 5% BSA diluted 1:500; or anti-g-globin (Santa Cruz, clone 51-7) in 5% milk diluted 1:500 and the membranes were washed with TBS-T for 30 min. Membranes labeled with primary antibodies were incubated with anti-rabbit HRP-conjugated antibody (Sigma-Aldrich) diluted 1:5000 for 30 min, anti-goat (1:3000) or anti-mouse (1:5000) and washed with TBS-T for 30 min. Membranes were visualized using the Immun-Star WesternC™ Chemiluminescence Kit (Bio-Rad) and images were captured using a ChemiDoc™ XRS+ System and processed using Image-Lab software (Bio-Rad).

[0311] ELISA. Serum-free culture media (OPTI-MEM) was collected and frozen at -80° C. Human IL-1ra secretion into culture media was quantified via enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's protocols (R&D Systems, Cat. No. DY280). The standard curve was prepared by diluting recombinant human IL-1ra in OPTI-MEM and the IL-1ra in culture media was measured undiluted. The samples were concentrated about 8 fold via centrifugation through 3 kDa MWCO filters for 20 min (Amicon Ultra, Cat # UFC500396). Reported values were corrected by the concentration factor for each sample.

[0312] Optical density was measured at 450 nm, with a wavelength correction at 540 nm. Each standard and sample was assayed in duplicate. The duplicate readings were averaged and normalized by subtracting the average zero standard optical density. A standard curve was generated by log-transforming the data and performing a linear regression of the IL-1ra concentration versus the optical density. Reported values are the mean and standard error of the mean from three independent experiments (n=3) that were performed on different days with technical duplicates that were averaged for each experiment.

[0313] qRT-PCR. Total RNA was isolated using the RNeasy Plus RNA isolation kit (Qiagen). cDNA synthesis was performed using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen). Real-time PCR using PerfeCTa® SYBR® Green FastMix was performed with the CFX96 Real-Time PCR Detection System (Bio-Rad) with oligonucleotide primers reported in Table 3 that were designed using Primer3Plus software and purchased from IDT.

TABLE 3

Sequences of primers used for qRT-PCR.					
Target	Forward Primer	SEQ		SEQ	
		ID NO	Reverse Primer	ID NO	
hASCL1	GGAGCTTCTCGACT TCACCA	41	AACGCCACTGACAA GAAAGC	53	
NANOG	GATTGTGGGCCTG AAGAAA	42	CAGATCCATGGAGG AAGGAA	54	
VEGFA	AAGGAGGAGGGCAG AATCAT	43	GGGTACTCCTGGAA GATGTCC	55	
TERT	AAACCTTCCCTCAGC TATGCC	44	GTTTGCAGCGCATG TTCCTC	56	
IL1B	AGCTGATGGCCCTA AACAGA	45	AAGCCCTTGCTGTA GTGGTG	57	
IL1R2	CAGGAGGACTCTGG CACCTA	46	CGGCAGGAAAGCAT CTGTAT	58	
IL1RN	GGAATCCATGGAGG GAAGAT	47	TGTTCTCGCTCAGG TCAGTG	59	
HBG1/2	GCTGAGTGAAGTGC ACTGTGA	48	GAATCTTTTGCCGA AATGGA	60	
MYOD1	CTCTCTGCTCCTTT GCCACA	49	GTGCTCTTCGGGTT TCAGGA	61	
GAPDH	CAATGACCCCTTCA TTGACC	50	TTGATTTTGAGGGG ATCTCG	62	
mASCL1	GGAACAAGAGCTGC TGGACT	51	GTTTTTCTGCCTCC CCATTT	63	
mGAPDH	AACCTTGGCATTGT GGAAGG	52	GGATGCAGGGATGA TGTCTCT	64	

[0314] Primer specificity was confirmed by agarose gel electrophoresis and melting curve analysis. Reaction efficiencies over the appropriate dynamic range were calculated to ensure linearity of the standard curve (FIG. 10). The results are expressed as fold-increase mRNA expression of the gene of interest normalized to GAPDH expression by the $\Delta\Delta C_T$ method. Reported values are the mean and standard error of the mean from three independent experiments

performed on different days (n=3) with technical duplicates that were averaged for each experiment.

[0315] RNA-Seq. RNA seq libraries were constructed. Briefly, first strand cDNA was synthesized from oligo dT Dynabead® (Invitrogen) captured mRNA using Super-Script® VILO™ cDNA Synthesis Kit (Invitrogen). Second strand cDNA was synthesized using DNA Polymerase I (New England Biolabs). cDNA was purified using Agencourt AMPure XP beads (Beckman Coulter) and Nextera transposase (Illumina; 5 min at 55° C.) was used to simultaneously fragment and insert sequencing primers into the double-stranded cDNA. Transposition reactions were halted using QG buffer (Qiagen) and fragmented cDNA was purified on AMPure XP beads. Indexed sequencing libraries were generated by 6 cycles of PCR.

[0316] Libraries were sequenced using 50-bp single end reads on two lanes of an Illumina HiSeq 2000 instrument, generating between 29 million and 74 million reads per library. Reads were aligned to human RefSeq transcripts using Bowtie (Langmead et al. *Genome biology* 10, R25 (2009)). The statistical significance of differential expression, including correction for multiple hypothesis testing, was calculated using DESeq (Anders et al. *Genome biology* 11, R106 (2010)). Raw RNA-seq reads and the number of reads aligned to each RefSeq transcript have been deposited for public access in the Gene Expression Omnibus (GEO), with accession number currently pending.

[0317] Immunofluorescence staining. For detection of Tuj1 and MAP2 expression, transfected MEFs were fixed at day 10 of culture in N3 medium with 4% PFA (EMS, Hatfield, Pa.) at room temperature (RT) for 20 min. Cells were then incubated in blocking buffer containing 0.2% Triton X-100, 3% w/v BSA, and 10% goat serum (Sigma-Aldrich, Saint Louis, Mo.) for two hrs at room temperature with rabbit anti-Tuj1 (Covance, Princeton, N.J., clone TUJ1 1-15-79, 1:500) and mouse anti-MAP2 (BD, clone Ap20, 1:500), or for an additional 24 hrs at 4° C. with mouse anti-Ascl1 (BD clone 24B72D11.1, 1:100). The cells were then washed three times with PBS, incubated for 1 hr at room temperature in blocking buffer with Alexa Fluor 488 goat anti-mouse IgG and Alexa Fluor 594 goat anti-rabbit IgG (Invitrogen, 1:200), and washed three times with PBS. Stained MEFs were then scanned with a Nikon Eclipse TE2000-U inverted fluorescence microscope with a ProScanII motorized stage (Prior Scientific, Rockland, Mass.) to produce large mosaic images of each complete culture area. These mosaics were processed with a FIJI macro to automatically and uniformly threshold each image according to local contrast, exclude small debris, and to count the number of Tuj1⁺ cells in each well.

[0318] Statistics. Statistical analysis was performed by Tukey's test with alpha equal to 0.05 in JMP 10 Pro.

Example 2

Results

[0319] To create a CRISPR/Cas9-based transcriptional activation system, catalytic residues of Cas9 (D10A, H840A) were mutated to create iCas9 and genetically fused with a C-terminal VP64 acidic transactivation domain (FIG. 1A, FIG. 1). Robust expression of iCas9-VP64 was observed from the transfected plasmid in human embryonic kidney (HEK) 293T cells by western blot of the N-terminal Flag epitope tag (FIG. 3). The CRISPR system recognizes its

target through base pairing of a 20 bp sequence in the gRNA to a complementary DNA target, which is followed by the NGG protospacer-adjacent motif (PAM) sequence, where N is any base pair. Combinations of synthetic transcription factors targeted to endogenous human promoters result in synergistic and robust activation of gene expression. Therefore four gRNA target sites followed by the NGG PAM sequence were identified in the promoter of the IL1RN gene within 500 bp of the transcriptional start site (FIG. 4, Table 2). To compare crRNA- and gRNA-based targeting strategies, the four target site sequences were introduced into crRNA and gRNA expression plasmids¹⁷ and co-transfected with the iCas9-VP64 expression plasmid into HEK293T cells. Although substantial induction of IL1RN expression was observed by qRT-PCR in samples treated with the combination of crRNAs, much higher levels were achieved with the combination of gRNAs (FIG. 1C). No changes to gene expression were observed in cells treated with gRNAs and an expression plasmid for iCas9 without VP64, demonstrating the critical role of the activation domain in modulating gene expression (FIG. 1C). Nuclease activity at these target sites was confirmed to have been abrogated in the iCas9-VP64 system by performing the Surveyor assay to detect DNA repair events in samples treated with iCas9-VP64 and wild-type Cas9 (FIG. 5). By transfecting each of the four gRNAs individually or in combination, targeting multiple sites in the promoter with combinations of gRNAs showed robust increases in gene expression (FIG. 1D). High levels of IL1RN expression were observed only when the gRNA combinations were co-transfected with iCas9-VP64 (FIG. 1D), as seen with other classes of engineered transcription factors. Similarly, production of the IL-1 receptor antagonist (IL-1ra) protein, encoded by the IL1RN gene, was only observed in three of the six samples treated with the combination of gRNAs across three different experiments, whereas it was never detected in samples treated with single gRNAs or control plasmid (FIG. 1E). To examine the specificity of gene activation by iCas9-VP64, global gene expression of HEK293T cells treated with the combination of four gRNAs by RNA-seq was assessed (FIG. 1F). Notably, the only genes with significantly increased expression relative to control (false discovery rate $\leq 3 \times 10^{-4}$) were the four isoforms expressed from the IL1RN locus (FIG. 4), indicating a high level of specificity of gene activation.

[0320] To demonstrate the general applicability of this system, four gRNAs were designed to target each of the promoters of eight other genes relevant to medicine and biotechnology, including ASCL1, NANOG, HBG1/2, MYOD1, VEGFA, TERT, IL1B, and IL1R2 (FIG. 4, Table 2). Forced expression of ASCL1 and MYOD1 leads to transdifferentiation of several cell types into neuronal and myogenic phenotypes, respectively. NANOG is a marker of pluripotency and that is also used in genetic reprogramming strategies. Activation of the homologs HBG1 and HBG2, which encode γ -globin during fetal development, can be used as a therapeutic strategy to compensate for β -globin mutations in sickle cell disease. Up-regulation of VEGFA by synthetic transcription factors has been explored as a strategy to enhance tissue regeneration and wound healing. The forced expression of telomerase, encoded by the TERT gene, can be used to immortalize cell lines. IL1B encodes the IL-1 β cytokine that mediates inflammation and autoimmunity. IL-1 β signaling can be blocked by expression of IL-1ra or the decoy receptor encoded by IL1R2. Expression of each

of these genes was enhanced by co-transfection of expression plasmids for iCas9-VP64 and the four gRNAs into HEK293T cells, as determined by qRT-PCR (FIGS. 2A-2H). In some cases expression of a single gRNA was sufficient to induce gene expression, but in all cases co-transfection of the four gRNAs led to synergistic effects (FIGS. 2A-2D). Notably, chromatin accessibility, as determined by DNase-seq, was not a predictor of successful gene activation (FIG. 4). RNA-seq was performed on cells transfected with iCas9-VP64 and the four gRNAs targeting HBG1, three of which also target HBG2. This revealed specific and reproducible increases in expression of both HBG1 and HBG2, which cannot be distinguished by RNA-seq, although statistical significance was not achieved due to low total expression levels (FIG. 6). Increases in protein expression of Ascl1 and γ -globin following treatment with iCas9-VP64 and the four gRNAs were detected by western blot (FIG. 7), corroborating higher mRNA levels observed by qRT-PCR (FIGS. 2A-2H). Low baseline levels of Ascl1 and γ -globin protein expression were detectable in empty vector controls. As preliminary evidence that the activation of gene targets by iCas9-VP64 can lead to secondary changes in gene networks and cell phenotypes, expression plasmids for iCas9-VP64 and the four gRNAs targeting ASCL1 were co-transfected into murine embryonic fibroblasts (MEFs) (FIGS. 8A-8H). Forced expression of Ascl1 in MEFs has been shown to partially activate the neuronal gene network, including the downstream target Tuj1. Because the gRNA target sites are conserved in the human and mouse ASCL1 promoters (FIG. 8A), activation of ASCL1 expression was also observed in MEFs treated with plasmids encoding iCas9-VP64 and the four gRNAs (FIG. 8B). Furthermore, cells expressing Ascl1 and the neuronal marker Tuj1 were readily detected by immunofluorescence staining 12 days after transfection in the iCas9-VP64/gRNA-treated samples (FIGS. 8C-8H). No Tuj1-positive cells were observed in the cells treated with the control plasmid.

[0321] Thus far there has not been any comprehensive survey of the specificity of Cas9/CRISPR activity in mammalian cells. Using RNA-seq, targeted gene activation was shown to be exquisitely specific with no detectable off-target gene activation (FIG. 1F, FIG. 6). IL1RN and HBG12 were chosen for this specificity analysis as the gene products, IL-1ra and γ -globin, may not generate secondary effects on gene expression in HEK293T cells. Exploiting the synergistic activity of multiple weak transcriptional activators, in contrast to using a single strong activator, may increase specific gene regulation since it is unlikely that multiple adjacent off-target sites would exist at another locus. Interestingly, the IL32 gene was moderately downregulated (false discovery rate <0.03) in both the samples treated with iCas9-VP64 and either the IL1RN- or HBG1/2-targeted gRNAs compared to control samples treated with only an empty expression plasmid (FIG. 1F, FIG. 6). Because both the IL1RN and HBG1/2-targeted samples were similarly affected, it is unlikely that this is the result of off-target iCas9-VP64 activity related to the identity of the target sequences.

[0322] To evaluate the specificity with which iCas9-VP64 binds the genome, ChIP sequencing was performed using an anti-HA antibody on cells treated with iCas9-VP64 and four gRNAs targeting the IL1RN promoter. The experiment revealed that iCas9 targets the IL1RN promoter (FIG. 15). Moreover, the experiment revealed an extremely high level

of specificity. The iCas9 had only 10 potential off-target binding sites ($FDR < 5\%$). To further query the specificity, RNA sequencing experiments were performed with iCas9 EGEMs and found that only IL1RN gene isoforms increased in expression relative to control ($FDR \leq 3 \times 10^{-4}$).

Example 3

CRISPRs Targeting the Dystrophin Gene—Methods and Materials

[0323] Plasmid constructs. The expression cassettes for the *S. pyogenes* sgRNA and human codon optimized Cas9 (hCas9) nuclease were used, as previously described (Perez-Pinera et al., *Nat Methods* 10:973-976 (2013)). In order to create a fluorescent reporter system to enrich CRISPR/Cas9-modified cells, a GeneBlock (IDT) was synthesized containing a portion of the 3' end of the Cas9 coding sequence fused to a T2A skipping peptide immediately upstream of a multiple cloning site and subsequently cloned into the hCas9 expression vector. An eGFP reporter gene was then cloned into the T2A vector to allow co-translation of Cas9 and eGFP proteins from the same expression vector (hCas9-T2A-GFP, SEQ ID NO: 116).

[0324] Cell culture and transfection. HEK293T cells were obtained from the American Tissue Collection Center (ATCC) through the Duke Cell Culture Facility and were maintained in DMEM supplemented with 10% bovine calf serum and 1% penicillin/streptomycin. Immortalized myoblasts (Mamchaoui, K. et al. *Skelet Muscle* 1, 1-11 (2011)) (one from a wild-type donor, and two Δ 48-50 DMD patient derived lines) were maintained in skeletal muscle media (PromoCell) supplemented with 20% bovine calf serum (Sigma), 50 μ g/ml fetuin, 10 ng/ml human epidermal growth factor (Sigma), 1 ng/ml human basic fibroblast growth factor (Sigma), 10 μ g/ml human insulin (Sigma), 1% GutaMAX (Invitrogen), and 1% penicillin/streptomycin (Invitrogen). Primary DMD dermal fibroblasts were obtained from the Coriell Cell repository (GM05162A, A46-50) and maintained in DMEM supplemented with 10% fetal bovine serum, 1 ng/ml human basic fibroblast growth factor, and 1% penicillin/streptomycin. All cell lines were maintained at 37° C. and 5% CO₂.

[0325] HEK293T cells were transfected with Lipofectamine 2000 (Invitrogen) with 400 ng of each expression vector according to the manufacturer's protocol in 24 well plates. Immortalized myoblasts and primary fibroblasts were transfected with 5 μ g of each expression vector by electroporation using the Gene Pulser XCell (BioRad) with PBS as an electroporation buffer using optimized conditions for each line (FIGS. 1A-1F) (Ousterout et al. *Mol Ther* 21:1718-1726 (2013)). Transfection efficiencies were measured by delivering an eGFP expression plasmid (pmaxGFP, Clontech) and using flow cytometry. These efficiencies were routinely $\geq 95\%$ for HEK293T and $\geq 70\%$ for the primary fibroblasts and immortalized myoblasts. For all experiments, the indicated mass of electroporated plasmid corresponds to the amount used for each CRISPR/Cas9-based system.

[0326] Cel-I Quantification of Endogenous Gene Modification (Surveyor Assay).

[0327] CRISPR/Cas9-based system-induced lesions at the endogenous target site were quantified using the Surveyor nuclease assay (Guschin, D. Y. et al. *Meth Mol Biol* 649, 247-256 (2010)), which can detect mutations characteristic of nuclease-mediated NHEJ. After transfection, cells were

incubated for 3 or 10 days at 37° C. and genomic DNA was extracted using the DNeasy Blood and Tissue kit (QIAGEN). The target locus was amplified by 35 cycles of PCR with the AccuPrime High Fidelity PCR kit (Invitrogen)

using primers specific to each locus (see Table 4), such as 5'-GAGTTTGGCTCAAATTGTTACTCTT-3' (SEQ ID NO: 626) and 5'-GGGAAATGGTCTAGGAGAGTAAAGT-3' (SEQ ID NO: 627).

TABLE 4

Summary of top 10 off target sites predicted in silico and activity at each site as detected by the Surveyor assay in HEK293T cells transfected with Cas9 and the indicated sgRNA expression cassettes.										
SEQ ID NO.	Guide	Target	Sequence	PAM	Score	Chr	Gene	Intron/Exon	#MMs	% indels
67	CR3	Guide	GCCTACTCAGACTGTTACTC	—	—	—	—	—	—	—
150		Target	tCCTACTCAGACTGTTACTC	TGG	—	X	DMD	Exon	1	13.0
151		OT1	tCCTACTCacACTGTTACTC	AGG	7.4	1	STRIP1	Intron	2	9.3
152		OT2	aCCTgCTCAcACTGTTACTC	CAG	2.5	2	ARHGAP25	Intron	3	n.d.
153		OT3	GCaTtCTCAaACTGTTACTC	AGG	2.4	13	None	None	3	n.d.
154		OT4	GgaTtCTCAcACTGTTACTC	GGG	1.3	14	PGPEP1	Exon	4	n.d.
155		OT5	aCaTACTtAtACTGTTACTC	TAG	1.3	19	MDGA2	Intron	4	n.d.
156		OT6	tatTcCTaAGACTGTTACTC	AAG	0.9	8	LPPR1	Intron	5	n.d.
157		OT7	aaggACTaAGACTGTTACTC	GGG	0.9	9	RNF122	Intron	5	n.d.
158		OT8	GagctCTCatACTGTTACTC	TAG	0.8	3	DNMBP	Exon	5	n.d.
159		OT9	GCaaAaTgAGACTGTTACTC	CAG	0.8	5	SLC12A2	Intron	4	n.d.
160		OT10	cCtcAtTCAGACTGTTACTC	AAG	0.8	4	KCNIP4	Intron	4	n.d.
65	CR1	Guide	GATTGGCTTTGATTCCCTA	—	—	—	—	—	—	—
161		Target	cATTGGCTTTGATTCCCTA	GGG	—	X	DMD	Intron	1	8.3
162		OT1	aATTGGCATTGATTCCCTA	GAG	7.1	16	None	None	2	0.8
163		OT2	cATTGGCTTTaATTCCCTA	TAG	4.8	4	None	None	2	n.d.
164		OT3	GATaGGCTgTGATTCCCTA	GAG	3.9	9	None	None	2	n.d.
165		OT4	GAaTaGcCtTGATTCCCTA	AAG	2.4	1	None	None	3	n.d.
166		OT5	aATTtGCTTTGATTCCCTg	AGG	1.5	1	TIMM17A	Intron	3	n.d.
167		OT6	GATgtGCTTTGATTCCCTt	GGG	1.4	17	MYO1D	Intron	3	n.d.
168		OT7	aATTGGtTTTaATTCCCTA	AAG	1.1	8	PIK1A	Intron	3	n.d.
169		OT8	aATTGGgTTTGATTCCCTt	TGG	1.1	11	MS4A1	Intron	3	n.d.
170		OT9	GATgGgtTTTtATTCCCTA	GAG	1.0	11	None	None	3	n.d.
171		OT10	GAaTGGtTTTGATTCCCTg	GAG	1.0	11	None	None	3	n.d.
69	CR5	Guide	GCAGTTGCCTAAGAACTGGT	—	—	—	—	—	—	—
172		Target	aCAGTTGCCTAAGAACTGGT	GGG	—	X	DMD	Intron	1	14.0
173		OT1	cCAGTTGtCTAAGAACTGGg	GAG	1.5	5	NRG1	Intron	3	n.d.
174		OT2	GCAGTTGCCTgtGAACTGGT	AGG	1.4	X	None	None	2	n.d.
175		OT3	GCAGaTGcAgAAGAACTGGT	GAG	1.4	19	SMIM7	Intron	3	n.d.
176		OT4	GCAGTTcCagAAGAACTGGT	GAG	0.9	11	GLB1L2	Intron	3	n.d.
177		OT5	caAcTTGCCTAtGAACTGGT	AGG	0.7	8	ASAP1	Intron	4	n.d.
178		OT6	aCAcTGcCTAAGAACTGGa	GGG	0.7	11	None	None	4	n.d.
179		OT7	tCAGgTgGCTAAGAACTGGg	TGG	0.7	14	NIN	Intron	4	n.d.
180		OT8	GaAGTTGgCcAAGAACTGGa	GAG	0.6	7	None	None	4	n.d.
181		OT9	GctGcTGCCcAAGAACTGGc	AGG	0.6	11	AMOTL1	Intron	4	n.d.
182		OT10	tCAGcTgGCTAAGAACTGGT	AAG	0.6	7	ACTR3C	Intron	4	n.d.
70	CR6	Guide	GGGGCTCCACCCTCACGAGT	—	—	—	—	—	—	—
183		Target	aGGGCTCCACCCTCACGAGT	GGG	—	X	DMD	Intron	1	19.9
184		OT1	GcaGCTCagCCCTCACGAGT	CAG	0.8	3	None	None	4	n.d.
185		OT2	GGGGCTtCagCaTCACGAGT	GAG	0.8	8	None	None	3	n.d.
186		OT3	GGGGCTCtCCCTCACTAGT	GAG	0.6	8	None	None	3	n.d.
187		OT4	GGGGaTCCACCTTCACcAGT	CAG	0.6	2	None	None	3	n.d.
188		OT5	aGGGCTggACCCTCACAAGT	AAG	0.4	16	AXIN1	Intron	4	n.d.
189		OT6	tGGtCTCctCCcCACGAGT	GGG	0.4	2	None	None	4	n.d.
190		OT7	aGGGCTCCcACcCACGAGT	GAG	0.3	5	None	None	4	n.d.
191		OT8	GaGGCTCCAtaCTCACcAGT	GAG	0.3	11	None	None	4	n.d.
192		OT9	GGaGCTgCcCctTCACGAGT	GGG	0.3	3	None	None	4	n.d.
193		OT10	atGaCTCCACCCTCAaGAGT	AAG	0.3	8	AGPAT5	None	4	n.d.
100	CR36	Guide	GCCTTCTTTATCCCTATCG	—	—	—	—	—	—	—
194		Target	GCCTTCTTTATCCCTATCG	AGG	—	X	DMD	Intron	0	20.6
195		OT1	GtCTgCTgTgTCCCTATCG	GGG	1.3	21	None	None	4	n.d.
196		OT2	cCCTTCTcTATCCCTgTTCG	TGG	1.3	8	None	None	3	n.d.
197		OT3	GCCTTCTTTATCCCTcTCT	TGG	0.9	10	None	None	2	0.5
198		OT4	GCgcTCTTtTCCCTATCt	TAG	0.6	16	None	None	4	n.d.
199		OT5	GCCcTCTgTcTCCCTgTTCG	CAG	0.5	1	NFASC	None	4	n.d.
200		OT6	tCCATCTtTgTCCCTATtG	AGG	0.5	10	None	None	4	n.d.
201		OT7	acCtTCTCTcTCCCTATaG	AGG	0.5	5	LOC100996485	Intron	4	n.d.

TABLE 4-continued

Summary of top 10 off target sites predicted in silico and activity at each site as detected by the Surveyor assay in HEK293T cells transfected with Cas9 and the indicated sgRNA expression cassettes.										
SEQ ID NO.	Guide	Target	Sequence	PAM	Score	Chr	Gene	Intron/ Exon	#MMs	% indels
202		OT8	GttTTCTTTTTCCTTATgG	GAG	0.5	3	None	None	4	n.d.
203		OT9	tgCTTCTTaATCCCCTATCa	AAG	0.4	7	None	None	4	n.d.
204		OT10	aCCTTCTTAcTCCCCTATCc	GGG	0.4	10	ADARB2	None	4	n.d.

n.d.: not detected.

[0328] The resulting PCR products were randomly melted and reannealed in a thermal cycler with the program: 95° C. for 240 s, followed by 85° C. for 60 s, 75° C. for 60 s, 65° C. for 60 s, 55° C. for 60 s, 45° C. for 60 s, 35° C. for 60 s, and 25° C. for 60 s with a -0.3° C./s rate between steps. Following reannealing, 8 µL of PCR product was mixed with 1 µL of Surveyor Nuclease S and 1 µL of Enhancer S (Transgenomic) and incubated at 42° C. for 1 hr. After incubation, 6 µL of digestion product was loaded onto a 10% TBE polyacrylamide gel and run at 200V for 30 min. The gels were stained with ethidium bromide and quantified using ImageLab (Bio-Rad) by densitometry as previously described (Guschin, et al. *Meth Mol Biol* 649, 247-256 (2010)).

[0329] Fluorescence-activated cell sorting of myoblasts. DMD myoblasts were electroporated with 5 micrograms each of hCas9-T2A-GFP and sgRNA expression vectors and incubated at 37° C. and 5% CO₂. Three days after electroporation, cells were trypsinized and collected for FACS sorting using a FACSvantage II sorting machine. GFP-positive cells were collected and grown for analysis.

[0330] PCR-based assay to detect genomic deletions. The exon 51 or exon 45-55 loci were amplified from genomic DNA by PCR (Invitrogen AccuPrime High Fidelity PCR kit) using primers flanking each locus. The flanking primers were CellI-CR1/2-F and CellI-CR5-R for exon 51 or CellI-CR6-F and CellI-CR36-R for exon 45-55 analysis (Table 4). PCR products were separated on TAE-agarose gels and stained with ethidium bromide for analysis.

[0331] PCR-based detection of translocations. Loci with predicted possible translocations were amplified by a two-step nested PCR (Invitrogen AccuPrime High Fidelity PCR kit for each step) of genomic DNA from cells transfected with Cas9 alone (control) or Cas9 with sgRNA. In the first step, translocations that may occur at each on-target and

off-target sgRNA target site were amplified by 35 cycles of PCR using combinations of Surveyor primers for each locus that were modified to include restriction sites to facilitate cloning and sequencing analysis (Table 4). One microliter of each PCR reaction was subjected to a second round of amplification by 35 rounds of PCR using nested primer sets custom designed for each individual predicted translocation (Table 4). Each second nested PCR primer binds within the same approximate region within the primary amplicon; however, each pair was optimized using Primer3 online bioinformatics software to ensure specific detection of each translocation. PCR amplicons corresponding to the expected length of predicted translocations and only present in cells treated with sgRNA were purified (QIAGEN Gel Extraction kit) and analyzed by Sanger sequencing.

[0332] mRNA analysis. Immortalized myoblasts were differentiated into myofibers by replacing the growth medium with DMEM supplemented with 1% insulin-transferrin-selenium (Invitrogen #51500056) and 1% penicillin/streptomycin (Invitrogen #15140) for 5 days before the cells were trypsinized and collected. Total RNA was isolated from these cells using the RNeasy Plus Mini Kit (QIAGEN) according to the manufacturer's instructions. RNA was reverse transcribed to cDNA using the VILO cDNA synthesis kit (Life Technologies #11754) and 1.5 micrograms of RNA for 2 hrs at 42° C. according to the manufacturer's instructions. The target loci were amplified by 35 cycles of PCR with the AccuPrime High Fidelity PCR kit (Invitrogen) using primers annealing to exons 44 and 52 to detect exon 51 deletion by CR1/5 or CR2/5 or primers annealing to exons 44 and 60 to detect exon 45-55 deletion by CR6/36 (Table 4). PCR products were run on TAE-agarose gels and stained with ethidium bromide for analysis. The resolved PCR bands were cloned and analyzed by Sanger sequencing to verify the expected exon junctions. Table 5 lists the sequences of primers used in Example 4.

TABLE 5

SEQ ID NO.	Primer name	Primer sequence	Notes
205	CellI-CR1/2-F	GAGAGGTTATGTGGCTTTACCA	Forward Surveyor primer for CR1/2
206	CellI-CR1-R	AAAAATGCTTCCCACCTTTGC	Reverse Surveyor primer for CR1
207	CellI-CR2-R	CTCATCTCATGCCTGGACA	Reverse Surveyor primer for CR2
208	CellI-CR3-F	GAGTTTGGCTCAAATTGTTACTCTT	Forward Surveyor primer for CR3

TABLE 5-continued

Sd EQ ID	NO.	Primer name	Primer sequence	Notes
	209	Cell-CR3-R	GGGAAATGGTCTAGGAGAGTAAAGT	Reverse Surveyor primer for CR3
	210	Cell-CR4/31-F	GTTTGGCTCAAATGTTACTCTTCA	Forward Surveyor primer for CR4 or CR31
	211	Cell-CR4/31-R	GTGAGAGTAATGTGTTGCTGAGAG	Reverse Surveyor primer for CR4 or CR31
	212	Cell-CR5-F	CGGGCTTGGACAGAACTTAC	Forward Surveyor primer for CR5
	213	Cell-CR5-R	CTGCGTAGTGCCAAACAAA	Reverse Surveyor primer for CR5
	214	Cell-CR6-F	TAATTTTCATTGAAGAGTGGCTGAA	Forward Surveyor primer for CR6
	215	Cell-CR6-R	AAGCCCTGTGTGGTAGTAGTCAGT	Reverse Surveyor primer for CR6
	216	Cell-CR7-F	TGAGTCATGTTGGATAACCACTCT	Forward Surveyor primer for CR7
	217	Cell-CR7-R	GAAGGTCAGGAACATACAATTCAA	Reverse Surveyor primer for CR7
	218	Cell-CR10/11-F	GATATGGGCATGTGAGTTTCATAG	Forward Surveyor primer for CR10 or CR11
	219	Cell-CR10/11-R	TGCTGTTGATTAATGGTTGATAGG	Reverse Surveyor primer for CR10 or CR11
	220	Cell-CR12/13-F	TTTTAAATGCCATGTTTGTGTC	Forward Surveyor primer for CR12 or CR13
	221	Cell-CR12/13-R	ATGAATAACCTAATGGGCAGAAAA	Reverse Surveyor primer for CR12 or CR13
	222	Cell-CR14/15-F	TCAAGTCGCTTCATTTTGATAGAC	Forward Surveyor primer for CR14 or CR15
	223	Cell-CR14/15-R	CACAACAAAACATATAGCCAAAGC	Reverse Surveyor primer for CR14 or CR15
	224	Cell-CR16/17-F	TGCTGCTAAAATAACACAAATCAGT	Forward Surveyor primer for CR16 or CR17
	225	Cell-CR16/17-R	CTGTGCCTATTGTGGTTATCCTG	Reverse Surveyor primer for CR16 or CR17
	226	Cell-CR18/19-F	ATTGATCTGCAATACATGTGGAGT	Forward Surveyor primer for CR18 or CR19
	227	Cell-CR18/19-R	TTTGCCCTGCTATTACAGTATGG	Reverse Surveyor primer for CR18 or CR19
	228	Cell-CR20/21-F	TGTAGGGTGGTTGGCTAAAATAAT	Forward Surveyor primer for CR20 or CR21
	229	Cell-CR20/21-R	TTTTTGACAGTCAATAACACAAA	Reverse Surveyor primer for CR20 or CR21
	230	Cell-CR22/23-F	GGCTGGTCTCACAATTGTACTTTA	Forward Surveyor primer for CR22 or CR23
	231	Cell-CR22/23-R	CATTATGGACTGAAAACTCTAGCA	Reverse Surveyor primer for CR22 or CR23
	232	Cell-CR24/25-F	ATCATCCTAGCCATAACACAATGA	Forward Surveyor primer for CR24 or CR25
	233	Cell-CR24/25-R	TTCAGCTTTAACGTGATTTTCTGT	Reverse Surveyor primer for CR24 or CR25
	234	Cell-CR26/27-F	GGATTGAGAAGCTGTTTACGAAGT	Forward Surveyor primer for CR26 or CR27

TABLE 5-continued

Sd EQ ID	NO. Primer name	Primer sequence	Notes
	235 CellI-CR26/27-R	TTTAGCTGGATTGGAAAAACAAAT	Reverse Surveyor primer for CR26 or CR27
	236 CellI-CR28/29-F	AACTCACCCCATTTGTTGGTATATT	Forward Surveyor primer for CR28 or CR29
	237 CellI-CR28/29-R	CCTTGTCCAAATACCGAAATACAT	Reverse Surveyor primer for CR28 or CR29
	238 CellI-CR33-F	CACATAATTCATGAACTTGGCTTC	Forward Surveyor primer for CR33
	239 CellI-CR33-R	TAGTAGCTGGGGAGGAAGATACAG	Reverse Surveyor primer for CR33
	240 CellI-CR34-F	TTTTTGTTTTAATTGCGACTGTGT	Forward Surveyor primer for CR34
	241 CellI-CR34-R	AGAAAAGGGGTTTTCTTTTGACTT	Reverse Surveyor primer for CR34
	242 CellI-CR35-F	CATTGTGACTGGATGAGAAGAAAC	Forward Surveyor primer for CR35
	243 CellI-CR35-R	AACGGCTGTTATTAAAGTCCTCAG	Reverse Surveyor primer for CR35
	244 CellI-CR36-F	CAAGTCAGAAGTCACTTGCTTTGT	Forward Surveyor primer for CR36
	245 CellI-CR36-R	TTTATGTGCAGGAATCAGTCTGT	Reverse Surveyor primer for CR36
	246 Dys-E44-F	TGGCGGCGTTTTTCATTAT	Forward RT-PCR primer binding in exon 44
	247 Dys-E52-R	TTCGATCCGTAATGATTGTTCTAGCC	Reverse RT-PCR primer binding in exon 52
	248 Dys-E60-R	GGTCTTCAGAGTGCTGAGG	Reverse RT-PCR primer binding in exon 60
	249 CR3-CellI-OT1-F	TGTGTGCTTCTGTACACATCATCT	Forward Surveyor primer for CR3 off-target 1
	250 CR3-CellI-OT1-R	AGATTTC AACCTCAAAAAGTCTGAG	Reverse Surveyor primer for CR3 off-target 1
	251 CR3-CellI-OT2-F	TAAACTCTTTCTTTCCGCAATTC	Forward Surveyor primer for CR3 off-target 2
	252 CR3-CellI-OT2-R	CAAGGTGACCTGCTACCTAAAAAT	Reverse Surveyor primer for CR3 off-target 2
	253 CR3-CellI-OT3-F	TATGACCAAGGCTATGTGTTCACT	Forward Surveyor primer for CR3 off-target 3
	254 CR3-CellI-OT3-R	ACAGCCTCTCTCCAGTAACATTCT	Reverse Surveyor primer for CR3 off-target 3
	255 CR3-CellI-OT4-F	TATTCTTGCACTGGTTTCACATTT	Forward Surveyor primer for CR3 off-target 4
	256 CR3-CellI-OT4-R	ATATTTTAAGCCAAGACCCAACAA	Reverse Surveyor primer for CR3 off-target 4
	257 CR3-CellI-OT5-F	CTTCAACTGTCTGTCTGATTGCT	Forward Surveyor primer for CR3 off-target 5
	258 CR3-CellI-OT5-R	AACAGCCTCTCTTCATTGTTCTCT	Reverse Surveyor primer for CR3 off-target 5
	259 CR3-CellI-OT6-F	CTCTGGAAGTTGTCTGTCTTGA	Forward Surveyor primer for CR3 off-target 6
	260 CR3-CellI-OT6-R	CTTTCCTGCGTTCTCATGTTACTA	Reverse Surveyor primer for CR3 off-target 6
	261 CR3-CellI-OT7-F	CCTTATATCCGTATCGCTCACTCT	Forward Surveyor primer for CR3 off-target 7

TABLE 5-continued

Sd EQ ID	NO. Primer name	Primer sequence	Notes
	262 CR3-CellI-OT7-R	CATATCTGTCTAACTCCGCACAC	Reverse Surveyor primer for CR3 off-target 7
	263 CR3-CellI-OT8-F	ACAGGTGTTATGTTGTCTGCATCT	Forward Surveyor primer for CR3 off-target 8
	264 CR3-CellI-OT8-R	ACTCCATTCCCAGATTAGTTATGC	Reverse Surveyor primer for CR3 off-target 8
	265 CR3-CellI-OT9-F	CTGTTTTCTTTGTGAGAGTGGAGA	Forward Surveyor primer for CR3 off-target 9
	266 CR3-CellI-OT9-R	TGTAAGGTGGTCAAACCTTGCTCTA	Reverse Surveyor primer for CR3 off-target 9
	267 CR3-CellI-OT10-F	TTTTTCCTAGTACCCACAGATTTTT	Forward Surveyor primer for CR3 off-target 10
	268 CR3-CellI-OT10-R	TCCCTGATTCTCTCATTGTGTGA	Reverse Surveyor primer for CR3 off-target 10
	269 CR1-CellI-OT1-F	TTGGGAACATCAGAGAAAGTATGA	Forward Surveyor primer for CR1 off-target 1
	270 CR1-CellI-OT1-R	ACAAATTACAGTCTCCTGGGAAAG	Reverse Surveyor primer for CR1 off-target 1
	271 CR1-CellI-OT2-F	AGTAGCTTACCTTGGCAGAGAAAA	Forward Surveyor primer for CR1 off-target 2
	272 CR1-CellI-OT2-R	TGACATACTGTTACCCCTTTCAGT	Reverse Surveyor primer for CR1 off-target 2
	273 CR1-CellI-OT3-F	GAAAGGCTCAGTGAATGTTTGT	Forward Surveyor primer for CR1 off-target 3
	274 CR1-CellI-OT3-R	CACTGCATCATCTCATTAAATCAA	Reverse Surveyor primer for CR1 off-target 3
	275 CR1-CellI-OT4-F	CCCATATATTCATGATTACCCACA	Forward Surveyor primer for CR1 off-target 4
	276 CR1-CellI-OT4-R	TATCAGAACGAGCACTAAAAGCAC	Reverse Surveyor primer for CR1 off-target 4
	277 CR1-CellI-OT5-F	TTGGGAGGCTGAGGTACAAG	Forward Surveyor primer for CR1 off-target 5
	278 CR1-CellI-OT5-R	GAATGAAAAACAAACAGAAGGTGA	Reverse Surveyor primer for CR1 off-target 5
	279 CR1-CellI-OT6-F	CTCCTCATCTGTACCCCTCAATCT	Forward Surveyor primer for CR1 off-target 6
	280 CR1-CellI-OT6-R	AGAGTGGCATCTAGTGTCAAGTGA	Reverse Surveyor primer for CR1 off-target 6
	281 CR1-CellI-OT7-F	TACCAAAAGCTTCTCCTGTTTACC	Forward Surveyor primer for CR1 off-target 7
	282 CR1-CellI-OT7-R	GTAAGTTGGATGGCCTATTCTTTG	Reverse Surveyor primer for CR1 off-target 7
	283 CR1-CellI-OT8-F	GAAGGAAATGCAAGGATACAAGAT	Forward Surveyor primer for CR1 off-target 8
	284 CR1-CellI-OT8-R	TGATTGAAAGAATCATTCCAGAAA	Reverse Surveyor primer for CR1 off-target 8
	285 CR1-CellI-OT9-F	TCAGAAGGAAAATTGAAATTGGTT	Forward Surveyor primer for CR1 off-target 9

TABLE 5-continued

Sd EQ ID	NO.	Primer name	Primer sequence	Notes
	286	CR1-CellI-OT9-R	CAGATGTGTTCTTCATCATTCCTC	Reverse Surveyor primer for CR1 off-target 9
	287	CR1-CellI-OT10-F	TTCTCTTAGGGAAAGCTCTCAAA	Forward Surveyor primer for CR1 off-target 10
	288	CR1-CellI-OT10-R	GGGTATAGATCATATGGAGGGAAG	Reverse Surveyor primer for CR1 off-target 10
	289	CR5-CellI-OT1-F	AGATGATCTGCCCACCTCAG	Forward Surveyor primer for CR5 off-target 1
	290	CR5-CellI-OT1-R	CTTCTTCCTCATTTAGTGGAAT	Reverse Surveyor primer for CR5 off-target 1
	291	CR5-CellI-OT2-F	ATGAATTGCAGATTGATGGTACTG	Forward Surveyor primer for CR5 off-target 2
	292	CR5-CellI-OT2-R	TCTCACCAAGAACCAATTGTCTA	Reverse Surveyor primer for CR5 off-target 2
	293	CR5-CellI-OT3-F	GTAGGATACCTTGGCAACAGCTTT	Forward Surveyor primer for CR5 off-target 3
	294	CR5-CellI-OT3-R	TTAACGAATTGTGAGATTGCTGT	Reverse Surveyor primer for CR5 off-target 3
	295	CR5-CellI-OT4-F	TCAGAAAGTCAAGTAGCACACACA	Forward Surveyor primer for CR5 off-target 4
	296	CR5-CellI-OT4-R	AGAAGCACACACTCAGGTAAAGC	Reverse Surveyor primer for CR5 off-target 4
	297	CR5-CellI-OT5-F	TCTTGGGGGAATAATGACTAAAA	Forward Surveyor primer for CR5 off-target 5
	298	CR5-CellI-OT5-R	TTTGGCATTTATGGGAATAAACT	Reverse Surveyor primer for CR5 off-target 5
	299	CR5-CellI-OT6-F	ACTAATTCTGGTCAAGCCCATCA	Forward Surveyor primer for CR5 off-target 6
	300	CR5-CellI-OT6-R	TTAAGACATCGGATGAACAGAAAG	Reverse Surveyor primer for CR5 off-target 6
	301	CR5-CellI-OT7-F	AGAAGCTTTCTGACATGATCTGC	Forward Surveyor primer for CR5 off-target 7
	302	CR5-CellI-OT7-R	TCAATTGCATTAGGACTTAGACCA	Reverse Surveyor primer for CR5 off-target 7
	303	CR5-CellI-OT8-F	GTTAAATTACCTGTGAAGCCCTTG	Forward Surveyor primer for CR5 off-target 8
	304	CR5-CellI-OT8-R	CGGAAACAGATCCACTTTATGAT	Reverse Surveyor primer for CR5 off-target 8
	305	CR5-CellI-OT9-F	AAATCCACTGGAAACATCTTGAGT	Forward Surveyor primer for CR5 off-target 9
	306	CR5-CellI-OT9-R	AGTCTCTTCAGAATCATGCCCTAT	Reverse Surveyor primer for CR5 off-target 9
	307	CR5-CellI-OT10-F	GCTTGGTGGCACATACCTGTAG	Forward Surveyor primer for CR5 off-target 10
	308	CR5-CellI-OT10-R	GGTAGGTAGATTGCTTGCTTGTT	Reverse Surveyor primer for CR5 off-target 10
	309	CR6-CellI-OT1-F	AGCTCTCAGCAGAGTAGGGATTA	Forward Surveyor primer for CR6 off-target 1

TABLE 5-continued

Sd EQ ID	NO. Primer name	Primer sequence	Notes
	310 CR6-CellI-OT1-R	GTGAGTCTACTGCACCCCATC	Reverse Surveyor primer for CR6 off-target 1
	311 CR6-CellI-OT2-F	TGACACTGTGAAGTCAATTCTGTC	Forward Surveyor primer for CR6 off-target 2
	312 CR6-CellI-OT2-R	TCAAGAACTTGACAATGAGCAAAT	Reverse Surveyor primer for CR6 off-target 2
	313 CR6-CellI-OT3-F	TATCCGATCCACTGTTGTGTGT	Forward Surveyor primer for CR6 off-target 3
	314 CR6-CellI-OT3-R	CAGGAGACCCAAAACCACTCTAC	Reverse Surveyor primer for CR6 off-target 3
	315 CR6-CellI-OT4-F	TTGTTCTACAAATAGGGCTTCCTT	Forward Surveyor primer for CR6 off-target 4
	316 CR6-CellI-OT4-R	TGTTAAGTTTGGGCTTATGTTCCCT	Reverse Surveyor primer for CR6 off-target 4
	317 CR6-CellI-OT5-F	CACAAGTCTCACTGCACAAACAT	Forward Surveyor primer for CR6 off-target 5
	318 CR6-CellI-OT5-R	TGACCCATGATTATCTCTCTTTGA	Reverse Surveyor primer for CR6 off-target 5
	319 CR6-CellI-OT6-F	TTCAGCTTCTGATTGGTTTTAATG	Forward Surveyor primer for CR6 off-target 6
	320 CR6-CellI-OT6-R	CCAATTCCTTAATTTCCCTACAG	Reverse Surveyor primer for CR6 off-target 6
	321 CR6-CellI-OT7-F	ATCTCAGACCAGGAGGGAGAC	Forward Surveyor primer for CR6 off-target 7
	322 CR6-CellI-OT7-R	CCTCAGGGTCAGTACATTTTTCAG	Reverse Surveyor primer for CR6 off-target 7
	323 CR6-CellI-OT8-F	TTCTTAGGACATTGCTCCACATAC	Forward Surveyor primer for CR6 off-target 8
	324 CR6-CellI-OT8-R	GCAAAACATAATGCAACTCGTAATC	Reverse Surveyor primer for CR6 off-target 8
	325 CR6-CellI-OT9-F	GCAAGGGAGTCTGTGTCTTTG	Forward Surveyor primer for CR6 off-target 9
	326 CR6-CellI-OT9-R	TCATTTAAGTGGCTGTTCTGTGTT	Reverse Surveyor primer for CR6 off-target 9
	327 CR6-CellI-OT10-F	ACAAAACAGAGAGAAAAGGCAGAG	Forward Surveyor primer for CR6 off-target 10
	328 CR6-CellI-OT10-R	GTTTTGATTTCTGGTGCTACAG	Reverse Surveyor primer for CR6 off-target 10
	329 CR36-CellI-OT1-F	ACTGAAGCTGAAGCCCAGTC	Forward Surveyor primer for CR36 off-target 1
	330 CR36-CellI-OT1-R	ACATGAGCTCTCAGGTTTCTGAC	Reverse Surveyor primer for CR36 off-target 1
	331 CR36-CellI-OT2-F	TCAAACCTAGATGGTTCCTATGTT	Forward Surveyor primer for CR36 off-target 2
	332 CR36-CellI-OT2-R	GTACCCTGAAAATGTAGGGTGACT	Reverse Surveyor primer for CR36 off-target 2
	333 CR36-CellI-OT3-F	CACTTCCCAAGTGAGGCAAT	Forward Surveyor primer for CR36 off-target 3

TABLE 5-continued

Sd EQ ID NO.	Primer name	Primer sequence	Notes
334	CR36-CellI-OT3-R	CTATACTTGGGGCTGACTTGCTAC	Reverse Surveyor primer for CR36 off-target 3
335	CR36-CellI-OT4-F	TCGTATAGGTTACTTTGGCTCACA	Forward Surveyor primer for CR36 off-target 4
336	CR36-CellI-OT4-R	AGGGATCTTTACTCCTCAGTGTGT	Reverse Surveyor primer for CR36 off-target 4
337	CR36-CellI-OT5-F	TGTAGAAGTTGGAATATCCTGCTG	Forward Surveyor primer for CR36 off-target 5
338	CR36-CellI-OT5-R	GTCAACAATTTGATCTCAGGCTTC	Reverse Surveyor primer for CR36 off-target 5
339	CR36-CellI-OT6-F	CTCAGTACTAAAGATGGACGCTTG	Forward Surveyor primer for CR36 off-target 6
340	CR36-CellI-OT6-R	AATCATTTTCAGTCTTCCCAACAAT	Reverse Surveyor primer for CR36 off-target 6
341	CR36-CellI-OT7-F	GGGAATCACAGTAGATGTTTGTCA	Forward Surveyor primer for CR36 off-target 7
342	CR36-CellI-OT7-R	AGACCAGGAGGTAAGAACATTTTG	Reverse Surveyor primer for CR36 off-target 7
343	CR36-CellI-OT8-F	CCACATAGAAAAGAGACTTGCAGAA	Forward Surveyor primer for CR36 off-target 8
344	CR36-CellI-OT8-R	AGAGATGCCAAAAGAACAGTCAAT	Reverse Surveyor primer for CR36 off-target 8
345	CR36-CellI-OT9-F	TGTGCCTTAGGCTATGTAACTGT	Forward Surveyor primer for CR36 off-target 9
346	CR36-CellI-OT9-R	AAACCCTTGTAACCAAAATTACCA	Reverse Surveyor primer for CR36 off-target 9
347	CR36-CellI-OT10-F	TAACTGCATCAGAAGTCCTTGCTA	Forward Surveyor primer for CR36 off-target 10
348	CR36-CellI-OT10-R	GGAGACCAAGCTGTAAAGTCA	Reverse Surveyor primer for CR36 off-target 10
349	CellI-CR3-F-nested	GTGGTGccgcggGAGTTGGCTCAAAT TGTTACTCTT	Nested PCR first round primers
350	CellI-CR3-R-nested	GTGGTGccgcggGGGAAATGGTCTAGG AGAGTAAAGT	Nested PCR first round primers
351	CellI-CR1-F-nested	GTGGTGccgcggGAGAGTTATGTGGC TTTACCA	Nested PCR first round primers
352	CellI-CR1-R-nested	GTGGTGccgcggCTCATTCATGCCTG GACA	Nested PCR first round primers
353	CellI-CR5-F-nested	GTGGTGccgcggCGGGCTTGACAGAA CTTAC	Nested PCR first round primers
354	CellI-CR5-R-nested	GTGGTGccgcggCTGCGTAGTGCCAAA ACAAA	Nested PCR first round primers
355	CellI-CR6-F-nested	GTGGTGccgcggTAATTTTCATTGAAGA GTGGCTGAA	Nested PCR first round primers
356	CellI-CR6-R-nested	GTGGTGccgcggAAGCCCTGTGTGGTA GTAGTCAGT	Nested PCR first round primers
357	CellI-CR36-F-nested	GTGGTGccgcggCAAGTCAGAAGTCAC TTGCTTTGT	Nested PCR first round primers

TABLE 5-continued

Sd EQ ID	Primer name	Primer sequence	Notes
358	Cell-CR36-R-nested	GTGGTGccgcggTTTTATGTGCAGGAA TCAGTCTGT	Nested PCR first round primers
359	CR3-Cell-OT1-F-nested	GTGGTGccgcggGTGTGCTTCTGTACA CATCATCT	Nested PCR first round primers
360	CR3-Cell-OT1-R-nested	GTGGTGccgcggAGATTCAACCCTCA AAAACGTAG	Nested PCR first round primers
361	CR1-Cell-OT1-F-nested	GTGGTGccgcggTTGGGAACATCAGAG AAAGTATGA	Nested PCR first round primers
362	CR1-Cell-OT1-R-nested	GTGGTGccgcggACAAATTACAGTCTC CTGGGAAAG	Nested PCR first round primers
363	CR36-Cell-OT3-F-nested	GTGGTGccgcggCACTTCCCAAGTGAG GCAAT	Nested PCR first round primers
364	CR36-Cell-OT3-R-nested	GTGGTGccgcggCTATACTTGGGGCTG ACTTGCTAC	Nested PCR first round primers
365	CR3-P1/P3-F	GTGGTGccgcggTTGGCTCTTTAGCTTG TGTTTC	Nested PCR second round primers
366	CR3-P1/P3-R	GTGGTGccgcggTGAGACTCCCAAAGG CAATC	Nested PCR second round primers
367	CR3-P1/P4-F	GTGGTGccgcggTTGGCTCTTTAGCTTG TGTTTC	Nested PCR second round primers
368	CR3-P1/P4-R	GTGGTGccgcggACTGAGGGGTGATCT TGGTG	Nested PCR second round primers
369	CR3-P2/P3-F	GTGGTGccgcggGCAGAGAAAGCCAGT CGGTA	Nested PCR second round primers
370	CR3-P2/P3-R	GTGGTGccgcggTGAGACTCCCAAAGG CAATC	Nested PCR second round primers
371	CR3-P2/P4-F	GTGGTGccgcggGCAGAGAAAGCCAGT CGGTA	Nested PCR second round primers
372	CR3-P2/P4-R	GTGGTGccgcggACTGAGGGGTGATCT TGGTG	Nested PCR second round primers
373	CR1-P1/P5-F	GTGGTGccgcggCCAGAGTTCTAGGG CAGAG	Nested PCR second round primers
374	CR1-P1/P5-R	GTGGTGccgcggAGCTAGTCCCCACAT TCCAC	Nested PCR second round primers
375	CR1-P1/P6-F	GTGGTGccgcggCCAGAGTTCTAGGG CAGAG	Nested PCR second round primers
376	CR1-P1/P6-R	GTGGTGccgcggGGTGGAGGAACTT TAGGC	Nested PCR second round primers
377	CR1-P2/P5-F	GTGGTGccgcggCTCATCTCATGCCTG GACA	Nested PCR second round primers
378	CR1-P2/P5-R	GTGGTGccgcggAGCTAGTCCCCACAT TCCAC	Nested PCR second round primers
379	CR1-P2/P6-F	GTGGTGccgcggTCTCATGCCTGGACA AGTAACT	Nested PCR second round primers
380	CR1-P2/P6-R	GTGGTGccgcggGGTGGAGGAACTT TAGGC	Nested PCR second round primers
381	CR5-P3/P5-F	GTGGTGccgcggGGCTTGACAGAACT TACCG	Nested PCR second round primers

TABLE 5-continued

Sd EQ ID	NO. Primer name	Primer sequence	Notes
	382 CR5-P3/P5-R	GTGGTGccgcggCACCCTGTCTGCCT AAGGA	Nested PCR second round primers
	383 CR5-P4/P6-F	GTGGTGccgcggGGCTTGGACAGAACT TACCG	Nested PCR second round primers
	384 CR5-P4/P6-R	GTGGTGccgcggGGTGGAGGAACTT TAGGC	Nested PCR second round primers
	385 CR5-P3/P5-F	GTGGTGccgcggCGTAGTGCCAAAACA AACAGT	Nested PCR second round primers
	386 CR5-P3/P5-R	GTGGTGccgcggCACCCTGTCTGCCT AAGGA	Nested PCR second round primers
	387 CR5-P4/P6-F	GTGGTGccgcggCGTAGTGCCAAAACA AACAGT	Nested PCR second round primers
	388 CR5-P4/P6-R	GTGGTGccgcggGGTGGAGGAACTT TAGGC	Nested PCR second round primers
	389 CR6-P1/P5-F	GTGGTGccgcggGCGAGGGCTACTTG ATATG	Nested PCR second round primers
	390 CR6-P1/P5-R	GTGGTGccgcggCTTCCAAGTGAGGC AATGC	Nested PCR second round primers
	391 CR6-P1/P6-F	GTGGTGccgcggACGTTTTGTGCTGCTG TAACA	Nested PCR second round primers
	392 CR6-P1/P6-R	GTGGTGccgcggCTGCAGGCACATTCT CTTCC	Nested PCR second round primers
	393 CR6-P2/P5-F	GTGGTGccgcggGCCCTGTGTGGTAGT AGTCA	Nested PCR second round primers
	394 CR6-P2/P5-R	GTGGTGccgcggCTTCCAAGTGAGGC AATGC	Nested PCR second round primers
	395 CR6-P2/P6-F	GTGGTGccgcggCAGTATTAAGGGGTG GGAGCT	Nested PCR second round primers
	396 CR6-P2/P6-R	GTGGTGccgcggTCTCTTCTCACACAG CTGA	Nested PCR second round primers
	397 CR36-P3/P5-F	GTGGTGccgcggGGAGCTTGAGGGAA GAGAA	Nested PCR second round primers
	398 CR36-P3/P5-R	GTGGTGccgcggCTTCCAAGTGAGGC AATGC	Nested PCR second round primers
	399 CR36-P4/P6-F	GTGGTGccgcggATGGATGGGAAGAC ACTGG	Nested PCR second round primers
	400 CR36-P4/P6-R	GTGGTGccgcggCTGCAGGCACATTCT CTTCC	Nested PCR second round primers
	401 CR36-P3/P5-F	GTGGTGccgcggGGATGAAACAGGGCA GGAAC	Nested PCR second round primers
	402 CR36-P3/P5-R	GTGGTGccgcggTTCCAAGTGAGGCA ATGC	Nested PCR second round primers
	403 CR36-P4/P6-F	GTGGTGccgcggTTTGACAGCCATGA TGAGG	Nested PCR second round primers
	404 CR36-P4/P6-R	GTGGTGccgcggCGACAGCCAAAACAG CCG	Nested PCR second round primers

[0333] Western blot analysis. To assess dystrophin protein expression, immortalized myoblasts were differentiated into myofibers by replacing the growth medium with DMEM supplemented with 1% insulin-transferrin-selenium (Invitrogen) and 1% antibiotic/antimycotic (Invitrogen) for 4-7 days, such as 6 or 7 days. Fibroblasts were transdifferentiated into myoblasts by inducing MyoD overexpression and incubating the cells in DMEM supplemented with 1% insulin-transferrin-selenium (Invitrogen), 1% antibiotic/antimycotic (Invitrogen) and 3 µg/mL doxycycline for 15 days. Dystrophin expression was assessed at 3 days after transfecting HEK293T cells. Cells were trypsinized, collected and lysed in RIPA buffer (Sigma) supplemented with a protease inhibitor cocktail (Sigma) and the total protein amount was quantified using the bicinchoninic acid assay according to the manufacturer's instructions (Pierce). Samples were then mixed with NuPAGE loading buffer (Invitrogen) and 5% β-mercaptoethanol and heated to 85° C. for 10 minutes. Twenty-five micrograms of protein were separated on 4-12% NuPAGE Bis-Tris gels (Invitrogen) with MES buffer (Invitrogen). Proteins were transferred to nitrocellulose membranes for 1-2 hrs in transfer buffer containing 10-20% methanol, such as 10% methanol, and 0.01% SDS. The blot was then blocked for 1 hr with 5% milk-TBST at room temperature. Blots were probed with the following primary antibodies: MANDYS8 to detect dystrophin (1:100, Sigma D8168) and rabbit anti-GAPDH (1:5000, Cell Signaling 2118S). Blots were then incubated with mouse or rabbit horseradish peroxidase-conjugated secondary antibodies (Santa Cruz) and visualized using the ChemiDoc chemiluminescent system (BioRad) and Western-C ECL substrate (BioRad).

[0334] Transplantation into immunodeficient mice. All animal experiments were conducted under protocols approved by the Duke Institutional Animal Care & Use Committee. Cells were trypsinized, collected and washed in 1× Hank's Balanced Salt Solution (HBSS, Sigma). Two million cells were pelleted and resuspended in five µL 1×HBSS (Sigma) supplemented with cardiotoxin (Sigma #C9759) immediately prior to injection. These cells were transplanted into the hind limb tibialis anterior (TA) muscle of NOD.SCID.gamma (NSG) mice (Duke CCIF Breeding Core) by intramuscular injection. Four weeks after injection, mice were euthanized and the TA muscles were harvested.

[0335] Immunofluorescence staining. Harvested TA muscles were incubated in 30% glycerol overnight at 4° C. before mounting and freezing in Optimal Cutting Tempera-

ture compound. Serial 10 micron sections were obtained by cryosectioning of the embedded muscle tissue at -20° C. Cryosections were then washed in PBS to remove the OCT compound and subsequently blocked for 30-60 minutes at room temperature in PBS containing 10% heat-inactivated fetal bovine serum for spectrin detection or 5% heat-inactivated fetal bovine serum for dystrophin detection. Cryosections were incubated overnight at 4° C. with the following primary antibodies that are specific to human epitopes only: anti-spectrin (1:20, Leica NCL-SPEC1) or anti-dystrophin (1:2, Leica NCL-DYS3). After primary staining, spectrin or dystrophin expression was detected using a tyramide-based immunofluorescence signal amplification detection kit (Life Technologies, TSA Kit #22, catalog #T-20932,). Briefly, cryosections were incubated with 1:200 goat anti-mouse biotin-XX secondary (Life Technologies #B2763) in blocking buffer for 1 hr at room temperature. The signal was then amplified using streptavidin-HRP conjugates (1:100, from TSA Kit) in blocking buffer for 1 hr at room temperature. Finally, cryosections were incubated with tyramide-AlexaFluor488 conjugates (1:100, TSA kit) in manufacturer-provided amplification buffer for 10 minutes at room temperature. Stained cryosections were then mounted in ProLong AntiFade (Life Technologies #P36934) and visualized with conventional fluorescence microscopy.

[0336] Cytotoxicity assay. To quantitatively assess potential sgRNA or SpCas9 nuclease-associated cytotoxicity, HEK293T cells were transfected with 10 ng of a GFP reporter and 100 ng SpCas9 expression vector and 100 ng sgRNA expression vector using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). The percentage of GFP positive cells was assessed at 2 and 5 days by flow cytometry. The survival rate was calculated as the decrease in GFP positive cells from days 2 to 5 and normalized to cells transfected with an empty nuclease expression vector as described (Cornu et al., Meth Mol Biol 649:237-245 (2010)).

Example 4

CRISPRs Targeting the Dystrophin Gene—Results

[0337] The CRISPR/Cas9-based system was designed to target the dystrophin gene. Various gRNAs were chosen to target different regions of the human and mouse dystrophin gene based on NNNNN NNNNN NNNNN NNNNN NGG (SEQ ID NO: 677) and GNNNN NNNNN NNNNN NNNNN NGG (SEQ ID NO: 678) (see Tables 6, 7 and 8).

TABLE 6

Name (SEQ ID NO)	Species	Gene	Target	Strand	19 bp for chimeric (add G on 5' end)	PAM
DCR1 (628)	Human	DMD	Intron 50	+	attggtcttgatttcacct (SEQ ID NO: 65)	GGG
DCR2 (66)	Human	DMD	Intron 50	-	tgttagagtaagtcagccta (SEQ ID NO: 679)	TGG
DCR3 (629)	Human	DMD	Exon 51-55'	+	cctactcagactgttactc (SEQ ID NO: 67)	TGG
DCR4 (68)	Human	DMD	Exon 51-53'	+	ttggacagaaacttaccgac (SEQ ID NO: 680)	TGG

TABLE 6-continued

Name (SEQ ID NO)	Species	Gene	Target	Strand	19 bp for chimeric (add G on 5' end)	PAM
DCR5 (630)	Human	DMD	Intron 51	-	cagttgcctaagaactggt (SEQ ID NO: 69)	GGG
DCR6 (631)	Human	DMD	Intron 44	-	GGGCTCCACCCTCACGAG T (SEQ ID NO: 70)	GGG
DCR7 (71)	Human	DMD	Intron 55	+	TTTGCTTCGCTATAAAACG (SEQ ID NO: 681)	AGG
DCR8 (72)	Human	DMD	Exon 41	+	TCTGAGGATGGGGCCGCA A (SEQ ID NO: 682)	TGG
DCR9 (73)	Human	DMD	Exon 44	-	GATCTGTCAAATCGCCTGC (SEQ ID NO: 683)	AGG
DCR10 (74)	Human	DMD	Exon 45	+	CCAGGATGGCATTGGGCA G (SEQ ID NO: 684)	CGG
DCR11 (75)	Human	DMD	Exon 45	+	CTGAATCTGCGGTGGCAG G (SEQ ID NO: 685)	AGG
DCR12 (76)	Human	DMD	Exon 46	-	TTCTTTTGTCTTCTAGCc (SEQ ID NO: 686)	TGG
DCR13 (77)	Human	DMD	Exon 46	+	GAAAAGCTTGAGCAAGTC A (SEQ ID NO: 687)	AGG
DCR14 (78)	Human	DMD	Exon 47	+	GAAGAGTTGCCCTGCGC C (SEQ ID NO: 688)	AGG
DCR15 (79)	Human	DMD	Exon 47	+	ACAAATCTCCAGTGGATA A (SEQ ID NO: 689)	AGG
DCR16 (80)	Human	DMD	Exon 48	-	TGTTTCTCAGGTAAAGCTC (SEQ ID NO: 690)	TGG
DCR17 (81)	Human	DMD	Exon 48	+	GAAGGACCATTGACGTTa (SEQ ID NO: 691)	AGG
DCR18 (82)	Human	DMD	Exon 49	-	AACTGCTATTTCAGTTTCc (SEQ ID NO: 692)	TGG
DCR19 (83)	Human	DMD	Exon 49	+	CCAGCCACTCAGCCAGTG A (SEQ ID NO: 693)	AGG
DCR20 (84)	Human	DMD	Exon 50	+	gtatgcttttctgttaaag (SEQ ID NO: 694)	AGG
DCR21 (85)	Human	DMD	Exon 50	+	CTCCTGGACTGACCACTAT (SEQ ID NO: 695)	TGG
DCR22 (86)	Human	DMD	Exon 52	+	GAACAGAGGCGTCCCCAG T (SEQ ID NO: 696)	TGG
DCR23 (87)	Human	DMD	Exon 52	+	GAGGCTAGAACAATCATT A (SEQ ID NO: 697)	CGG
DCR24 (88)	Human	DMD	Exon 53	+	ACAAGAACACCTTCAGAA C (SEQ ID NO: 698)	CGG
DCR25 (89)	Human	DMD	Exon 53	-	GGTTTCTGTGATTTTCTTT (SEQ ID NO: 699)	TGG
DCR26 (90)	Human	DMD	Exon 54	+	GGCCAAAGACCTCCGCCA G (SEQ ID NO: 700)	TGG
DCR27 (91)	Human	DMD	Exon 54	+	TTGGAGAAGCATTATATA A (SEQ ID NO: 701)	AGG
DCR28 (92)	Human	DMD	Exon 55	-	TCGCTCACTACCctgcaa (SEQ ID NO: 702)	AGG

TABLE 6-continued

Name (SEQ ID NO)	Species	Gene	Target	Strand	19 bp for chimeric (add G on 5' end)	PAM
DCR29 (93)	Human	DMD	Exon 55	+	AAAAGAGCTGATGAAACA A (SEQ ID NO: 703)	TGG
DCR30 (94)	Human	DMD	5'UTR/Exon 1	+	TACTTTTTCaAAATGCTT (SEQ ID NO: 704)	TGG
DCR31 (95)	Human	DMD	Exon 51	+	gagatgatcatcaagcaga (SEQ ID NO: 705)	AGG
DCR32 (96)	Mouse	DMD	mdx mutation	+	ctttgaaagagcaaTaaaa (SEQ ID NO: 706)	TGG
DCR33 (97)	Human	DMD	Intron 44	-	CACAAAAGTCAAATCGGA A (SEQ ID NO: 707)	TGG
DCR34 (98)	Human	DMD	Intron 44	-	ATTTCATATAAGATTCGG (SEQ ID NO: 708)	AGG
DCR35 (99)	Human	DMD	Intron 55	-	CTTAAGCAATCCGAATC (SEQ ID NO: 709)	TGG
DCR36 (632)	Human	DMD	Intron 55	-	CCTTCTTTATCCCTATCG (SEQ ID NO: 100)	AGG
DCR40 (104)	Mouse	DMD	Exon 23	-	aggccaaacctcggttac (SEQ ID NO: 710)	NNGRR
DCR41 (105)	Mouse	DMD	Exon 23	+	TTCGAAAATTCAGgtaag (SEQ ID NO: 711)	NNGRR
DCR42 (106)	Mouse	DMD	Exon 23	+	gcagaacaggagataacag (SEQ ID NO: 712)	NNGRR
DCR43 (107)	Mouse	ACVR2B	Exon 1	+	gcggccctcgcccttctct (SEQ ID NO: 713)	ggggat
DCR48 (108)	Human	DMD	Intron 45	-	TAGTGATCGTGGATACGA G (SEQ ID NO: 714)	AGG
DCR49 (109)	Human	DMD	Intron 45	-	TACAGCCCTCGGTGTATAT (SEQ ID NO: 715)	TGG
DCR50 (110)	Human	DMD	Intron 52	-	GGAAGGAATTAAGCCCGA A (SEQ ID NO: 716)	TGG
DCR51 (111)	Human	DMD	Intron 53	-	GGAACAGCTTTTCGTAGTTG (SEQ ID NO: 718)	AGG
DCR52 (112)	Human	DMD	Intron 54	+	ATAAAGTCCAGTGTGAT C (SEQ ID NO: 719)	AGG
DCR53 (113)			Intron 54	+	AAAACCAGAGCTTCGGTC A (SEQ ID NO: 720)	AGG
DCR54 (114)	Mouse	Rosa26	ZFN region	+	GAGTCTTCTGGGCAGGCTT AAAGGCTAACC (SEQ ID NO: 720)	TGG
DCR55 (115)	Mouse	Rosa26 mRNA		-	TCGGGTGAGCATGTCTTTA ATCTACCTCGA (SEQ ID NO: 721)	TGG
DCR49 (116)	Human	DMD	Ex 51	-	gtgtcaccagagtaacagt (SEQ ID NO: 722)	ctgagt
DCR50 (117)	Human	DMD	Ex 51	+	tgatcatcaagcagaaggt (SEQ ID NO: 723)	atgag
DCR60 (118)	Mouse	DMD	Exon 23	+	AACTTCGAAAATTCAGgta (SEQ ID NO: 724)	agccgagg
DCR61 (119)	Mouse	DMD	Intron 22	+	gaaactcatcaaatatgcgt (SEQ ID NO: 725)	gttagtgt

TABLE 6-continued

Name (SEQ ID NO)	Species	Gene	Target	Strand	19 bp for chimeric (add G on 5' end)	PAM
DCR62 (120)	Mouse	DMD	Intron 22	-	tcatttacactaacacgcat (SEQ ID NO: 726)	atttgatg
DCR63 (121)	Mouse	DMD	Intron 22	+	gaatgaaactcatcaaatat (SEQ ID NO: 727)	gcgtgtta
DCR64 (122)	Mouse	DMD	Intron 23	-	tcataaatatctttgaagga (SEQ ID NO: 728)	ctctgggt
DCR65 (123)	Mouse	DMD	Intron 23	-	tgttttcataggaaaaatag (SEQ ID NO: 729)	gcaagttg
DCR66 (124)	Mouse	DMD	Intron 23	+	aattggaaaatgtgatggga (SEQ ID NO: 730)	aacagata
DCR67 (125)	Human	DMD	Exon 51	+	atgatcatcaagcagaaggt (SEQ ID NO: 731)	atgagaaa
DCR68 (126)	Human	DMD	Exon 51	+	agatgatcatcaagcagaag (SEQ ID NO: 732)	gtatgaga
DCR69 (127)	Human	DMD	Exon 51	-	cattttttctcataccttct (SEQ ID NO: 733)	gcttgatg
DCR70 (128)	Human	DMD	Exon 51	+	tctactcagactgttactc (SEQ ID NO: 734)	tggtgaca
DCR71 (129)	Human	DMD	Exon 51	-	acaggttggtgcaccagagt (SEQ ID NO: 735)	aacagtct
DCR72 (130)	Human	DMD	Exon 51	-	ttatcattttttctcatacc (SEQ ID NO: 736)	ttctgctt
DCR73 (131)	Human	DMD	Intron 51	-	ttgcctaagaactgggtggga (SEQ ID NO: 737)	aatggtct
DCR74 (132)	Human	DMD	Intron 51	-	aaacagttgcctaagaactg (SEQ ID NO: 738)	gtgggaaa
DCR75 (133)	Human	DMD	Intron 51	+	tttccaccagttcttaggc (SEQ ID NO: 739)	aactgttt
DCR76 (134)	Human	DMD	Intron 50	+	tggctttgatattccctaggg (SEQ ID NO: 740)	tccagctt
DCR77 (135)	Human	DMD	Intron 50	-	tagggaaatcaaagccaatg (SEQ ID NO: 741)	aaacgttc
DCR78 (136)	Human	DMD	Intron 50	-	gaccctagggaatcaaagc (SEQ ID NO: 742)	caatgaaa
DCR79 (137)	Human	DMD	Intron 44	-	TGAGGGCTCCACCCTCAC GA (SEQ ID NO: 743)	GTGGGTTT
DCR80 (138)	Human	DMD	Intron 44	-	AAGGATTGAGGGCTCCAC CC (SEQ ID NO: 744)	TCACGAGT
DCR81 (139)	Human	DMD	Intron 44	-	GCTCCACCCTCACGAGTG GG (SEQ ID NO: 745)	TTTGGTTC
DCR82 (140)	Human	DMD	Intron 55	-	TATCCCTATCGAGGAAA CC (SEQ ID NO: 746)	ACGAGTTT
DCR83 (141)	Human	DMD	Intron 55	+	GATAAAGAAGGCCTATTT CA (SEQ ID NO: 747)	TAGAGTTG
DCR84 (142)	Human	DMD	Intron 55	-	AGGCCTTCTTTATCCCCTA T (SEQ ID NO: 748)	CGAGGAAA
DCR85 (143)	Human	DMD	Intron 44	-	TGAGGGCTCCACCCTCAC GA (SEQ ID NO: 749)	GTGGGT

TABLE 6-continued

Name (SEQ ID NO)	Species	Gene	Target	Strand	19 bp for chimeric (add G on 5' end)	PAM
DCR86 (144)	Human	DMD	Intron 55	+	GATAAAGAAGGCCTATT CA (SEQ ID NO: 750)	TAGAGT

TABLE 7

Name	Notes	% Mod
DCR1	Delete exon 51	6.6
DCR2	Delete exon 51	10.3
DCR3	Frameshift	13
DCR4	Delete exon 51	11.9
DCR5	Delete exon 51	12.4
DCR6	As close to exon 44 as possible in intron 44 (in case of patient deletions)	16.1
DCR7	As close to exon 56 as possible in intron 55 (in case of patient deletions)	6.8
DCR8	Can correct exon 42-43 deletion (-1/+2) only, (-2/+1) is not correctable by this	17.3
DCR9	Skip exon 44 (5')	14.4
DCR10	Frameshift	14.9
DCR11	Correct downstream of exon 45	<1
DCR12	5' splice acceptor/frameshift	<1
DCR13	Correct downstream of exon 46	16.9
DCR14	Frameshift	17.2
DCR15	Correct downstream of exon 47	15.4
DCR16	Frameshift	11.5
DCR17	Correct downstream of exon 48	<1
DCR18	5' splice acceptor/frameshift	1.8
DCR19	Correct downstream of exon 49	33.7
DCR20	5' splice acceptor	14.9
DCR21	Correct downstream of exon 50	24.1

TABLE 7-continued

Name	Notes	% Mod
DCR22	Frameshift	25.9
DCR23	Correct downstream of exon 52	25.2
DCR24	Frameshift (can only correct +1 frame)	24.8
DCR25	Correct downstream of exon 53	2.6
DCR26	Frameshift	24.5
DCR27	Correct downstream of exon 54	13.4
DCR28	5' splice acceptor	21.6
DCR29	Correct downstream of exon 55	19.2
DCR30	Integrate minidys in exon 1	not tested
DCR31	Correct downstream of exon 51	18.9
DCR32	Delete stop codon	not tested
DCR33	Alternative to CR6	1.3
DCR34	Alternative to CR6	13.2
DCR35	Alternative to CR7	22.5
DCR36	Alternative to CR7	26.4
DCR40	Disrupt exon 23 5' splice donor (correct mdx mutation)	
DCR41	Disrupt exon 23 5' splice donor (correct mdx mutation)	
DCR42	Delete exon 53 mdx4cv mutation	
DCR43	Disrupt myostatin receptor	

TABLE 8

Name	Cas9	Notes	Cas9 used
DCR49	<i>S. Aureus</i>	Frameshift in exon 51	SaCas9 (from Zhang pX441) (NNGRRT PAM)
DCR50	<i>S. Aureus</i>	Disrupt 5' end of exon 51	SaCas9 (from Zhang pX441) (NNGRR PAM)
DCR60	<i>N. Meningitidis</i>	NNNNGANN Target 3' splice donor of exon 23 to bypass mdx mutation	NmCas9 (NNNNGANN PAM)
DCR61	<i>N. Meningitidis</i>	NNNNGNNT Delete exon 23 and bypass mdx mutation	NmCas9 (NNNNGNNT PAM)
DCR62	<i>N. Meningitidis</i>	NNNNGANN Delete exon 23 and bypass mdx mutation	NmCas9 (NNNNGANN PAM)
DCR63	<i>N. Meningitidis</i>	NNNNGTTN Delete exon 23 and bypass mdx mutation	NmCas9 (NNNNGTTN PAM)
DCR64	<i>N. Meningitidis</i>	NNNNGNNT Delete exon 23 and bypass mdx mutation	NmCas9 (NNNNGNNT PAM)
DCR65	<i>N. Meningitidis</i>	NNNNGTTN Delete exon 23 and bypass mdx mutation	NmCas9 (NNNNGTTN PAM)
DCR66	<i>N. Meningitidis</i>	NNNNGANN Delete exon 23 and bypass mdx mutation	NmCas9 (NNNNGANN PAM)
DCR67	<i>N. Meningitidis</i>	NNNNGANN Target 3' splice donor of exon 51 to skip exon	NmCas9 (NNNNGANN PAM)
DCR68	<i>N. Meningitidis</i>	NNNNGANN Target 3' splice donor of exon 51 to skip exon	NmCas9 (NNNNGANN PAM)

TABLE 8-continued

Name	Cas9	Notes	Cas9 used
DCR69	<i>N. Meningitidis</i>	NNNNGANN Target 3' splice donor of exon 51 to skip exon	NmCas9 (NNNNGANN PAM)
DCR70	<i>N. Meningitidis</i>	NNNNGANN Frameshift in exon 51	NmCas9 (NNNNGANN PAM)
DCR71	<i>N. Meningitidis</i>	NNNNGNNT Frameshift in exon 51	NmCas9 (NNNNGNNT PAM)
DCR72	<i>N. Meningitidis</i>	NNNNGNNT Target 3' splice donor of exon 51 to skip exon	NmCas9 (NNNNGNNT PAM)
DCR73	<i>N. Meningitidis</i>	NNNNGNNT Delete exon 51 (bind as close to DCR5 as possible)	NmCas9 (NNNNGNNT PAM)
DCR74	<i>N. Meningitidis</i>	NNNNGANN Delete exon 51 (bind as close to DCR5 as possible)	NmCas9 (NNNNGANN PAM)
DCR75	<i>N. Meningitidis</i>	NNNNGTTN Delete exon 51 (bind as close to DCR5 as possible)	NmCas9 (NNNNGTTN PAM)
DCR76	<i>N. Meningitidis</i>	NNNNGNNT Delete exon 51 (bind as close to DCR1/2 as possible)	NmCas9 (NNNNGNNT PAM)
DCR77	<i>N. Meningitidis</i>	NNNNGTTN Delete exon 51 (bind as close to DCR1/2 as possible)	NmCas9 (NNNNGTTN PAM)
DCR78	<i>N. Meningitidis</i>	NNNNGANN Delete exon 51 (bind as close to DCR1/2 as possible)	NmCas9 (NNNNGANN PAM)
DCR79	<i>N. Meningitidis</i>	NNNNGNNT Delete exons 45-55 - overlaps NNNNGTTN PAM, bind as close to DCR6 as possible	NmCas9 (NNNNGNNT PAM)
DCR80	<i>N. Meningitidis</i>	NNNNGANN Delete exons 45-55, bind as close to DCR6 as possible	NmCas9 (NNNNGANN PAM)
DCR81	<i>N. Meningitidis</i>	NNNNGTTN Delete exons 45-55, bind as close to DCR6 as possible	NmCas9 (NNNNGTTN PAM)
DCR82	<i>N. Meningitidis</i>	NNNNGNNT Delete exons 45-55, bind as close to DCR36 as possible - overlaps NNNNGTTN PAM	NmCas9 (NNNNGNNT PAM)
DCR83	<i>N. Meningitidis</i>	NNNNGTTN Delete exons 45-55, bind as close to DCR36 as possible	NmCas9 (NNNNGTTN PAM)
DCR84	<i>N. Meningitidis</i>	NNNNGANN Delete exons 45-55, bind as close to DCR36 as possible	NmCas9 (NNNNGANN PAM)
DCR85	<i>S. Aureus</i>	NNGRRT Delete exons 45-55, bind as close to DCR6 as possible	SaCas9 (from Zhang pX441) (NNGRRT PAM)
DCR86	<i>S. Aureus</i>	NNGRRT Delete exons 45-55, bind as close to DCR36 as possible	SaCas9 (from Zhang pX441) (NNGRRT PAM)

[0338] In particular, 400 ng of Cas9 was co-transfected into HEK 293T cells with either 400 ng of empty vector or gRNA that targets the region encompassing Exon 51, i.e., CR1, CR2, CR3, CR4, and CR5 (see FIG. 11B). Genomic DNA was harvested at 2 days post-transfection and analyzed using the Surveyor assay (see FIG. 11A and FIG. 11C).

[0339] The CRISPR/Cas9-based system was used in DMD 8036 (del148-50) cells to determine if the system could repair a mutant dystrophin gene. 5 µg of Cas9 was co-transfected into DMD 8036 (del148-50) cells with either 7.5 µg of empty vector or gRNA. In particular, 7.5 µg of CR1 ("DCR1"), 7.5 µg of CR5 ("DCR5"), 15 µg of CR3

("DCR3") or 7.5 µg of a combination of CR1 and CR5 (DCR1+DCR5) were used. Genomic DNA was harvested at 3 days post-transfection and analyzed using the Surveyor assay (FIG. 12) or PCR analysis across the entire locus (FIG. 13). This locus was amplified by PCR using primers flanking the region containing the genomic targets for CR1 and CR5 (the forward primer: 5'-gagaggttatgtggtttacca (SEQ ID NO:457), the reverse primer: 5'-ctgcgtagtgcacaaacaaa (SEQ ID NO:458)), resulting in a 1447 bp band for the wild-type locus or an expected size of approximately 630 bp for the deleted locus. After 7 days of differentiation, western blot of the treated cells shows expression of dystrophin protein (see FIG. 14).

Example 5

Targeting CRISPR/Cas9 to Hotspot Mutations in the Human Dystrophin Gene

[0340] To utilize the CRISPR/Cas9 gene editing platform for correcting a wide range of dystrophin mutations, dozens of sgRNAs targeted to the hotspot mutation region between exons 45-55 were created (FIGS. 16 A-16D). The *S. pyogenes* system that utilizes a human-codon optimized SpCas9 nuclease and a chimeric single-guide RNA (sgRNA) expression vector to guide efficient site-specific gene editing was used. Similar to Example 4 targeting exon 51 with TALENs, protospacers were selected to target the 5' and 3' ends of exons 45 through 55 which meet the 5'-NRG-3' PAM requirement of SpCas9. Small insertions or deletions created by NHEJ-based DNA repair within these exons can generate targeted frameshift mutations that address various dystrophin mutations surrounding each exon (FIGS. 16A-16B). For example, CR3 was designed to correct dystrophin mutations or deletions surrounding exon 51 by introducing small insertions or deletions in the 5' end of exon 51 to restore the downstream dystrophin reading frame (FIG. 16B). Additionally, sgRNAs were designed to employ the multiplex capability of the CRISPR/Cas9 system and specifically delete individual exons or a series of exons to restore the dystrophin reading frame, similar to the methods of oligonucleotide-based exon skipping. For this purpose, sgRNAs were targeted to the intronic regions surrounding exon 51 (FIG. 16C) or exons 45-55 (FIG. 16D). These sgRNAs were intentionally targeted to sites nearest to the downstream or upstream exon intended to be included in the resulting transcript to minimize the likelihood that the background patient deletion would include the intronic sgRNA target sites.

Example 6

Screening of sgRNAs Targeted to the Dystrophin Gene in Human Cells

[0341] Gene editing frequency in the human HEK293T cell line was assessed to rapidly determine different sgRNA targeting efficiencies. HEK293 Ts were transfected with constructs encoding human codon-optimized SpCas9 and the indicated sgRNA. Each sgRNA was designed to modify the dystrophin gene as indicated. The frequency of gene modification at day 3 or day 10 post-transfection was determined by the Surveyor assay. The ratio of measured Surveyor signal at day 3 and day 10 was calculated to quantify the stability of gene editing frequencies for each sgRNA in human cells. As quantified by the Surveyor assay 3 days post-transfection, 29/32 (~90%) of the sgRNAs tested were able to mediate highly efficient gene modification at the intended locus (Table 9, FIG. 17). The gene editing frequencies were stable for almost all of the sgRNAs (<25% signal change from day 3 to day 10, Table 9, FIG. 18), indicating that gene editing mediated by each individual sgRNA was well-tolerated. A notable exception is CR33, which had no detectable activity at day 10, although activity may be below the sensitivity of the Surveyor assay (est. ~1%).

TABLE 9

Measured activity of sgRNAs in human cells				
Target	sgRNA #	% modified alleles at day 3	% modified alleles at day 10	% change day 10/day 3
Multiplex deletion of exon 51				
Int 50	CR1	6.6	9.3	41.8
Int 50	CR2	10.3	14.0	36.2
Ex 51	CR4	11.9	14.4	21.3
Int 51	CR5	12.4	13.3	7.8
Multiplex deletion of exons 45-55				
Int 44	CR6	16.1	16.9	4.3
Int 44	CR33	1.3	<1	n.d.
Int 44	CR34	13.2	11.0	-16.6
Int 55	CR7	6.8	7.1	5.3
Int 55	CR35	22.5	20.9	-7.1
Int 55	CR36	26.4	24.7	-6.4
Targeted frameshifts				
Ex 45	CR10	14.9	16.3	9.3
Ex 45	CR11	<1	<1	n.d.
Ex 46	CR12	<1	<1	n.d.
Ex 46	CR13	16.9	18.4	9.2
Ex 47	CR14	17.2	17.6	2.9
Ex 47	CR15	15.4	15.3	-0.9
Ex 48	CR16	11.5	10.9	-5.0
Ex 48	CR17	<1	<1	n.d.
Ex 49	CR18	1.8	2.2	20.1
Ex 49	CR19	33.7	38.4	13.9
Ex 50	CR20	14.9	13.7	-7.6
Ex 50	CR21	24.1	20.8	-13.5
Ex 51	CR3	13.0	16.7	28.0
Ex 51	CR31	18.9	16.9	-10.2
Ex 52	CR22	25.9	20.3	-21.6
Ex 52	CR23	25.2	24.0	-4.8
Ex 53	CR24	24.8	23.6	-4.6
Ex 53	CR25	2.6	2.9	9.5
Ex 54	CR26	24.5	22.0	-10.1
Ex 54	CR27	13.4	12.6	-5.9
Ex 55	CR28	21.6	19.8	-8.4
Ex 55	CR29	19.2	19.6	2.2

Example 7

Enrichment of Gene-Edited Cells Using a Fluorescence-Based Reporter System

[0342] sgRNAs were selected to correct specific mutations in DMD patient myoblast cell lines. After transfection into DMD myoblasts, unexpectedly low or undetectable gene modification activity was observed as measured by the Surveyor assay (FIG. 19C, bulk population). Flow cytometry was used to select for transfected cells co-expressing GFP through a 2A ribosomal skipping peptide linked to the SpCas9 protein (FIG. 19A). The addition of this fluorescent reporter to the SpCas9 expression vector did not seem to significantly impact gene editing activity in HEK293T cells (FIG. 19B). A low percentage of transfected myoblasts (~0.5-2%) expressed the fluorescent reporter at 3 days after electroporation, despite high transfection efficiencies of control GFP expression plasmids (typically >70%, FIG. 19D, pmaxGFP). Given the high levels of CRISPR/Cas9 activity in the easily transfected HEK293T line, inefficient transgene expression after electroporation of SpCas9-T2A-GFP and sgRNA constructs into the DMD cells may explain the low observed gene editing efficiencies in unsorted cells. After sorting the GFP-positive DMD myoblasts, a substantial increase was observed in detectable activity at most sgRNA target loci (FIG. 19C). Therefore, all subsequent experiments used cells sorted for SpCas9 expression by expression of this fluorescent reporter.

Example 8

Restoration of Dystrophin Expression by Targeted Frameshifts

[0343] Small insertions and deletions created by NHEJ DNA repair may be used to create targeted frameshifts to correct aberrant reading frames. A sgRNA, CR3, was designed to restore the dystrophin reading frame by introducing small insertions and deletions within exon 51 (FIG. 16B and FIG. 20A). The types of insertions and deletions generated by CRISPR/Cas9 at this locus were assessed by Sanger sequencing of alleles from the genomic DNA of HEK293T cells co-transfected with expression plasmids for SpCas9 and the CR3 sgRNA (FIG. 20B). Notably, the insertions and deletions resulted in conversion to all three reading frames (FIG. 20B, FIG. 20C). To demonstrate genetic correction in a relevant patient cell line, expression plasmids for SpCas9 and the CR3 sgRNA were electroporated into a DMD myoblast line with a deletion of exons 48-50 that is correctable by creating frameshifts in exon 51. The treated cells were sorted, verified to have gene modification activity by the Surveyor assay (CR3, FIG. 19C sorted population), and differentiated into myotubes to test for restored dystrophin expression. Expression of dystrophin protein was observed concomitant with the detectable nuclease activity (FIG. 20D). The *S. pyogenes* CRISPR/Cas9 system presents a powerful method to quickly generate targeted frameshifts to address a variety of patient mutations and restore expression of the human dystrophin gene.

Example 9

Multiplex CRISPR/Cas9 Gene Editing Mediates Genomic Deletion of Exon 51 and Rescues Dystrophin Protein Expression

[0344] The multiplexing capability of the CRISPR/Cas9 system presents a novel method to efficiently generate genomic deletions of specific exons for targeted gene correction. DMD patient myoblasts with background deletions correctable by exon 51 skipping were treated with two combinations of sgRNAs flanking exon 51 (CR1/CR5 or CR2/CR5) and sorted to enrich for gene-edited cells as in FIGS. 19A-19D. As detected by end-point PCR of the genomic DNA from these treated cells, the expected genomic deletions were only present when both sgRNAs were electroporated into the cells with SpCas9 (FIG. 21A). Sanger sequencing confirmed the expected junction of the distal chromosomal segments (FIG. 21B) for both deletions. After differentiating the sorted myoblasts, a deletion of exon 51 from the mRNA transcript was detected only in the cells treated with both sgRNAs (FIG. 21C). Finally, restored dystrophin protein expression was detected in the treated cells concomitant with observed genome- and mRNA-level deletions of exon 51 (FIG. 21D).

Example 10

Dystrophin Rescue by a Multi-Exon Large Genomic Deletion

[0345] Although addressing patient-specific mutations is a powerful use of the CRISPR/Cas9 system, it would be advantageous to develop a single method that can address a myriad of common patient deletions. For example, a prom-

ising strategy is to exclude the entire exon 45-55 region as a method to correct up to 62% of known patient deletions. Multiplex CRISPR/Cas9-based gene editing was tested to determine if it may be able to generate efficient deletion of the exon 45-55 locus in human cells. After transfection into HEK293T cells, the expected deletion of ~336,000 bp was detected by PCR of the genomic DNA (FIG. 22A). Similarly, this deletion was detected by PCR of the genomic DNA from SpCas9/sgRNA-treated DMD patient cells harboring a background deletion of exons 48-50 of unknown length (FIG. 22A). Sanger sequencing of this deletion band from the genomic DNA of treated DMD cells revealed the expected junctions of intron 44 and intron 55 immediately adjacent to the sgRNA target sites (FIG. 22B). After differentiation of treated DMD cells, the expected deletion of exons 45-55 was detected in the dystrophin mRNA transcript and verified to be a fusion of exons 44 and 56 by Sanger sequencing (FIG. 22C). Restored protein expression was observed by western blot in the sorted cell populations containing the CRISPR/Cas9-induced deletion of exons 45-55 from the genome and resulting mRNA transcripts (FIG. 22D). These data demonstrate that multiplex CRISPR/Cas9 editing presents a single universal method to restore the dystrophin reading frame in more than 60% of DMD patient mutations.

Example 11

Transplantation of Corrected Myoblasts into Immunodeficient Mice

[0346] A promising method for DMD therapy is to correct a population of autologous patient muscle progenitor cells that can be engrafted into the patient's skeletal muscle tissue to rescue dystrophin expression. To demonstrate the ability of the corrected cells to express human dystrophin *in vivo*, a population of DMD myoblasts that were treated with sgRNAs CR1 and CR5, which flank exon 51, was transplanted and sorted for expression of GFP as before (FIGS. 19A-19D, FIG. 23). After 4 weeks, muscle fibers positive for human spectrin, which is expressed by both corrected and uncorrected cells, were detected in cryosections of injected muscle tissue (FIG. 24). A number of these fibers were also positive for human dystrophin with expression localized to the sarcolemma, demonstrating functional gene correction in these cells (FIG. 24, FIGS. 25A-25F). No fibers positive for human dystrophin were observed in sections from mice injected with the untreated DMD myoblasts (FIG. 24, FIGS. 25A-25F), indicating that the CRISPR/Cas9-modified cells were the source of human dystrophin expression.

Example 12

Off-Target and Cytotoxicity Analysis

[0347] The relative cytotoxicity of the CRISPR/Cas9 system was assessed in human cells for select sgRNAs by adapting a flow cytometry-based GFP retention assay as previously described (Ousterout et al., Mol Ther 21:1718-1726 (2013)). Minimal cytotoxicity was observed for SpCas9 co-expressed with or without sgRNAs after transfection into human cells (FIG. 26A). Publicly available tools are available to assess and prioritize potential CRISPR/Cas9 activity at off-target loci based on predicted positional bias of a given mismatch in the sgRNA protospacer sequence and the total number of mismatches to the intended target site

(Hsu et al., Nat Biotechnol 31:827-826 (2013)). This public webserver was used to predict the most likely off-target sites for the sgRNAs used to correct the dystrophin gene in this study (Table 4). The top ten potential off-target sites were assessed by the Surveyor assay in HEK293T cells treated with SpCas9 and individual sgRNA expression cassettes for CR1, CR3, CR5, CR6, or CR36. CR1, CR3 and CR36 each had one of these ten predicted off-target loci demonstrate significant levels of gene modification (Table 4 and FIG. 27). Interestingly, the CR3 off-target sequence had substantial homology and similar modification frequencies to the intended on-target (9.3% at OT-1 vs. 13.3% at intended site (Table 4 and FIG. 27). Notably, CR3-OT1 was the only one of these three off-target sites to show significant levels of activity in the sorted hDMD cells by the Surveyor assay (FIG. 26B).

[0348] Nuclease activity at off-target sites may cause unintended chromosomal rearrangements by distal re-ligation between cleaved target and off-target loci on distinct chromosomes. This presents a significant concern for deletion-based gene correction strategies due to the increased potential for off-target activity by using two or more nucleases, such as in multiplex CRISPR/Cas9 gene editing. Potential translocations were probed for using a highly sensitive nested genomic PCR assay to detect translocations at the validated off-target loci (Table 4) during both single and multiplex CRISPR/Cas9 editing strategies. Using this assay, translocations were readily detected between on-target and off-target sites in the model HEK293T cell line that also shows high levels of off-target activity (FIG. 26C and FIG. 28A, 28B). Sanger sequencing of the PCR amplicons confirmed the identity of the predicted translocation event for each primer pair (FIGS. 29-30). A subset of the translocations detected in the HEK293T cells were also detectable by nested PCR in the sorted hDMD myoblasts, although the signal was considerably weaker and the sequence identity was not confirmed due to low yield of product (FIG. 26D, FIG. 28A, and FIG. 28C). Translocations were not detected using this assay in HEK293T or sorted hDMD cells treated with CR6 or CR6/CR36, respectively, (FIGS. 28A-28C) that had low levels of off-target activity at CR6-OT3 only in HEK293T cells (Table 4). These results underscore the importance of selecting highly specific sgRNAs, particularly for multiplex editing applications, and show that this approach can benefit from ongoing efforts to improve the specificity of the CRISPR/Cas9 system. These data suggest that the selected sgRNAs are able to correct the dystrophin gene without significant toxicity and with only a single strongly predicted off-target site with detectable levels of activity.

Example 13

Discussion

[0349] Genome editing is a powerful tool for correcting genetic disease and the recent development of the CRISPR/Cas9 system is dramatically accelerating progress in this area. The correction of DMD, the most common genetic

disease that also currently has no approved therapeutic options, was demonstrated. Many gene- and cell-based therapies for DMD are in preclinical development and clinical trials, and genome editing methods are compatible with many of these approaches. For example, genome editing may be combined with patient-specific cell-based therapies for DMD. The CRISPR/Cas9 system may function in human pluripotent stem cells and other human cell lines, as well as human skeletal myoblasts, as shown. Importantly, gene editing with CRISPR/Cas9 did not abolish the myogenic capacity of these cells, as demonstrated by efficient dystrophin expression in vitro and in vivo after transplantation into immunodeficient mice. Thus, this strategy should be compatible with cell-based therapies for DMD.

[0350] Additionally, an enriched pool of gene-corrected cells demonstrated expression of human dystrophin in vivo following engraftment into immunodeficient mice. CRISPR/Cas9 gene editing did not have significant toxic effects in human myoblasts as observed by stable gene editing frequencies and minimal cytotoxicity of several sgRNAs. However, gene editing activity was confirmed at three out of 50 predicted off-target sites across five sgRNAs and CRISPR/Cas9-induced chromosomal translocations between on-target and off-target sites were detectable. The CRISPR/Cas9 technology is an efficient and versatile method for correcting a significant fraction of dystrophin mutations and can serve as a general platform for treating genetic disease.

[0351] Additionally, direct transfection of the sgRNA and Cas9 mRNA, in contrast to the plasmid-based delivery method used here, may be used to increase specificity and safety by reducing the duration of Cas9 expression and eliminating the possibility of random plasmid integration. Alternatively, delivery of the CRISPR/Cas9 system directly to skeletal and/or cardiac muscle by viral, plasmid, or RNA delivery vectors may be used for in vivo genome editing and translation of this approach. The large size of *S. pyogenes* Cas9 gene (~4.2 kilobases) presents a challenge to its use in size-restricted adeno-associated viral vectors. However, Cas9 genes from other species, such as *N. meningitidis* and *S. thermophilus*, are short enough to efficiently package both Cas9 and sgRNA expression cassettes into single AAV vectors for in vivo gene editing applications.

[0352] The CRISPR/Cas9 system enabled efficient modification of nearly 90% of tested targets, consistent with other reports of robust activity of this system at diverse loci. The robustness and versatility of this technology is a significant advancement towards at-will creation of patient-specific gene editing. Low levels of dystrophin, including as little as 4% of wild-type expression, may be sufficient to improve survival, motor function, and cardiac function in a mouse model. The levels of CRISPR/Cas9 activity may be sufficient for therapeutic benefit.

[0353] The use of multiplexing with CRISPR/Cas9 to delete exons also presents a unique set of opportunities and challenges. The deletion of complete exons from the genome to restore dystrophin expression was performed, in contrast to restoring the reading frame of the dystrophin gene with

small indels generated by NHEJ-based DNA repair following the action of a single nuclease. The protein product of the edited gene is predictable and already characterized in Becker muscular dystrophy patients with the naturally occurring deletion, in contrast to the random indels created by intraexonic action of a single nuclease that will lead to the creation of novel epitopes from each DNA repair event. Furthermore, the product resulting from the exon deletions will lead to restored dystrophin for every successful gene editing event, whereas modifying the gene with random indels within exons will only restore the reading frame in the one-third of editing events that leads to the correct reading frame.

[0354] All of the sgRNAs tested were not associated with significant cytotoxic effects in human cells. Three potential off-target sites out of 50 total tested sites for the five sgRNAs used were identified to restore dystrophin expression. Furthermore, chromosomal translocations between the intended on-target sites and these off-target sites were detectable by highly sensitive nested PCR assays in HEK293T cells expressing high levels of Cas9 and sgRNAs. Notably, the off-target activity and translocations identified in HEK293T cells, which is an immortalized and aneuploid cell line that expresses very high levels of Cas9 and sgRNA, did not occur at as high a level and in some cases were undetectable in the hDMD myoblasts. Importantly, this level of specificity may be tolerable given the severity of DMD, the lack of an apparent cytotoxic effect in human cells

Example 14

[0355] An engineered AAV capsid, termed SASTG (FIG. 34; SEQ ID NOs: 436 and 437), was developed for enhanced cardiac and skeletal muscle tissue tropism (Piacentino et al. (2012) *Human Gene Therapy* 23:635-646). A ZFN targeting the Rosa26 locus ("Rosa26 ZFN"; FIG. 33; SEQ ID NO: 434 and 435) was shown to be highly active in mouse cells (Perez-Pinera et al. *Nucleic Acids Research* (2012) 40:3741-3752). AAV-SASTG vectors encoding the Rosa26 ZFN protein were designed and subsequently generated and purified by the UNC Viral Vector Core. The Surveyor assay (Guschin et al., *Methods Mol Biol* 649, 247-256 (2010)) was used to demonstrate NHEJ mutagenesis at the Rosa26 locus following delivery of AAV-SASTG Rosa26 ZFNs in cultured C2C12 myoblasts that were actively cycling or forced into differentiation by serum removal (not shown).

[0356] To verify that adult post-mitotic skeletal muscle were efficiently targeted by the Rosa26 ZFN following AAV transfer, AAV-SASTG vectors encoding the Rosa26 ZFN were injected directly into the tibialis anterior (TA) muscle of 6 week old C57BL/6J mice at titers of 1×10^{10} vector genomes (vg) or 2.5×10^{10} vg per muscle. Mice were sacrificed 4 weeks after injection and the TA muscles were harvested and partitioned into several fragments for genomic DNA extraction and analysis (FIG. 31). Genomic DNA was PCR amplified and subjected to the Surveyor assay to detect NHEJ mutations characteristic of ZFN mutagenesis at the Rosa26 target site (FIGS. 32A-32C). FIGS. 32A-32C shows Surveyor analysis of Rosa26 ZFN activities in skeletal muscle in vitro and in vivo following delivery of AAV-SASTG-ROSA. Proliferating C2C12s were transduced with the indicated amount of virus and harvested at 4 days

post-infection (FIG. 32A). C2C12s were incubated in differentiation medium for 5 days and then transduced with the indicated amount of AAV-SASTG-ROSA virus in 24 well plates (FIG. 32B). Samples were collected at 10 days post-transduction. The indicated amount of AAV-SASTG-ROSA was injected directly into the tibialis anterior of C57BL/6J mice and muscles were harvested 4 weeks post-infection. The harvested TA muscles were partitioned into 8 separate pieces for genomic DNA analysis, each shown in a separate lane (FIG. 32C). Notably, high levels of gene modification were detected in all fragments at the highest dose (2.5×10^{10} vg).

Example 15

AAV-CRISPR Constructs for Targeting Mutant Dystrophin Genes

[0357] AAV constructs are designed to therapeutically correct mutations of the dystrophin gene that cause Duchenne muscular dystrophy and skeletal and cardiac muscle degeneration. CRISPR/Cas9 systems can be delivered using the AAV to restore the dystrophin reading frame by deleting exon 51, deleting exons 45-55, disrupting splice donor or acceptor sites, or creating frameshifts within exon 51 (Ousterout et al., *Molecular Therapy* 2013) to restore the dystrophin reading frame and protein expression. The CRISPR/Cas9 system will include a Cas9 having a sequence of SEQ ID NO: 64 or 114 (See FIGS. 40 and 41). gRNAs that could be combined with these Cas9s, targeting their respective PAM sequences, are provided (see FIGS. 40 and 41; see also Tables 2 and 3).

Example 16

Generation of Induced Neurons (iNs)

[0358] The generation of induced neurons (iNs) from other cell lineages has potential applications in regenerative medicine and the study of neurological diseases. The direct conversion of mouse embryonic fibroblasts (MEFs) to functional neuronal cells may occur through the delivery of a cocktail of three neuronal transcription factors—BRN2, ASCL1, and MYT1L (BAM factors, FIG. 48). Other methods may include additional factors to induce various neuronal subtypes. These experiments require transcription factors to be delivered ectopically, and the activation of the corresponding endogenous loci to sustain the neuronal phenotype. The CRISPR/Cas9 system was engineered as a versatile transcription factor to activate endogenous genes in mammalian cells with the capacity to target any promoter in the genome through an RNA-guided mechanism (FIGS. 49A-49B).

[0359] Materials & Methods. The CRISPR/Cas9-transcription factor was used to activate the endogenous genes encoding ASCL1 and BRN2 to directly reprogram MEFs to functional induced neurons.

[0360] Cell Culture: MEFs were seeded in either 24-well TCPS plates or on poly-D-lysine/laminin-coated coverslips. Following transduction of dCas9-VP64 and transfection of the gRNAs (see Tables 10 and 11 for sequences of gRNAs), the cells were cultured in MEF medium (Adler et al. *Mol Ther Nucleic Acids* 1:e32 (2012)) for 24 hrs and then transferred to N3 neural induction medium (Vierbuchen et al. *Nature* 463:1035-1041 (2010)) for the duration of the experiment (FIG. 49B).

TABLE 10

gRNAs for mouse ASCL1 (CR13):				
Oligo (5' to 3')	5' overhang	ASCL1 Target Sequence		SEQ ID NO
CR13-1_S:	cacc	G CAGCCGCTCGTCGAGCAG (SEQ ID NO: 468)		492
CR13-1_AS:	AAAC	CTGCTGCAGCGAGCGGCTG (SEQ ID NO: 469)	C	493
CR13-2_S:	cacc	G GCTGGGTGTCCTTGAAG (SEQ ID NO: 470)		494
CR13-2_AS:	AAAC	TTTCAATGGGACACCCAGC (SEQ ID NO: 471)	C	495
CR13-3_S:	cacc	G GTTTATTTCAGCCGGGAGTC (SEQ ID NO: 472)		496
CR13-3_AS:	AAAC	GACTCCCGGCTGAATAAAC (SEQ ID NO: 473)	C	497
CR13-4_S:	cacc	G TGGAGAGTTTGCAAGGAGC (SEQ ID NO: 474)		498
CR13-4_AS:	AAAC	GCTCCTTGCAAACTCTCCA (SEQ ID NO: 475)	C	499
CR13-5_S:	cacc	G CCCTCCAGACTTTCCACCT (SEQ ID NO: 476)		500
CR13-5_AS:	AAAC	AGGTGGAAAGTCTGGAGGG (SEQ ID NO: 477)	C	501
CR13-6_S:	cacc	G AATTTCTTCCAAGTTCTC (SEQ ID NO: 478)		502
CR13-6_AS:	AAAC	GAGAACTTGAAGAAAATT (SEQ ID NO: 479)	C	503
CR13-7_S:	cacc	G CTGCGGAGAGAAGAAAGGG (SEQ ID NO: 480)		504
CR13-7_AS:	AAAC	CCCTTTCTTCTCTCCGAG (SEQ ID NO: 481)	C	505
CR13-8_S:	cacc	G AGAGCCACCCCTGGCTCC (SEQ ID NO: 482)		506
CR13-8_AS:	AAAC	GGAGCCAGGGGTGGCTCT (SEQ ID NO: 483)	C	507
CR13-9_S:	cacc	G cgaagccaaccgcggcgagg (SEQ ID NO: 484)		508
CR13-9_AS:	AAAC	cccgcgcgggttggtcttcg (SEQ ID NO: 485)	C	509
CR13-10_S:	cacc	G agagggaagacgatcgccc (SEQ ID NO: 486)		510
CR13-10_AS:	AAAC	gggcgatcgtcttccctct (SEQ ID NO: 487)	C	511
CR13-11_S:	cacc	G cccctttaactttcctccg (SEQ ID NO: 488)		512
CR13-11_AS:	AAAC	cggaggaaagttaagggg (SEQ ID NO: 489)	C	513
CR13-12_S:	cacc	G gcagccccgcttcttctaa (SEQ ID NO: 490)		514
CR13-12_AS:	AAAC	ttgaaggaagcggggctgc (SEQ ID NO: 491)	C	515

TABLE 11

gRNAs for mouse BRN2 (CR16):				
Oligo (5' to 3')	5' overhang	BRN2 Target Sequence		SEQ ID NO
CR16-1_S:	cacc	G CGAGAGCGAGAGGAGGAG (SEQ ID NO: 516)		540
CR16-1_AS:	AAAC	CTCCTCTCTCGTCTCG (SEQ ID NO: 517)	C	541
CR16-2_S:	cacc	G GAGAGAGCTTGAGAGCGCG (SEQ ID NO: 518)		542
CR16-2_AS:	AAAC	CGCGCTCTCAAGCTCTCTC (SEQ ID NO: 519)	C	543
CR16-3_S:	cacc	G GGTGGAGGGGCGGGGCC (SEQ ID NO: 520)		544
CR16-3_AS:	AAAC	GGGCCCCGCCCTCCACC (SEQ ID NO: 521)	C	545

TABLE 11-continued

gRNAs for mouse BRN2 (CR16):				
Oligo (5' to 3')	5' overhang	BRN2 Target Sequence		SEQ ID NO
CR16-4_S:	cacc	G GGTATCCACGTAAATCAAA (SEQ ID NO: 522)		546
CR16-4_AS:	AAAC	TTTGATTACGTGGATACC (SEQ ID NO: 523)	C	547
CR16-5_S:	cacc	G CCAATCACTGGCTCCGGTC (SEQ ID NO: 524)		548
CR16-5_AS:	AAAC	GACCGGAGCCAGTGATTGG (SEQ ID NO: 525)	C	549
CR16-6_S:	cacc	G GGCGCCCGAGGGAAGAAGA (SEQ ID NO: 526)		550
CR16-6_AS:	AAAC	TCTTCTTCCCTCGGGCGCC (SEQ ID NO: 527)	C	551
CR16-7_S:	cacc	G GGGTGGGGGTACCAGAGGA (SEQ ID NO: 528)		552
CR16-7_AS:	AAAC	TCCTCTGGTACCCCCACCC (SEQ ID NO: 529)	C	553
CR16-8_S:	cacc	G CCGGGGACAGAAGAGAGGG (SEQ ID NO: 530)		554
CR16-8_AS:	AAAC	CCCTCTTCTGTCCCCGG (SEQ ID NO: 531)	C	555
CR16-9_S:	cacc	G gagagagagtgggagaagc (SEQ ID NO: 532)		556
CR16-9_AS:	AAAC	gcttctcccactctctctc (SEQ ID NO: 533)	C	557
CR16-10_S:	cacc	G aaagtaactgtcaaatgcg (SEQ ID NO: 534)		558
CR16-10_AS:	AAAC	cgcatttgacagttacttt (SEQ ID NO: 535)	C	559
CR16-11_S:	cacc	G ttaaccagagcgcccagtc (SEQ ID NO: 536)		560
CR16-11_AS:	AAAC	gactgggcgctctggttaa (SEQ ID NO: 537)	C	561
CR16-12_S:	cacc	G cgtcggagctgcccgctag (SEQ ID NO: 538)		562
CR16-12_AS:	AAAC	ctagcgggcagctccgacg (SEQ ID NO: 539)	C	563

[0361] qRT-PCR & IF: Activation of endogenous ASCL1 was assessed by qRT-PCR and immunofluorescence in MEFs on day 3 following delivery of either dCas9-VP64 and gRNAs, ASCL1 cDNA, or a negative control vector encoding luciferase. The generation of iNs was evaluated by TUJ1 and MAP2 co-staining and identification of cells with neuronal morphology and extended processes.

[0362] Live Cell Reporters: After 7-8 days in N3 medium, MEFs cultured on poly-D-lysine/laminin-coated coverslips were transduced with viruses carrying hSyn-RFP and MAP2-GCaMP5 reporters to identify the most mature iNs for functional characterization via calcium imaging and electrophysiology (FIG. 49B).

[0363] Results. dCas9-VP64 and gRNAs targeted to the ASCL1 promoter activated the endogenous gene in MEFs. Co-delivery of 8 gRNAs activated the endogenous gene 400-fold, a significant increase ($p < 0.05$) over the 100-fold activation induced by the co-delivery of 4 gRNAs (FIG. 50A). Nuclear-localized Ascl1 protein was detected by immunofluorescence in MEFs. Ectopic Ascl1 expression produced more Ascl1 protein than dCas9-VP64 with either gRNA cocktail, but did not activate the endogenous locus by day 3 (FIGS. 50A-50B). TUJ1 and MAP2 co-positive cells with extended processes were identified by immunofluorescence after 13 days in neurogenic medium following delivery of dCas9-VP64 and gRNAs targeting the ASCL1 and BRN2 promoters (FIG. 51A first row). A similar number of

TUJ1 and MAP2 co-positive cells were identified with ectopic expression of the BAM factors (FIG. 51A second row). Cells with a neuronal morphology expressing the hSyn-RFP reporter were visible in culture as early as day 11 in neurogenic medium (FIG. 51B). A cell expressing the MAP2-GCaMP5 calcium indicator exhibited KCl-induced depolarization detected with a fluorescent microscope (FIGS. 52A-52B).

[0364] The direct conversion of mouse embryonic fibroblasts to TUJ1 and MAP2 co-positive cells with a neuronal morphology was accomplished through activation of endogenous BRN2 and ASCL1 by CRISPR/Cas9-based transcription factors. Though dCas9-VP64 produces less protein than ectopic expression of ASCL1 (FIG. 50B), the generation of neuronal-like cells is similar. The activation of the endogenous loci may induce a reprogramming cascade of events that is not mechanistically identical to that generated with ectopic expression.

[0365] dCas9-VP64 was able to penetrate heterochromatin and activated stably silenced endogenous genes, a characteristic of only a subset of “pioneer” transcription factors. As a result, converting cell lineage with CRISPR/Cas9-transcription factors may better overcome epigenetic barriers to reprogramming than ectopic expression of transcription factors, particularly in hard-to-reprogram cell-types, such as adult human cells. This may have clinical importance in the

field of regenerative medicine, as it is often desired to use autologous sources in cell replacement therapies.

Example 17

Multiplex CRISPR/Cas9-Based Genome Engineering—Materials and Methods

[0366] Plasmid constructs. The expression cassettes for the *S. pyogenes* sgRNA and human codon optimized Cas9 (hCas9) nuclease were used, as described above. Additional promoters for mU6 (Ohshima et al., *Nucleic Acids Res* 9:5145-5158 (1981)), H1 (Myslinski et al., *Nucleic Acids Res* 29:2502-2509 (2001)), and 7SK (Murphy et al., *Cell* 51:81-87 (1987)) pol-III promoters were synthesized using GeneBlocks (IDT) and cloned in place of the hU6 sgRNA expression cassette. A GeneBlock (IDT) was cloned onto the 3' end of the Cas9 coding sequence to fuse a T2A skipping peptide and eGFP gene immediately after Cas9 to monitor vector expression. The coding region for hCas9-T2A-GFP (SEQ ID NO: 145) was then transferred into a lentiviral expression vector containing the human ubiquitin C (hUbc) promoter to drive expression of hCas9-T2A-GFP, as well as restriction sites to facilitate Golden Gate cloning of sgRNA expression cassettes immediately upstream of the hUbc promoter (FIG. 42A).

[0367] Protocol for assembly of custom lentiviral vectors. Assembly of custom lentiviral vectors expressing up to four sgRNAs of choice and active Cas9, dCas9, or dCas9-VP64 was accomplished in less than five days. The cloning method used the Golden Gate cloning and type IIS restriction enzymes that cleave outside their recognition sequence to create unique overhangs. Golden Gate assembly expedited cloning as all four expression cassettes were ligated into the final lentiviral vector in one step. The lentiviral vector expressed active Cas9, cCas9, or dCas9-VP64 in addition to one, two, three, or four sgRNAs expressed from independent promoters.

[0368] Step 1: Single stranded oligos containing each 20 bp protospacer were annealed in such a fashion to create sticky ends and were ligated into the desired pZDonor-promoter vector. Order two single stranded oligos for each desired genomic target. To anneal the complimentary oligos, mix 8 μ L sense oligo+8 μ L antisense oligo (both at 10 mM)+2 μ L 10 $\times$ ligase buffer. The oligos are melted and reannealed in a PCR machine with the program: 96° C. for 300 seconds, followed by 85° C. for 20 seconds, 75° C. for 20 seconds, 65° C. for 20 seconds, 55° C. for 20 seconds, 45° C. for 20 seconds, 35° C. for 20 seconds, and 25° C. for 20 seconds with a -0.3° C./second rate between steps. To phosphorylate the sticky ends, add 1 μ L 25 mM ATP+1 microliter T4 Polynucleotide Kinase (NEB) and incubate at 37° C. for 60 minutes followed by 65° C. for 20 minutes to heat inactivate the enzyme. Each protospacer was ligated into the desired expression vector using T4 DNA ligase (NEB) incubated at 16° C. for 60 minutes using 50 ng of vector and 1 μ L of annealed oligonucleotides in a 10 μ L reaction volume according to manufacturer's instructions. Five microliters of each ligation was transformed into XL1 blue chemically competent bacteria (Agilent) following the manufacturer's instructions. Plate transformation onto LB agar plates containing 50 μ g/mL kanamycin (Sigma) and incubate overnight at 37° C. In our experience, >90% of the colonies will contain the desired ligation product. Sequenc-

ing using the M13 reverse standard sequencing primer was performed to validate each final sgRNA construct prior to moving onto step 2.

[0369] Step 2: Construct the four promoter-gRNA cassettes into a lentiviral destination vector using Golden Gate assembly. After completion of step 1, there are four independent plasmids each expressing a different sgRNA from a different promoter. To assemble the four different promoter-sgRNA constructs into the desired destination vector, combine 200 ng of each sgRNA expression plasmid and desired lentiviral destination vector with 1 μ L of T4 DNA ligase (NEB), 1 μ L BsmBI FastDigest (Fisher Scientific), and 2 μ L 10 $\times$ T4 ligase buffer (NEB) in a 20 μ L reaction volume. Incubate the reaction as follows: 37° C. for 10 minutes, 16° C. for 15 minutes, 37° C. for 30 minutes, 80° C. for 5 minutes. Transform 5 μ L of ligation reaction into SURE 2 chemically competent cells (Agilent) following the manufacturer's instructions. Plate transformations onto LB agar plates containing 100 μ g/mL ampicillin and incubate overnight at 37° C. Optionally, colonies can be screened by lacZ-based blue/white screening using IPTG and X-gal; however, in our experience, >90% of the transformants contain the proper ligation product. Due to the inverted repeats formed by the opposing sgRNA expression cassettes, the final constructs may be unstable and thus we recommend maintaining these plasmids in the SURE 2 cell line and screening the final plasmid with a test PCR using the sense primer 5'-TCGGGTTTATTACAGGGACAGCAG-3' (SEQ ID NO:464) and antisense primer 5'-TCTAAGGCCGAGTCTTATGAGCAG-3' (SEQ ID NO:465). These primers amplify across the four promoter-gRNA region. Due to the repetitive nature, a distinct banding pattern should be observed with the largest product approximately 1800 bp in size.

[0370] Cell culture and transfection. HEK293T cells were obtained from the American Tissue Collection Center (ATCC, Manassas, Va.) through the Duke University Cancer Center Facilities and were maintained in DMEM supplemented with 10% FBS and 1% penicillin streptomycin. Primary human dermal fibroblasts (Catalog ID: GM03348) were obtained from Coriell Institute (Camden, N.J.) and were maintained in DMEM supplemented with 10% FBS and 1% penicillin streptomycin. All cells were cultured at 37° C. with 5% CO₂. HEK293T cells were transfected with Lipofectamine 2000 (Life Technologies) with 200 ng of each sgRNA expression vector (800 ng total pDNA) according to the manufacturer's protocol in 24 well plates.

[0371] Viral production and transduction. All lentiviral vectors used in this study are second generation and were produced using standard viral production methods. Briefly, 3.5 million HEK293 Ts were plated per 10 cm dish. The following day, cells were transfected by the calcium phosphate transfection method with 20 μ g of transfer vector, 6 μ g of pMD2G, and 10 μ g psPAX2. The media was changed 12-14 hrs post transfection. The viral supernatant was collected 24 and 48 hrs after this media change, passed through a 0.45 micron filter and pooled. For transduction, the cell medium was replaced with viral supernatant supplemented with 4 μ g/mL polybrene. The viral supernatant was exchanged for fresh medium 12-24 hrs later.

[0372] Reverse Transcription PCR. RNA was isolated using the miRNeasy Mini RNA isolation kit (Qiagen). DNase digestion was performed using the DNA-free Kit (Applied Biosystems). cDNA synthesis was performed

using the SuperScript VILO cDNA Synthesis Kit (Invitrogen). cDNA was PCR amplified using Taq DNA polymerase (NEB) and the resulting product was run on TAE agarose gels. Images were captured using a ChemiDoc XRS+ System and processed using ImageLab software (Bio-Rad).

[0373] Quantitative Real Time PCR. RNA was isolated using the RNeasy Plus RNA isolation kit (Qiagen). cDNA synthesis was performed using the SuperScript VILO cDNA Synthesis Kit (Invitrogen). Real-time PCR using PerfeCTa SYBR Green FastMix (Quanta Biosciences) was performed with the CFX96 Real-Time PCR Detection System (Bio-Rad). Primer specificity was confirmed by agarose gel electrophoresis and melting curve analysis. Reaction efficiencies over the appropriate dynamic range were calculated to ensure linearity of the standard curve. The results are expressed as fold-increase mRNA expression of the gene of interest normalized to β -Actin expression using the $\Delta\Delta Ct$ method. Reported values are the mean and S.E.M. from two independent experiments (n=2) where technical replicates were averaged for each experiment.

[0374] Western Blot. Cells were lysed in RIPA Buffer (Sigma) supplemented with protease inhibitor cocktail (Sigma). Protein concentration was measured using BCA protein assay reagent (Thermo Scientific) and BioTek Synergy 2 Multi-Mode Microplate Reader. Lysates were mixed with loading buffer and boiled for 5 min; 25 μ g of protein were run in NuPage 10% Bis-Tris Gel polyacrylamide gels (Bio-Rad) and transferred to nitrocellulose membranes. Nonspecific antibody binding was blocked with TBST (50 mM Tris, 150 mM NaCl and 0.1% Tween-20) with 5% nonfat milk for 1 hr at room temperature. The membranes were incubated with primary antibodies: (anti-MyoD (1:250, Santa Cruz Sc-32758) in 5% BSA in TBST overnight at 4° C.; anti-Myogenin (1:250, Santa Cruz Sc-12732) in 5% BSA overnight at 4° C.; anti-FLAG-HRP (1:1000, Cell Signaling 2044) in 5% milk in TBST for 60 min at room temperature; anti-GAPDH (1:5000, Cell Signaling, clone 14C10) in 5% milk in TBST for 30 min at room temperature. Membranes were then washed three times with TBST for 15 minutes total. Membranes were incubated with anti-rabbit HRP-conjugated antibody (Sigma, A 6154) or anti-mouse HRP-conjugated antibody (Santa Cruz, SC-2005) diluted 1:5,000 for 30 min and washed with TBST three times for 15 min each. Membranes were visualized using the ImmunoStar WesternC Chemiluminescence Kit (Bio-Rad) and images were captured using a ChemiDoc XRS+ System and processed using ImageLab software (Bio-Rad).

[0375] Cel-I quantification of endogenous gene modification. CRISPR/Cas9 nuclease lesions at the endogenous target site were quantified using the Surveyor nuclease assay, which can detect mutations characteristic of nuclease-mediated NHEJ. After transfection or transduction, cells were incubated for 3-10 days at 37° C. and genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen). The target locus was amplified by 35 cycles of PCR with the AccuPrime High Fidelity PCR kit (Invitrogen). The resulting PCR products were randomly melted and reannealed in a PCR machine with the program: 95° C. for 240 seconds, followed by 85° C. for 60 seconds, 75° C. for 60 seconds, 65° C. for 60 seconds, 55° C. for 60 seconds, 45° C. for 60 seconds, 35° C. for 60 seconds, and 25° C. for 60 seconds with a -0.3° C./second rate between steps. Following re-annealing, 8 μ L of PCR product was mixed with 1 μ L of Surveyor Nuclease S and 1 μ L of Enhancer S

(Transgenomic, Omaha, Nebr.) and incubated at 42° C. for 1 hr. After incubation, 6 μ L of digestion product was loaded onto a 10% TBE polyacrylamide gel and run at 200 V for 30 minutes. The gels were stained with ethidium bromide and quantified using ImageLab (Bio-Rad) by densitometry (Perez-Pinera et al., *Nucleic Acids Res* 40:3741-3751 (2012)).

[0376] Statistical Analysis. At least two independent experiments were compiled as means and standard errors of the mean. Effects were evaluated with multivariate ANOVA and Dunnett's post hoc test using JMP 10 Pro.

Example 18

Development of a Single Lentiviral Vector for Multiplex CRISPR/Cas9 Applications

[0377] A limitation of current CRISPR/Cas9 gene editing systems, particularly transactivator systems, is the simultaneous and efficient delivery of multiple sgRNAs and Cas9 protein for multiplex gene editing and synergistic gene activation applications, especially in difficult to transfect cell types. To overcome this limitation, we developed a single lentiviral vector that efficiently expresses Cas9 and up to four sgRNAs. In order to maximize the expression efficiency of each sgRNA, this vector expresses four sgRNAs from four independent pol III promoters (human U6 promoter, mouse U6 promoter, 7SK, and H1). We validated sgRNA expression from each promoter using end-point RT-PCR to detect a sgRNA targeting the AAVS1 locus (FIG. 42A). To test the activity of each sgRNA expression construct, we co-transfected each promoter construct expressing an sgRNA targeting AAVS1 independently with an active Cas9 expression construct into human HEK293T cells. Notably, we detected consistent and high levels of gene modification at the target locus for each sgRNA that are comparable to a well-characterized zinc-finger nuclease with high activity at the AAVS1 locus (FIG. 42B). Furthermore, lentiviral delivery of different Cas9-based constructs, including an active Cas9 nuclease, dead Cas9, and dead Cas9 fused to the VP64 transactivator domain, resulted in expression of full-length Cas9 protein in HEK293T cells as determined by western blot (FIG. 42C).

[0378] Using these components, we developed a Golden Gate cloning method to facilitate rapid and efficient cloning of multiple sgRNA expression cassettes into a single lentiviral vector expressing the desired Cas9 effector (FIG. 43). In the first step, oligonucleotides encoding sgRNA proto-spacer sequences are cloned independently into different expression vectors, each with a distinct promoter driving sgRNA expression. In the second step, each sgRNA expression construct is subcloned into a lentiviral Cas9 expression vector of choice by Golden Gate assembly. This strategy allows for robust and rapid cloning of up to four sgRNAs into a single lentiviral vector for gene editing or activation applications. To express less than four sgRNAs, a PolyT terminator sequence is cloned down-stream of unused promoters to prevent transcription from the unused promoters. Each vector co-expresses the choice Cas9 with eGFP via a 2A skipping peptide to enable fluorescence-activated flow sorting and enrichment of cells with a high multiplicity of infection. Finally, the entire region containing the sgRNA and Cas9 expression cassettes is flanked by loxP sites to mediate removal by Cre-lox excision.

Example 19

Validation of a Single Lentiviral sgRNA/Cas9
Expression Vector for Multiplex Genome
Engineering

[0379] To validate the independent activity of each sgRNA, we assembled a single lentiviral vector expressing active Cas9 and four sgRNAs, each targeting an independent loci (FIG. 44A). As control vectors, we assembled constructs expressing only one sgRNA along with polyT protospacers in the other three positions. We transduced HEK293 Ts and primary fibroblasts with lentiviral vectors expressing the indicated sgRNAs and monitored gene modification frequencies at 7 or 10 days post-transduction, respectively (FIG. 44B). In both cell types, the single lentiviral vector mediated highly efficient multiplex editing at all four loci (FIG. 44B). Interestingly, expression of all four sgRNAs together resulted in higher modification frequencies than a single sgRNA alone at 3 out of 4 loci in fibroblasts (FIG. 44B). We observed efficient multiplex gene editing in fibroblasts, which are conventionally a difficult to transfect cell type. These data demonstrate that a single lentivirus can express four active sgRNAs efficiently and that this lentiviral platform can be used to target four distinct loci for multiplex CRISPR/Cas9 gene editing.

Example 20

Transient RNA-Guided Gene Activation in Cell
Lines Stably Expressing a Lentiviral Cas9-Based
Transactivator

[0380] Next, we were interested in developing a system that enables transient gene activation by transfecting sgRNAs into model cell lines stably expressing Cas9. HEK293 Ts were transduced with different Cas9-T2A-GFP and GFP expression was monitored using flow cytometry. Following normal passaging every 2-3 days, each cell line exhibited stable GFP expression for up to 35 days post transduction. Transduced HEK293 Ts were then transfected with one to four separate sgRNA expression constructs targeting either the IL1RN or HBG1 promoter. Transient transfection of these sgRNA constructs in stable dCas9-VP64 expressing cells lines resulted in tunable endogenous gene activation (FIGS. 45A-45B). Gene activation following transient transfection of sgRNA constructs in cells expressing dCas9-VP64 reached a maximum level of activation approximately 3-6 post-transfection and fell to undetectable levels by 20 days

post-transfection (FIGS. 45C-45D). Furthermore, we were able to re-activate each gene by a second transfection of all four sgRNA constructs targeting each promoter, although activation levels were significantly lower than observed from the first transfection (FIGS. 45C-45D). This reduction in activity after the second transfection may be due to reduced vector expression or competitive growth of untransduced cells. Despite this, these data demonstrate that lentiviral Cas9 combined with transient sgRNA delivery can be used as a versatile system to tunably and transiently activate and re-activate target genes in a Cas9 stably transduced cell line.

Example 21

Stable Gene Activation in HEK293T Cells Using a
Single Lentiviral sgRNA/Cas9 Transactivator
Expression Vector

[0381] Lentiviral delivery may enable stable, long-term gene activation by CRISPR/Cas9 transactivation. To test this, HEK293 Ts were transduced using a single lentiviral vector encoding dCas9-VP64 and one to four sgRNA expression cassettes. Similar to our transient transfection results (FIGS. 45A-45D), we were able to tunably and robustly activate expression of endogenous IL1RN and HBG1 genes (FIGS. 46A-46B). Gene activation induced by co-transfection of HEK293T cells with dCas9-VP64 and four sgRNAs targeted to the IL1RN and HBG1 promoters peaked three-five days post-transfection and gene expression returned to background levels 15-20 days post-transfection (FIGS. 46A-46B). In contrast, lentiviral delivery of dCas9-VP64 and the same four IL1RN or HBG1-targeted sgRNAs induced sustained gene activation for more than 20 days post-transduction (FIGS. 46C-46D). Thus, single lentiviral delivery of multiplex dCas9-VP64 transactivators is a useful platform to efficiently and stably upregulate target endogenous genes.

Example 22

dCas9-KRAB—Targeting the HS2 Enhancer

[0382] The HS2 enhancer is a well-characterized distal regulatory element necessary for activation of the globin gene locus. dCas9-KRAB with gRNAs targeted to the HS2 enhancer were delivered to determine if this system would repress γ -, ϵ -, and β -globin expression in the K562 human erythroid leukemia cell line (FIG. 54). A panel of gRNAs was created targeting different sites along the core region of the HS2 enhancer SEQ ID NO: 467). See Table 12.

TABLE 12

HS2 gRNA Target Sequences	
Cr#Protospacer	Complete gRNA Sequence
1 gagacacacagaaatgtaac (SEQ ID NO: 564)	gagacacacagaaatgtaacgtttTAGAGCTAGAAATAGCAAGTTAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGG TGCTTTTTTCC (SEQ ID NO: 585)
2 ggtggggcactgaccccgac (SEQ ID NO: 565)	ggtggggcactgaccccgacgtttTAGAGCTAGAAATAGCAAGTTAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGG TGCTTTTTTCC (SEQ ID NO: 586)
3 ctagagtgatgactcctatc (SEQ ID NO: 566)	ctagagtgatgactcctatcgtttTAGAGCTAGAAATAGCAAGTTAAATA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGT GCTTTTTTCC (SEQ ID NO: 587)

TABLE 12-continued

HS2 gRNA Target Sequences	
Cr#Protospacer	Complete gRNA Sequence
4 gactaaaactccacctcaaa (SEQ ID NO: 567)	gactaaaactccacctcaaagtttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 588)
5 aatatgtcacattctgtctc (SEQ ID NO: 568)	aatatgtcacattctgtctcggttTAGAGCTAGAAATAGCAAGTTAAATAAG GCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT CTTTTTTTCC (SEQ ID NO: 589)
6 ggactatgggaggtcactaa (SEQ ID NO: 569)	ggactatgggaggtcactaagttttTAGAGCTAGAAATAGCAAGTTAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGG TGCTTTTTTTCC (SEQ ID NO: 590)
7 gctcatgcttgactatggg (SEQ ID NO: 570)	gctcatgcttgactatgggggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 591)
8 gttctggccaggccctgtc (SEQ ID NO: 571)	gttctggccaggccctgtcggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 592)
9 agtgcacccaccccgcttc (SEQ ID NO: 572)	agtgcacccaccccgcttcggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 593)
10 gtggggcactgaccccgaca (SEQ ID NO: 573)	gtggggcactgaccccgacagttTAGAGCTAGAAATAGCAAGTTAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGG TGCTTTTTTTCC (SEQ ID NO: 594)
11 aaccttctaagcaaaccttc (SEQ ID NO: 574)	aaaccttctaagcaaaccttcggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 595)
12 gttacacagaaccagaaggc (SEQ ID NO: 575)	gttacacagaaccagaaggcggttTAGAGCTAGAAATAGCAAGTTAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGG TGCTTTTTTTCC (SEQ ID NO: 596)
13 gaaggttacacagaaccaga (SEQ ID NO: 576)	gaaggttacacagaaccagagttTAGAGCTAGAAATAGCAAGTTAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGG TGCTTTTTTTCC (SEQ ID NO: 597)
14 agtcatgatgatgatgctg (SEQ ID NO: 577)	agtcatgatgatgatgctgggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 598)
15 gatgagtcagctgagggctt (SEQ ID NO: 578)	gatgagtcagctgagggcttggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 599)
16 actctaggctgagaacatct (SEQ ID NO: 579)	actctaggctgagaacatctggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 600)
17 gtccccagcaggatgcttac (SEQ ID NO: 580)	gtccccagcaggatgcttacggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 601)
18 gccctgtaagcatcctgctg (SEQ ID NO: 581)	gccctgtaagcatcctgctgggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 602)
19 cagggcagatggcaaaaaaa (SEQ ID NO: 582)	cagggcagatggcaaaaaagtttTAGAGCTAGAAATAGCAAGTTAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGG TGCTTTTTTTCC (SEQ ID NO: 603)
20 gaggtggagttttagtcagg (SEQ ID NO: 583)	gaggtggagttttagtcagggttTAGAGCTAGAAATAGCAAGTTAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGT GCTTTTTTTCC (SEQ ID NO: 604)
21 aaacggcatcataaagaaaa (SEQ ID NO: 584)	aaacggcatcataaagaaaagtttTAGAGCTAGAAATAGCAAGTTAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGG TGCTTTTTTTCC (SEQ ID NO: 605)

[0383] Screening single gRNAs at the globin locus by CRISPR/dCas9. dCas9 and dCas9-KRAB effectors were delivered lentivirally 5-8 days prior to electroporation with 5 µg of a plasmid encoding the U6-sgRNA expression (FIG. 55A). Cells that were not electroporated with gRNA (no gRNA) and cells treated with a gRNA targeting a different locus (IL1RN) were included as controls. Multiple gRNAs effected potent repression of ε-, γ-, and β-globin genes when assayed 3 days post-transfection, with up to 80% knock-down achieved (FIGS. 55B, 55C, 55D). Expressing a gRNA with either dCas9 or dCas9-KRAB inhibited gene expression at the globin locus. Generally, treatment with dCas9-KRAB resulted in stronger repression for a given gRNA compared to dCas9 alone, suggesting an important role for the KRAB domain in recruiting heterochromatin factors that enhance repression. The repression levels achieved are dependent on the amount of gRNA plasmid delivered by transfection only in the dCas9-KRAB treated cells (FIGS. 56A, 56B, 56C). Increasing the dose of Cr4 gRNA plasmid up to 10 µg increases silencing levels of the globin genes in dCas9-KRAB-treated cells.

[0384] Stable silencing of globin genes by dCas9-KRAB. dCas9/dCas9-KRAB were co-expressed with single gRNAs lentivirally in K562s (FIG. 57A). Cells that were not treated with lentivirus (NT), treated with dCas9/dCas9-KRAB without a gRNA (no gRNA), and with dCas9/dCas9-KRAB and gRNA targeting a different locus (IL1RN) were included as controls. Cells treated with lentivirus were selected from days 4 to 7. Multiple gRNAs effected potent transcriptional repression of ε-, γ-, and β-globin genes when assayed 7 days after transduction, with up to 95% knockdown achieved

(FIGS. 57B, 57C, 57D). Expression of β-globin was silenced the most in response gRNAs targeted to the HS2 enhancer. Treatment with dCas9-KRAB with a gRNA resulted in dramatically more repression than treatment with dCas9 and gRNA.

[0385] These findings demonstrate that dCas9-KRAB targeted to the HS2 enhancer by gRNAs effects potent repression of the distal globin genes. This is the first example of targeted epigenetic control of distal regulatory elements in mammalian cells by the CRISPR/Cas9 system. Enhancers regulate development and disease, and this disclosure provides a method to probe and control enhancer function and may be used to determine the effects of dCas9-KRAB on local chromatin accessibility and genome-wide expression.

Example 23

dCas9-p300

[0386] A dCas9-p300 fusion protein was designed and compared to dCas9-VP64 fusion protein (see FIG. 59). The amino acid constructs of dCas9 constructions are shown in FIGS. 61A-61C. Cells from the Human Embryonic Kidney tissue culture line HEK293T (ATCC; CRL-11268) were seeded at a density of 1.5e5 cells per well in 24-well tissue culture dishes one day prior to transfection with Lipofectamine 2000 transfection reagent (Life Technologies). 24 hrs later cells were transfected with 1 µL Lipofectamine 2000, 375 ng dCas9 expression construct (dCas9, dCas9VP64, or dCas9p300 respectively), and 125 ng of pooled gRNA expression plasmids (4 each at equimolar ratios). Table 13 shows the gRNA information.

TABLE 13

gRNA information				
Target Location	Protospacer Sequence (5'-3')	Genomic Location (GRCh38 Primary Assembly)	Reference	
IL1RN Promoter	TGTACTCTCTGAGGTGCTC (SEQ ID NO: 606)	Chr2: 113117865-113117883	Perez-Pinera et al., Nat Methods, 2013	
IL1RN Promoter	ACGCAGATAAGAACCAGTT (SEQ ID NO: 607)	Chr2: 113117714-113117732	Perez-Pinera et al., Nat Methods, 2013	
IL1RN Promoter	CATCAAGTCAGCCATCAGC (SEQ ID NO: 608)	Chr2: 113117781-113117799	Perez-Pinera et al., Nat Methods, 2013	
IL1RN Promoter	GAGTCACCCTCCTGGAAAC (SEQ ID NO: 609)	Chr2: 113117749-113117767	Perez-Pinera et al., Nat Methods, 2013	
MyoD Promoter	CCTGGGCTCCGGGCGTTT (SEQ ID NO: 610)	Chr11: 17719509-17719527	Perez-Pinera et al., Nat Methods, 2013	
MyoD Promoter	GGCCCTGCGGCCACCCCG (SEQ ID NO: 611)	Chr11: 17719422-17719440	Perez-Pinera et al., Nat Methods, 2013	
MyoD Promoter	CTCCCTCCCTGCCCCGTAG (SEQ ID NO: 612)	Chr11: 17719350-17719368	Perez-Pinera et al., Nat Methods, 2013	

TABLE 13-continued

gRNA information			
Target Location	Protospacer Sequence (5'-3')	Genomic Location (GRCh38 Primary Assembly)	Reference
MyoD Promoter	AGGTTTGGAAAGGCGTGC (SEQ ID NO: 613)	Chr11: 17719290-17719308	Perez-Pinera et al., Nat Methods, 2013
Oct4 Promoter	ACTCCACTGCACTCCAGTCT (SEQ ID NO: 614)	Chr6: 31170953-31170934	Hu et al., Nucleic Acids Res, 2014
Oct4 Promoter	TCTGTGGGGACCTGCACTG (SEQ ID NO: 615)	Chr6: 31170885-31170866	Hu et al., Nucleic Acids Res, 2014
Oct4 Promoter	GGGGCGCCAGTTGTGTCTCC (SEQ ID NO: 616)	Chr6: 31170855-31170836	Hu et al., Nucleic Acids Res, 2014
Oct4 Promoter	ACACCATTGCCACCACCAT (SEQ ID NO: 617)	Chr6: 31170816-31170797	Hu et al., Nucleic Acids Res, 2014

[0387] The 3 days post-transfection cells were harvested and assayed by RT-QPCR for mRNA expression. The RT-QPCR primer sequences are listed in Table 14.

TABLE 14

RT-QPCR Primers		
Primer Target	Primer Sequence (5'-3')	Reference
GAPDH Forward	CAATGACCCCTTCATTGACC (SEQ ID NO: 618)	Perez-Pinera et al., Nat Methods, 2013
GAPDH Reverse	TTGATTTTGGAGGGATCTCG (SEQ ID NO: 619)	Perez-Pinera et al., Nat Methods, 2013
IL1RN Forward	GGAATCCATGGAGGGAAGAT (SEQ ID NO: 620)	Perez-Pinera et al., Nat Methods, 2013
IL1RN Reverse	TGTTCTCGCTCAGGTCACTG (SEQ ID NO: 621)	Perez-Pinera et al., Nat Methods, 2013
MyoD Forward	CTCTCTGCTCCTTTGCCACA (SEQ ID NO: 622)	Perez-Pinera et al., Nat Methods, 2013
MyoD Reverse	GTGCTCTTCGGGTTTCAGGA (SEQ ID NO: 623)	Perez-Pinera et al., Nat Methods, 2013
Oct4 Forward	CGAAAGAGAAAGCGAACCAGTAT (SEQ ID NO: 624)	Hu et al., Nucleic Acids Res, 2014
Oct4 Reverse	CGTTGTGCATAGTCGCTGCTTGA (SEQ ID NO: 625)	Hu et al., Nucleic Acids Res, 2014

[0388] RT-QPCR was normalized to GAPDH expression using the $\Delta\Delta C_t$ method. Results are expressed as fold-increase expression of the gene of interest relative to cells

treated with Lipofectamine only without DNA transfected ("No DNA") (See FIGS. 60A-60C).

[0389] FIG. 62 shows that mutating residues in the p300 HAT domain causes a loss of its ability to activate gene expression. FIGS. 63A-63B shows that multiple gRNAs work synergistically with dCas-9-p300, as showed with dCas-9-VP64.

Example 24

[0390] FIGS. 64A-64C shows TALEN mediated integration of minidystrophin at the 5'UTR of the Dp427m skeletal muscle isoform of dystrophin in skeletal myoblast cell lines derived from human DMD patients carrying different deletions in the dystrophin gene. DMD patient cells were electroporated with constructs encoding a TALEN pair active at the 5'UTR locus and a donor template carrying the minidystrophin gene. FIG. 64A shows a schematic of how minidystrophin was integrated into the 5'UTR. FIG. 64B shows that hygromycin-resistant clonal cell lines were isolated and screened by PCR for successful site-specific integrations at the 5'UTR using the primers shown in FIG. 64A. Asterisks indicate clones selected for further analysis in FIG. 64C. FIG. 64C shows that clonally isolated DMD myoblasts with detected integration events were differentiated for 6 days and assessed for expression of an HA tag fused to the C terminus of minidystrophin.

[0391] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents.

[0392] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the invention, may be made without departing from the spirit and scope thereof.

APPENDIX

hCas9-T2A-GFP DNA sequence: SEQ ID NO: 145 (SpCas9 human optimized sequence, , T2A peptide, eGFP sequence)

gcccacATGGACAAAGAGTACTCCATTGGGCTCGATATCGGCACAAACAGCGTCGGCTGGGCCGTCATTACGGACAGGTA
CAAGGTGCCGAGCAAAAATTTCAAAGTTCTGGGCAATATACCGATCGCCACAGATAAAGAGAAACCTATTGGCGCCCTCT
TGTTTCGACTCGGGGAGACGCCGCAAGCCAGCGGCTCAAAAGCAAGCAGCGCGAGATATATCCCGCAAGAAGATCGG
ATCTGCTACCTGCGAGGAGATCTTTAGTAATGAGATGGCTAAGTGGATGACTCTTTCTTCCATAGGCTGGAGGAGTCTTT
TTTGGTGGGAGGAGATAAAAAGCAGCGGCCCAACCAATCTTTGGCAATATCGTGAGCAGGAGTGCGCTACCATGAAAAGT
ACCCAACTATATATCATCTGAGGAAGAAGCTTGTAGACAGTACTGTAAGGCTGACTTCGCGTGATCTATTCTCGCGCTG
GCGCATATGATCAAATTTCCGGGACACTTCTCATCGAGGGGGACCTGAACCAGACAAACAGCGATGTGCACAAACTCTT
TATCCAATCGGTTTCAGACTTACAATCAGCTTTTGGAGGAAGACCCGATCAACGCATCTCCGGATTCAGCCGCAAGAACCTC
TGAGCGCTAGGCTGTCCAAATCCCGCGGCTGAAAACCTCATCGACAGCTCTCTGGTGGAGAAGAAAGACAGCGCTGTTT
GGTAATCTTATCGCCCTGTCACTCGGCTGACCCCCAACCTTTAAATCTAACTTCGACCTTGGCGGAAGATGCCAAGCTTCA
ACTGAGCAAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCAGATCGGCCAGCAGTACAGACCTTTTCTTGG
CGGCAAGAAGACCTGTGACAGCCATTCTGCTGAGTGATATTCTGCGAGTGAAACAGGAGATACCAAAAGCTCCGCTGAG
GCTAGTATGATCAAGCCGCTATGATGAGCACCCACAGACTTGACTTTGCTGAAAGCCCTTGTGAGACAGCAACTGCCTGA
GAAGTACAAGGAATTTCTTCGATCAGTCTAAAAATGGCTACGCGCGGATCTGAGCGCGGAGCAAGCCAGGGAAT
TTTACAAATTTTATAGCCCATTTGGAAAAATGACCGCAGCAGGAGACTGCTGGGTAAAGCTTAAACAGAGAACTCTG
TTGCGCAACAGCGCACTTTGCAACAATGGAAGCATCCCCACCAGATTCACCTGGGCGAAGCTGCACGCTATCCTCAGCGC
GAGAAGAGATTTTACCCCTTTTGAAGATAACAGGGAAAAAGATTGAGAAAAATCCTCACATTTCCGATACCCCTCATG
TAGGCCCCCTCGCCCGGGGAAATTCAGATTGCGGTGGATGACTCGCAATCAGAAGAGACCATCACTCCCTGGAACTCTG
GAGGAAGTCGTGGATAAGGGGGCTCTGCCAGTCTTTCATCGAAAGGATGACTAACTTTGATAAAAAATCTGCCTTAACGA
AAAGGTCCTTCTTAAACACTCTCTGCTGTACGAGTACTTCACAGTCTTATAACGAGCTCAACAGGTCAAAATACGTCACAG
AAGGATGAGAAGAGCCAGCATCTCTGTACGAGCAGAAAGAAAGTACTCTGGACTCTCTTCAAGACGAACCCGAA
GTTACCGTGAACAGCTCAAGAAAGACTATTTCAAAGATTGAATGTTTCGACTCTGTTGAAATCAGCGGATGGAGGA
TCGCTTCAACGCATCTCTCGGAAACGATACAGATCTCTCGAAATCTATAAAGACAAGGACTTCTCGGACATGGAGGAG
ACAGGAGACTTCTTGAGGACATTGCTCTCACTTCTGTTTGAAGTATGGGAGATGATTGAGAAGCGTTGAAAGCT
TACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAAGAGGCGCCGATATACAGGATGGGGCGGCTGTCAAGAAA
ACTGATCATGGGATTCGACAGAAGCAGAGTCAAGCAATCTCGGATTTTCTTAAAGTCGATGGATTGCCAAACCGGA
ACTTCATGCAAGTTCGATCATGATGACTCTCACTTTAAGAGGAGACTCCGAAAGACACATTTCTGGCCAGGGGAG
AGTCTTCAGAGACATCTCGTAACTTTGCAAGTAGCCAGCTATCAAAAAGGGAATATGCGAGACCGTTTAAAGTCTGTGA
TGAATCGTCAAGATATGCGGAAGGCAATAGCCCGAGAATCTGTTATCGAGATGGCCCGAGAGAACCAACTACCGAA
AGGAGCAAGAGAACAGTAGGGAAGAAGATGAAGAGATTGAAGAGGTTATAAAGAACTGGGCTCCCAATCTTAAGGA
CACCACAGTGAAGAACCCAGCTTCAGATGAGAAGCTTACTCTGTACTCTGCGAGACCGCAGGACATGTACTGTGA
TCAGGAAGTGGACATCAATCGCTCTCCGACTACGAGCTGGATCATATCTGTCGCCAGCTCTTCTTCAAGATGATTCTA
TTGATAATAAAGTGTGTGACAAAGATCCGATAAAAAATAGAGGGAAAGATGATAACGCTCCCTCAGAGAAGTTGTCAAGAAA
ATGAAAAATTTTGTGGCGCAGCTGCTGAACCGCAAACGATGATCAACAACCGAAGTTGATAATCTGACTAAAGGCTGAACG
AGGTGGCTGTCTGAGTTGGATAAAGCCGGCTTCACTCAAAGGCAAGCTTGTGAGACAGCCGAGATCACAAGCACGTTGG
CCCAAAATTTCTGATTCACGATAGAAACCAACCAAGTACGATGAAATGACAACTGATTCTGAGAGGTTGAAAGTTTATCTCTG
AAGTCTAAGCTGTGTCTGATGTTCAAGAAGCACTTTCAGTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCCGA
TGATCGCTACCTGAATCGAGCTGGTAGGCATGCACTTATCAAAAATATCCAAAGTGAATCTGAATTTGTTTACGAG
ACTATAAAGTGTACGATGTTAGGAAAAATGATCGCAAGTCTGAGCGAGAAATAGGCAAGGGCCACCGCTTAAGTATCTTT
TACAGCAATATATGTAATTTTTCAGACAGCGAGATTACACTGGCCAAATGGAGAGATTCCGAAGAGCGCACTTATCTGAAC
AACCGGAGAAGACAGGAGAAATCTGTGGGACAAGGTGAGGATTTCGCGACAGCTCCGGAAGGTCCTGATCTCCCGCAGG
TGAACATCGTTAAAAAGACCGAAGTACAGACCGGAGGCTTCTCAAGGAAAGTATCTCTCCGAAAAGGAACAGCGACAA
CTGATCGCACGCAAAAAGATTTGGGACCCCAAGAAATACGGCGGATTTGATTTCTCTCACTAGCTCTACAGTGTACTGGT
TGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAAATCTCAAAGCGTCAAGGAAGCTGTGGCATCACAATCATGGAAGTACGAT
CAAGCTTCCGAAAAAACCCCATCGACTTTCTCGAGGCGAAGGATATAAAGAGCTCAAAAGACCTCATCATTAAAGCT
CCCAAGTACTCTCTTTTAAATGTTTAAACCGGCGAAACGAATGCTCGTATGTGCGGCGAGCTCTCGAGAAAGGTTAACGA
CTGGGCATCGCTCTTAAATACGTTAATTTCTTGTATCTGGCCGAGCACTATGAAAAGTCTCAAAGGTTCTCCGAAAGTAT
ATGAGCAGAGCTGCTGACAGCTGATCTCGATGAAAGTGCTTTCTGCTTACAATAAGCAAGGATAGCCCATCAGGGAGCA
GGCAGAAACACTATTCACCTGTTTACTCTGACCAACTTGGGCGCGCTGCGAGCTTCAAGTCTGACACACCACATAG
ACAGAAAGCGGTACACCTCTACAAAGGAGGTCTCTGGAGCCCACTGATTTCATCAGTCAATTACGGGGCTCTATGAAACA
AGAATCGACCTCTTCAGCTCGGTGGAGACAGCAGGCTGACGCTGCTAGCTAGCGGCGACGAGG

AAV/Rosa26 construct (SEQ ID NO: 456)

ggggggggggggggggggggttggcgcctccctctctgcgcgctcgctcgctcactgaggccgggggaccaaaggctgcgcg
 agcccggggcttggccggggcgccctcactgagcgagcgagcgagaggggagtgcccaactccatcactagggtt
 cctagatctgaatttcgacccggttacataactacggttaaatggcccgctcgctgaccgcccaacgaccccccgcat
 tgacgtcaataatgacgtatgtcccatagtaacgccaatagggaacttccattgacgtcaatgggtggagtttaccg
 taactgcccaacttggcagtcatacgaattgatcattgccaaactgcgccctcattgacgtcaatgacggttaaatggcc
 cgctggcattatggcccgatcacatgacctatttgggaacttctacttgccgagtaacatcagttatttgctcgtgatt
 ccatgggtgacgggttttggcagtcatacgaatggcgctgaacgggttgactcagcgggatttccaagttctccacc
 attgacgtcaatgggagtttgttttggcacaataatcagggaacttccaaactcgtaaacactccgcccatggac
 gcaaatgggcggttaggcgtgacggtggagggtctatataagcagagctcgtttagtgaaacctgagatcgccctggagac
 gccatcaacgcgtgttttgacctcatagaagacacgggacgcctcacgcctccggactatagaggatccggtacgtcag
 gaactgaaaacacaaaagatttaactqtaaaatttgacttctttgtctttatttcgaqgtccgaqgtccqgtqgtgtgc

APPENDIX - continued

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APPENDIX - continued

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Lys	Tyr	Asp	Glu	Asn	Asp	Lys	Leu	Ile	Arg	Glu	Val	Lys	Val	Ile	Thr	980	985	990	
Leu	Lys	Ser	Lys	Leu	Val	Ser	Asp	Phe	Arg	Lys	Asp	Phe	Gln	Phe	Tyr	995	1000	1005	
Lys	Val	Arg	Glu	Ile	Asn	Asn	Tyr	His	His	Ala	His	Asp	Ala	Tyr		1010	1015	1020	
Leu	Asn	Ala	Val	Val	Gly	Thr	Ala	Leu	Ile	Lys	Lys	Tyr	Pro	Lys		1025	1030	1035	
Leu	Glu	Ser	Glu	Phe	Val	Tyr	Gly	Asp	Tyr	Lys	Val	Tyr	Asp	Val		1040	1045	1050	
Arg	Lys	Met	Ile	Ala	Lys	Ser	Glu	Gln	Glu	Ile	Gly	Lys	Ala	Thr		1055	1060	1065	
Ala	Lys	Tyr	Phe	Phe	Tyr	Ser	Asn	Ile	Met	Asn	Phe	Phe	Lys	Thr		1070	1075	1080	
Glu	Ile	Thr	Leu	Ala	Asn	Gly	Glu	Ile	Arg	Lys	Arg	Pro	Leu	Ile		1085	1090	1095	
Glu	Thr	Asn	Gly	Glu	Thr	Gly	Glu	Ile	Val	Trp	Asp	Lys	Gly	Arg		1100	1105	1110	
Asp	Phe	Ala	Thr	Val	Arg	Lys	Val	Leu	Ser	Met	Pro	Gln	Val	Asn		1115	1120	1125	
Ile	Val	Lys	Lys	Thr	Glu	Val	Gln	Thr	Gly	Gly	Phe	Ser	Lys	Glu		1130	1135	1140	
Ser	Ile	Leu	Pro	Lys	Arg	Asn	Ser	Asp	Lys	Leu	Ile	Ala	Arg	Lys		1145	1150	1155	
Lys	Asp	Trp	Asp	Pro	Lys	Lys	Tyr	Gly	Gly	Phe	Asp	Ser	Pro	Thr		1160	1165	1170	
Val	Ala	Tyr	Ser	Val	Leu	Val	Val	Ala	Lys	Val	Glu	Lys	Gly	Lys					

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1175	1180	1185
Ser Lys Lys Leu Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile 1190 1195 1200		
Met Glu Arg Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu 1205 1210 1215		
Ala Lys Gly Tyr Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu 1220 1225 1230		
Pro Lys Tyr Ser Leu Phe Glu Leu Glu Asn Gly Arg Lys Arg Met 1235 1240 1245		
Leu Ala Ser Ala Gly Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu 1250 1255 1260		
Pro Ser Lys Tyr Val Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu 1265 1270 1275		
Lys Leu Lys Gly Ser Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe 1280 1285 1290		
Val Glu Gln His Lys His Tyr Leu Asp Glu Ile Ile Glu Gln Ile 1295 1300 1305		
Ser Glu Phe Ser Lys Arg Val Ile Leu Ala Asp Ala Asn Leu Asp 1310 1315 1320		
Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp Lys Pro Ile Arg 1325 1330 1335		
Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu Thr Asn Leu 1340 1345 1350		
Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg 1355 1360 1365		
Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp Ala Thr Leu Ile 1370 1375 1380		
His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser 1385 1390 1395		
Gln Leu Gly Gly Asp Pro Ile Ala Gly Ser Lys Ala Ser Pro Lys 1400 1405 1410		
Lys Lys Arg Lys Val Gly Arg Ala Asp Ala Leu Asp Asp Phe Asp 1415 1420 1425		
Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp 1430 1435 1440		
Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu 1445 1450 1455		
Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Ile Asn 1460 1465 1470		
Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser 1475 1480		

<210> SEQ ID NO 2

<211> LENGTH: 350

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 2

gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag 60

ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga 120

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aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat    180
atgcttatcg taacttgaaa gtatttcgat ttcttggcctt tatatatctt gtggaaagga    240
cgaaacacccg ggtcttcgag aagacctgtt ttagagctag aaatagcaag ttaaaataag    300
gctagtcctg tatcaacttg aaaaagtggc accgagtcgg tgcttttttt    350

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<210> SEQ ID NO 3
<211> LENGTH: 338
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 3

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acccccggca gcagcccgcc ccgagcgcgc cgcctgttta ttcagccggg agtccggcac    60
gcgccaggcg cacgcactgc aacaacaaac ccagctgaat ggagagtttg caaggagcgg    120
gagaaaggaa cgggaggggg ggagaggaga ggagagggg gagtttaggg agtgggtggg    180
aggaagaggt aagaggaggg gggggagtg gggctgcagc cgctcgctgc agcagcgggg    240
agtggggggc gaggcggggc cagggctgcg cgtggggctg ggtgtcccat tgaaaaggcg    300
gacgcactcc ggcagcccag cactctctca cttctggc    338

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<210> SEQ ID NO 4
<211> LENGTH: 305
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 4

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ccgccccgag cgcgcggcct gtttattcag ccgggagtcg ggcacgcgc gggcgcacgc    60
actgcaacaa caaaccaggc tgaatggaga gtttgcaagg agcgggcgcg ggcaactgga    120
gggggggggg gcgagaggga gggagctgag gaggtggggg aagaggaggg gtagtggggg    180
ctgcagccgc tcgctgcagc agcggggaggt ggggggcgag gcggggccag ggctgcgcgt    240
ggggctgggt gtccattga aaaggcggcc gcaccgcagc cgcccagcag tctctcactt    300
ctggc    305

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<210> SEQ ID NO 5
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        oligonucleotide

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<400> SEQUENCE: 5

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gctgggtgtc ccattgaaa    19

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<210> SEQ ID NO 6
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        oligonucleotide

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<400> SEQUENCE: 6

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cagccgctcg ctgcagcag    19

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<210> SEQ ID NO 7
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 7

tgagagagttt gcaaggagc 19

<210> SEQ ID NO 8
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 8

gtttattcag ccgggagtc 19

<210> SEQ ID NO 9
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 9

cgccaggagg ggtgggtcta 20

<210> SEQ ID NO 10
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 10

ccttggtgag actggtaga 19

<210> SEQ ID NO 11
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 11

gtcttcaggt tctgttgct 19

<210> SEQ ID NO 12
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 12

atattcctga tttaaaagt 19

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<210> SEQ ID NO 13
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 13
ttaaagtcg gctgtagc 19

<210> SEQ ID NO 14
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 14
cgggccgggg gcggggtcc 19

<210> SEQ ID NO 15
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 15
gcccgagccg cgtgtggaa 19

<210> SEQ ID NO 16
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 16
ccttcattgc ggcgggctg 19

<210> SEQ ID NO 17
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 17
ccgacccctc ccgggtccc 19

<210> SEQ ID NO 18
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 18

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caggaccgcg cttcccaag 19

<210> SEQ ID NO 19
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 19

tgcaccctgg gagcgcgag 19

<210> SEQ ID NO 20
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 20

ccgcacgcac ctgttccca 19

<210> SEQ ID NO 21
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 21

aaaacagcga gggagaaac 19

<210> SEQ ID NO 22
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 22

ttaacttgat tgtgaaac 19

<210> SEQ ID NO 23
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 23

aaaacaatgc atatttgca 19

<210> SEQ ID NO 24
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 24

aaaatccagt attttaatg

19

<210> SEQ ID NO 25

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 25

accagcact gcagcctgg

19

<210> SEQ ID NO 26

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 26

aacttatgcg gcgtttcct

19

<210> SEQ ID NO 27

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 27

tcactttaaa accacctct

19

<210> SEQ ID NO 28

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 28

gcacatctttt ctctttaat

19

<210> SEQ ID NO 29

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 29

tgtactctct gaggtgctc

19

<210> SEQ ID NO 30

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 30

acgcagataa gaaccagtt 19

<210> SEQ ID NO 31
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 31

catcaagtca gccatcagc 19

<210> SEQ ID NO 32
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 32

gagtcaccct cctggaaac 19

<210> SEQ ID NO 33
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 33

gctagggatg aagaataaa 19

<210> SEQ ID NO 34
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 34

ttgaccaata gccttgaca 19

<210> SEQ ID NO 35
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 35

tgcaaataatc tgtctgaaa 19

<210> SEQ ID NO 36
<211> LENGTH: 19
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 36

aaattagcag tatectctt 19

<210> SEQ ID NO 37
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 37

cctgggctcc ggggcgttt 19

<210> SEQ ID NO 38
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 38

ggccctgcg gccaccccg 19

<210> SEQ ID NO 39
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 39

ctccctccct gcccggtag 19

<210> SEQ ID NO 40
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 40

aggtttggaa agggcgtgc 19

<210> SEQ ID NO 41
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 41

ggagcttctc gacttcacca 20

<210> SEQ ID NO 42

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 42

gatttgtggg cctgaagaaa 20

<210> SEQ ID NO 43
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 43

aaggaggagg gcagaatcat 20

<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 44

aaaccttcct cagctatgcc c 21

<210> SEQ ID NO 45
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 45

agctgatggc cctaaacaga 20

<210> SEQ ID NO 46
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 46

caggaggact ctggcaccta 20

<210> SEQ ID NO 47
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 47

ggaatccatg gaggaagat 20

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<210> SEQ ID NO 48
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 48
gctgagtgaa ctgcactgtg a 21

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 49
ctctctgctc ctttgccaca 20

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 50
caatgacccc ttcattgacc 20

<210> SEQ ID NO 51
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 51
ggaacaagag ctgctggact 20

<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 52
aactttggca ttgtggaagg 20

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 53

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aacgccactg acaagaaagc 20

<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 54

cagatccatg gaggaaggaa 20

<210> SEQ ID NO 55
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 55

gggtactcct ggaagatgtc c 21

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 56

gtttgcgacg catgttcctc 20

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 57

aagcccttgc ttagtggtg 20

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 58

cggcaggaaa gcatctgtat 20

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

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<400> SEQUENCE: 59

tggttcctcgct caggtcagtg 20

<210> SEQ ID NO 60

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 60

gaattctttg ccgaaatgga 20

<210> SEQ ID NO 61

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 61

gtgctcttcg ggtttcagga 20

<210> SEQ ID NO 62

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 62

ttgattttgg agggatctcg 20

<210> SEQ ID NO 63

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 63

gtttttctgc ctccccattt 20

<210> SEQ ID NO 64

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 64

ggatgcaggg atgatgttct 20

<210> SEQ ID NO 65

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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oligonucleotide

<400> SEQUENCE: 65

gattggcttt gatttccta 20

<210> SEQ ID NO 66
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 66

gtgtagagta agtcagccta tgg 23

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 67

gcctactcag actgttactc 20

<210> SEQ ID NO 68
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 68

gttgacaga acttaccgac tgg 23

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 69

gcagttgcct aagaactggt 20

<210> SEQ ID NO 70
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 70

ggggctccac cctcacgagt 20

<210> SEQ ID NO 71
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 71

gtttgcttcg ctataaaaacg agg 23

<210> SEQ ID NO 72
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 72

gtctgaggat ggggccgcaa tgg 23

<210> SEQ ID NO 73
<211> LENGTH: 23
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 73

ggatctgtca aatcgctgc agg 23

<210> SEQ ID NO 74
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 74

gccaggatgg cattgggcag cgg 23

<210> SEQ ID NO 75
<211> LENGTH: 23
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 75

gctgaatctg cggtaggcagg agg 23

<210> SEQ ID NO 76
<211> LENGTH: 23
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 76

gttcttttgt tcttctagcc tgg 23

<210> SEQ ID NO 77
<211> LENGTH: 23

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 77

ggaaaagctt gagcaagtca agg 23

<210> SEQ ID NO 78
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 78

ggaagagttg cccctgcgcc agg 23

<210> SEQ ID NO 79
<211> LENGTH: 23
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 79

gacaaatctc cagtggataa agg 23

<210> SEQ ID NO 80
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 80

gtgttttctca ggtaaagctc tgg 23

<210> SEQ ID NO 81
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 81

ggaaggacca ttgacgtta agg 23

<210> SEQ ID NO 82
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 82

gaactgctat ttcagtttcc tgg 23

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<210> SEQ ID NO 83
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<212> TYPE: DNA
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<220> FEATURE:
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oligonucleotide

<400> SEQUENCE: 83

gccagccact cagccagtga agg 23

<210> SEQ ID NO 84
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 84

ggtatgcttt tctgttaaag agg 23

<210> SEQ ID NO 85
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 85

gctcctggac tgaccactat tgg 23

<210> SEQ ID NO 86
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 86

ggaacagagg cgtccccagt tgg 23

<210> SEQ ID NO 87
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 87

ggaggctaga acaatcatta cgg 23

<210> SEQ ID NO 88
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 88

gacaagaaca ccttcagaac cgg 23

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<210> SEQ ID NO 89
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 89
gggtttctgt gattttcttt tgg 23

<210> SEQ ID NO 90
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 90
gggccaaaga cctccgccag tgg 23

<210> SEQ ID NO 91
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 91
gttgagaag cattcataaa agg 23

<210> SEQ ID NO 92
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 92
gtcgctcact caccctgcaa agg 23

<210> SEQ ID NO 93
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 93
gaaaagagct gatgaaacaa tgg 23

<210> SEQ ID NO 94
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 94

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gtacactttt caaaatgctt tgg 23

<210> SEQ ID NO 95
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 95

ggagatgata atcaagcaga agg 23

<210> SEQ ID NO 96
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 96

gctttgaaag agcaataaaa tgg 23

<210> SEQ ID NO 97
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 97

gcacaaaagt caaatcgga tgg 23

<210> SEQ ID NO 98
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 98

gatttcaata taagattcg agg 23

<210> SEQ ID NO 99
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 99

gcttaagcaa tccgaactc tgg 23

<210> SEQ ID NO 100
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 100

gccttcttta tcccctatcg

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<210> SEQ ID NO 101

<400> SEQUENCE: 101

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<210> SEQ ID NO 102

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<210> SEQ ID NO 103

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<210> SEQ ID NO 104

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (21)..(22)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 104

gaggccaaac ctcggcttac nngrr

25

<210> SEQ ID NO 105

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (21)..(22)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 105

gttcgaaaat ttcaggttaag nngrr

25

<210> SEQ ID NO 106

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (21)..(22)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 106

ggcagaacag gagataacag nngrrt

26

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<210> SEQ ID NO 107
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 107

ggcggccctc gcccttctct ggggat 26

<210> SEQ ID NO 108
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 108

gtagtgatcg tggatacgag agg 23

<210> SEQ ID NO 109
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 109

gtacagccct cgggttatat tgg 23

<210> SEQ ID NO 110
<211> LENGTH: 23
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 110

gggaaggaat taagcccgaa tgg 23

<210> SEQ ID NO 111
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 111

gggaacagct ttcgtagttg agg 23

<210> SEQ ID NO 112
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 112

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gataaaagtcc agtgtcgatc agg 23

<210> SEQ ID NO 113
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 113

gaaaaccaga gcttcggtca agg 23

<210> SEQ ID NO 114
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 114

ggagtcttct gggcaggctt aaaggctaac ctgg 34

<210> SEQ ID NO 115
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 115

gtcgggtgag catgtcttta atctacctcg atgg 34

<210> SEQ ID NO 116
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 116

ggtgtcacca gagtaacagt ctgagt 26

<210> SEQ ID NO 117
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 117

gtgatcatca agcagaaggt atgag 25

<210> SEQ ID NO 118
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 118

gaacttcgaa aatttcaggt aagccgagg

29

<210> SEQ ID NO 119

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 119

ggaaactcat caaatatgcg tgtagtgt

29

<210> SEQ ID NO 120

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 120

gtcatttaca ctaacacgca tatttgatg

29

<210> SEQ ID NO 121

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 121

ggaatgaaac tcatcaaata tgcgtgtta

29

<210> SEQ ID NO 122

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 122

gtcatcaata tctttgaagg actctgggt

29

<210> SEQ ID NO 123

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 123

gtgttttcat aggaaaaata ggcaagttg

29

<210> SEQ ID NO 124

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 124

gaattggaaa atgtgatggg aaacagata 29

<210> SEQ ID NO 125
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 125

gatgatcatc aagcagaagg tatgagaaa 29

<210> SEQ ID NO 126
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 126

gagatgatca tcaagcagaa ggtatgaga 29

<210> SEQ ID NO 127
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 127

gcattttttc tcataccttc tgcttgatg 29

<210> SEQ ID NO 128
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 128

gtcctactca gactgttact ctggtgaca 29

<210> SEQ ID NO 129
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 129

gacagggtgt gtcaccagag taacagtct 29

<210> SEQ ID NO 130
<211> LENGTH: 29
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 130

gttatcatatt tttctcatatc cttctgctt 29

<210> SEQ ID NO 131
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 131

gttgcctaag aactggtggg aaatggtct 29

<210> SEQ ID NO 132
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 132

gaaacagttg cctaagaact ggtgggaaa 29

<210> SEQ ID NO 133
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 133

gtttccacc agttcttagg caactgttt 29

<210> SEQ ID NO 134
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 134

gtggctttga tttccctagg gtccagctt 29

<210> SEQ ID NO 135
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 135

gtagggaat caaagccaat gaaacgttc 29

<210> SEQ ID NO 136

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<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 136

ggaccctagg gaaatcaaag ccaatgaaa 29

<210> SEQ ID NO 137
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 137

gtgagggtc caccctcacg agtgggttt 29

<210> SEQ ID NO 138
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 138

gaaggattga gggctccacc ctacagagt 29

<210> SEQ ID NO 139
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 139

ggctccacc tcacgagtgg gtttggttc 29

<210> SEQ ID NO 140
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 140

gtatccccta tcgaggaaac cacgagttt 29

<210> SEQ ID NO 141
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 141

ggataaagaa ggcctatttc atagagttg 29

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<210> SEQ ID NO 142
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 142

gaggccttct ttatccccta tcgaggaaa 29

<210> SEQ ID NO 143
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 143

gtgagggctc caccctcacg agtgggt 27

<210> SEQ ID NO 144
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 144

ggataaagaa ggcctatttc atagagt 27

<210> SEQ ID NO 145
<211> LENGTH: 4932
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polynucleotide

<400> SEQUENCE: 145

gccaccatgg acaagaagta ctccattggg ctcgatatcg gcacaaacag cgtcggtctg 60

gccgtcatta cggacgagta caaggtgccg agcaaaaaat tcaaagttct gggcaatacc 120

gatcgccaca gcataaagaa gaacctcatt ggcgcctcc tgttcgactc cggggagacg 180

gccgaagcca cgcgggtcaa aagaacagca cggcgagat ataccgcag aaagaatcgg 240

atctgctacc tgcaggagat ctttagtaat gagatggcta aggtggatga ctctttcttc 300

cataggctgg aggagtcctt tttggtggag gaggataaaa agcacgagcg ccaccaatc 360

tttggaata tcgtggacga ggtggcgtag catgaaaagt acccaacat atatcatctg 420

aggaagaagc ttgtagacag tactgataag gctgacttgc ggttgatcta tctcgcgctg 480

gcgcatatga tcaaatctcg gggacacttc ctcacgaggg gggacctgaa ccagacaac 540

agcgatgtcg acaaaactctt tatccaactg gttcagactt acaatcagct ttctgaagag 600

aaccgcatca acgcatccgg agttgacgcc aaagcaatcc tgagcgctag gctgtccaaa 660

tcccgggcgc tcgaaaacct catcgcacag ctccctgggg agaagaagaa cggcctgttt 720

ggtaatctta tcgccctgtc actcgggctg acccccaact ttaaatctaa cttcgacctg 780

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gccgaagatg ccaagcttca actgagcaaa gacacctacg atgatgatct cgacaatctg	840
ctggcccaga tcggcgacca gtacgcagac ctttttttgg cggcaaagaa cctgtcagac	900
gccattctgc tgagtgatat tctgcgagtg aacacggaga tcaccaaagc tccgctgagc	960
gctagtatga tcaagcgcta tgatgagcac caccaagact tgactttgct gaaggccctt	1020
gtcagacagc aactgcctga gaagtacaag gaaattttct tcgatcagtc taaaaatggc	1080
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<211> LENGTH: 23

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<210> SEQ ID NO 152

<211> LENGTH: 23

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<211> LENGTH: 23

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<400> SEQUENCE: 153

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<210> SEQ ID NO 154

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<212> TYPE: DNA

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 166

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<400> SEQUENCE: 170

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<400> SEQUENCE: 172

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<400> SEQUENCE: 176

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<220> FEATURE:
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agggtccac cctcacgagt ggg 23

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gcagctcagc cctcacgagt cag 23

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agggtggac cctcacaagt aag 23

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<210> SEQ ID NO 193

<211> LENGTH: 23

<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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atgactccac cctcaagagt aag 23

<210> SEQ ID NO 194

<211> LENGTH: 23

<212> TYPE: DNA

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<210> SEQ ID NO 195

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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<400> SEQUENCE: 195

gtctgctgtg tccctatcg ggg 23

<210> SEQ ID NO 196
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 196

cccttctcta tccctgtcg tgg 23

<210> SEQ ID NO 197
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<400> SEQUENCE: 197

gccttcttta tccctctct tgg 23

<210> SEQ ID NO 198
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<400> SEQUENCE: 198

gcgctctttt tccctatct tag 23

<210> SEQ ID NO 199
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 199

gccctctgtc tccctgtcg cag 23

<210> SEQ ID NO 200
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 200

tccatctttg tccctattg agg 23

<210> SEQ ID NO 201
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 201

accttctctc tcccctatag agg 23

<210> SEQ ID NO 202
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<400> SEQUENCE: 203

tgcttcttaa tcccctatca aag 23

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<220> FEATURE:
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<400> SEQUENCE: 204

accttcttac tcccctatcc ggg 23

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<220> FEATURE:
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<400> SEQUENCE: 205

gagagggttat gtggctttac ca 22

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<400> SEQUENCE: 206

aaaaatgctt cccactttgc 20

<210> SEQ ID NO 207
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<212> TYPE: DNA
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<400> SEQUENCE: 207

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<400> SEQUENCE: 208

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 209

gggaaatggt ctaggagagt aaagt 25

<210> SEQ ID NO 210
<211> LENGTH: 25
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<220> FEATURE:
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<400> SEQUENCE: 210

gtttggctca aattgttact ctcca 25

<210> SEQ ID NO 211
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primer

<400> SEQUENCE: 211

gtgagagtaa tgtgttggt gagag 25

<210> SEQ ID NO 212
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<220> FEATURE:
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primer

<400> SEQUENCE: 212

cgggcttgga cagaacttac 20

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<400> SEQUENCE: 213

ctgcgtagtg ccaaaacaaa 20

<210> SEQ ID NO 214
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taatttcatt gaagagtggc tgaa 24

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<400> SEQUENCE: 215

aagccctgtg tggtagtagt cagt 24

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<400> SEQUENCE: 216

tgagtcattg tggataacca gtct 24

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<400> SEQUENCE: 217

gaaggtcagg aacatacaat tcaa 24

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<400> SEQUENCE: 218

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<400> SEQUENCE: 219
tgctgttgat taatggttga tagg 24

<210> SEQ ID NO 220
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<400> SEQUENCE: 220
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<210> SEQ ID NO 221
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<220> FEATURE:
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<400> SEQUENCE: 221
atgaataacc taatgggcag aaaa 24

<210> SEQ ID NO 222
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<400> SEQUENCE: 222
tcaagtcgct tcattttgat agac 24

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<400> SEQUENCE: 223
cacaacaaaa catatagcca aagc 24

<210> SEQ ID NO 224
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tgctgctaaa ataacacaaa tcagt 25

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<400> SEQUENCE: 225

ctgtgcctat tgtggttatc ctg 23

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<400> SEQUENCE: 226

attgatctgc aatacatgtg gagt 24

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<400> SEQUENCE: 227

tttgctctg ctattacagt atgg 24

<210> SEQ ID NO 228
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<400> SEQUENCE: 228

tgtagggtgg ttggctaaaa taat 24

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<400> SEQUENCE: 229

tttttgcaca gtcaataaca caaa 24

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<400> SEQUENCE: 230

ggctggtctc acaattgtac ttta 24

<210> SEQ ID NO 231

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<212> TYPE: DNA

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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cattatggac tgaaaatctc agca 24

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 233

ttcagcttta acgtgatttt ctgt 24

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<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 234

ggattcagaa gctgtttacg aagt 24

<210> SEQ ID NO 235

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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<211> LENGTH: 24

<212> TYPE: DNA

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 236

aactcacccc attgttggtta tatt 24

<210> SEQ ID NO 237

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 237

ccttgtccaa ataccgaaat acat 24

<210> SEQ ID NO 238

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 238

cacataattc atgaacttgg cttc 24

<210> SEQ ID NO 239

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 239

tagtagctgg ggaggaagat acag 24

<210> SEQ ID NO 240

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 240

tttttgtttt aattgcgact gtgt 24

<210> SEQ ID NO 241

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 241

agaaaagggg ttttcttttg actt 24

<210> SEQ ID NO 242

<211> LENGTH: 24

<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 242

cattgtgact ggatgagaag aaac 24

<210> SEQ ID NO 243
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 243

aacggctggt attaaagtcc tcag 24

<210> SEQ ID NO 244
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 244

caagtcagaa gtcacttgct ttgt 24

<210> SEQ ID NO 245
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 245

ttttatgtgc aggaatcagt ctgt 24

<210> SEQ ID NO 246
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 246

tggcggcggt ttcattat 18

<210> SEQ ID NO 247
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 247

ttcgatccgt aatgattggt ctagcc 26

<210> SEQ ID NO 248

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 248

ggtcttccag agtgctgagg 20

<210> SEQ ID NO 249
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 249

tgtgtgcttc tgtacacatc atct 24

<210> SEQ ID NO 250
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 250

agatttcaac cctcaaaaac tgag 24

<210> SEQ ID NO 251
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 251

taaactcttt cttttccgca attc 24

<210> SEQ ID NO 252
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 252

caaggtgacc tgctacctaa aaat 24

<210> SEQ ID NO 253
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 253

tatgaccaag gctatgtgtt cact 24

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<210> SEQ ID NO 254
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 254

acagcctctc tccagtaaca ttct 24

<210> SEQ ID NO 255
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 255

tattcttgca gtggtttcac attt 24

<210> SEQ ID NO 256
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 256

atattttaag ccaagaccca acaa 24

<210> SEQ ID NO 257
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 257

ctttcaactg tctgtctgat tgct 24

<210> SEQ ID NO 258
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 258

aacagcctct cttcattggt ctct 24

<210> SEQ ID NO 259
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 259

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ctctggaact tgtctctgtc ttga 24

<210> SEQ ID NO 260
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 260

ctttctctgcg ttctcatggt acta 24

<210> SEQ ID NO 261
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 261

ccttatatcc gtatcgctca ctct 24

<210> SEQ ID NO 262
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 262

catatctgtc taacttccgc acac 24

<210> SEQ ID NO 263
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 263

acaggtgtta tgttgtctgc atct 24

<210> SEQ ID NO 264
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 264

actccattcc cagattagtt atgc 24

<210> SEQ ID NO 265
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

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<400> SEQUENCE: 265

ctgtttttctt tgtgagagtg gaga 24

<210> SEQ ID NO 266

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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tgtaaggtgg tcaaacttgc tcta 24

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<210> SEQ ID NO 268

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 268

tccctgattc tctcatttgt gtta 24

<210> SEQ ID NO 269

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 269

ttgggaacat cagagaaagt atga 24

<210> SEQ ID NO 270

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 270

acaaattaca gtctcctggg aaag 24

<210> SEQ ID NO 271

<211> LENGTH: 24

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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primer

<400> SEQUENCE: 271

agtagcttac cttggcagag aaaa 24

<210> SEQ ID NO 272

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 273

gaaaggctca gtgaatgttt gtt 23

<210> SEQ ID NO 274

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 275

cccatatatt catgattacc caca 24

<210> SEQ ID NO 276

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 276

tatcagaacg agcactaaaa gcac 24

<210> SEQ ID NO 277

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 277

ttgggaggct gaggtacaag 20

<210> SEQ ID NO 278
<211> LENGTH: 24
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 278

gaatgaaaaa caaacagaag gtga 24

<210> SEQ ID NO 279
<211> LENGTH: 24
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 279

ctcctcatct gtacccttca atct 24

<210> SEQ ID NO 280
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 280

agagtggcat ctagtgtag tgag 24

<210> SEQ ID NO 281
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 281

taccaaaaagc ttctctgtt tacc 24

<210> SEQ ID NO 282
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 282

gtaagttgga tggcctattc ttg 24

<210> SEQ ID NO 283
<211> LENGTH: 24

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 283

gaaggaaatg caaggataca agat 24

<210> SEQ ID NO 284
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primer

<400> SEQUENCE: 284

tgattgaaag aatcattcca gaaa 24

<210> SEQ ID NO 285
<211> LENGTH: 24
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 285

tcagaaggaa aattgaaatt gggt 24

<210> SEQ ID NO 286
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 286

cagatgtgtt cttcatcatt cctc 24

<210> SEQ ID NO 287
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 287

ttctctttag ggaaagctct caaa 24

<210> SEQ ID NO 288
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 288

gggtatagat catatggagg gaag 24

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<210> SEQ ID NO 289
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 289

agatgatctg cccacctcag 20

<210> SEQ ID NO 290
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 290

ctttcttcct catttagtgg caat 24

<210> SEQ ID NO 291
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 291

atgaattgca gattgatggg actg 24

<210> SEQ ID NO 292
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 292

tctcaccaag aaccaaattg tcta 24

<210> SEQ ID NO 293
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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primer

<400> SEQUENCE: 293

gtaggatacc ttggcaacag tctt 24

<210> SEQ ID NO 294
<211> LENGTH: 24
<212> TYPE: DNA
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 294

ttaacgaatt gtgagatttg ctgt 24

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<210> SEQ ID NO 295
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 295

tcagaaagtc aagtagcaca caca 24

<210> SEQ ID NO 296
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 296

agaagcacac actcaggtaa agc 23

<210> SEQ ID NO 297
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 297

tctttggggg aataatgact aaaa 24

<210> SEQ ID NO 298
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 298

tttggcattt atgggaataa aact 24

<210> SEQ ID NO 299
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 299

actaattctg gtcaagccca tca 23

<210> SEQ ID NO 300
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 300

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ttaagacatc ggatgaacag aaag 24

<210> SEQ ID NO 301
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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primer

<400> SEQUENCE: 301

agaagctttc tgacatgatc tgc 23

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 302

tcaattgcat taggacttag acca 24

<210> SEQ ID NO 303
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 303

gttaaattac ctgtgaagcc cttg 24

<210> SEQ ID NO 304
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 304

cggaaaacag atccacttta tgat 24

<210> SEQ ID NO 305
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 305

aaatccactg gaaacatctt gagt 24

<210> SEQ ID NO 306
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

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<400> SEQUENCE: 306

agtctcttca gaatcatgcc ctat 24

<210> SEQ ID NO 307

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 307

gcttggtggc acatacctgt ag 22

<210> SEQ ID NO 308

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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ggtaggtaga tttgcttgct tggt 24

<210> SEQ ID NO 309

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 309

agctctcagc agagtaggga tttt 24

<210> SEQ ID NO 310

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gtgagctctac tgcaccccat c 21

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<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 311

tgacactgtg aagtaattc tgtc 24

<210> SEQ ID NO 312

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 312

tcaagaactt gacaatgagc aaat 24

<210> SEQ ID NO 313
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 313

tatccgatcc actgttgtgt gt 22

<210> SEQ ID NO 314
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 314

caggagaccc aaaaccactc tac 23

<210> SEQ ID NO 315
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 315

ttgttctaca aatagggtt cctt 24

<210> SEQ ID NO 316
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 316

tgtaaagttt gggcttatgt tcct 24

<210> SEQ ID NO 317
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 317

cacaagtctc actgcacaaa cat 23

<210> SEQ ID NO 318
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 318

tgacccatga ttatctctct ttga 24

<210> SEQ ID NO 319
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 319

ttcagcttct gattggtttt aatg 24

<210> SEQ ID NO 320
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 320

ccaattcctt aattttccct acag 24

<210> SEQ ID NO 321
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 321

atctcagacc aggagggaga c 21

<210> SEQ ID NO 322
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 322

cctcagggtc agtacatttt tcag 24

<210> SEQ ID NO 323
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 323

ttcttaggac attgctccac atac 24

<210> SEQ ID NO 324

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<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 324

gcaaacataa tgcaactcgt aatc 24

<210> SEQ ID NO 325
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 325

gcaagggagt ctgtgtcttt g 21

<210> SEQ ID NO 326
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 326

tcatttaagt ggctgttctg tggt 24

<210> SEQ ID NO 327
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 327

acaaaacaga gagaaaaggc agag 24

<210> SEQ ID NO 328
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 328

gttttgattt ctggtgccta cag 23

<210> SEQ ID NO 329
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 329

actgaagctg aagcccagtc 20

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<210> SEQ ID NO 330
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 330

acatgagctc tcaggtttct gac 23

<210> SEQ ID NO 331
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 331

tcaaacttag atggttcctt atgtt 25

<210> SEQ ID NO 332
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 332

gtaccctgaa aatgtagggt gact 24

<210> SEQ ID NO 333
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 333

cacttcccaa gtgaggcaat 20

<210> SEQ ID NO 334
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 334

ctatacttgg ggctgacttg ctac 24

<210> SEQ ID NO 335
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 335

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tcgtataggt tactttggct caca 24

<210> SEQ ID NO 336
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 336

agggatcttt actctcagt gtgt 24

<210> SEQ ID NO 337
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 337

tgtagaagtt ggaatatoct gctg 24

<210> SEQ ID NO 338
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 338

gtcaacaatt tgatctcagg cttc 24

<210> SEQ ID NO 339
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 339

ctcagtacta aagatggacg ctgt 24

<210> SEQ ID NO 340
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 340

aatcatttca gtcttcccaa caat 24

<210> SEQ ID NO 341
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

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<400> SEQUENCE: 341

gggaatcaca gtagatgttt gtca 24

<210> SEQ ID NO 342

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 342

agaccaggag gtaagaacat ttg 24

<210> SEQ ID NO 343

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 343

ccacatagaa agagacttgc agaa 24

<210> SEQ ID NO 344

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 344

agagatgccaa aaagaacagt caat 24

<210> SEQ ID NO 345

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 345

tgtgccttag gctatgtaaa ctgt 24

<210> SEQ ID NO 346

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 346

aaacccttgt aacaaaaatt acca 24

<210> SEQ ID NO 347

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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primer

<400> SEQUENCE: 347

taactgcac agaagtcctt gcta 24

<210> SEQ ID NO 348
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 348

ggagaccaag ctgctaaagt ca 22

<210> SEQ ID NO 349
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 349

gtggtgccgc gggagtttgg ctcaaattgt tactctt 37

<210> SEQ ID NO 350
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 350

gtggtgccgc gggggaaatg gtctaggaga gtaaagt 37

<210> SEQ ID NO 351
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 351

gtggtgccgc gggagagggt atgtggcttt acca 34

<210> SEQ ID NO 352
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 352

gtggtgccgc ggctcattct catgcctgga ca 32

<210> SEQ ID NO 353
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 353

gtggtgccgc gccgggcttg gacagaactt ac 32

<210> SEQ ID NO 354
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 354

gtggtgccgc ggctgcgtag tgccaaaaca aa 32

<210> SEQ ID NO 355
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 355

gtggtgccgc ggtaatttca ttgaagagtg gctgaa 36

<210> SEQ ID NO 356
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 356

gtggtgccgc ggaagccctg tgtggtagta gtcagt 36

<210> SEQ ID NO 357
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 357

gtggtgccgc ggcaagtcag aagtcacttg ctttgt 36

<210> SEQ ID NO 358
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 358

gtggtgccgc gggttttatgt gcaggaatca gtctgt 36

<210> SEQ ID NO 359
<211> LENGTH: 36

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 359

gtggtgccgc ggtgtgtgct tctgtacaca tcattc 36

<210> SEQ ID NO 360
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 360

gtggtgccgc ggagatttca accctcaaaa actgag 36

<210> SEQ ID NO 361
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 361

gtggtgccgc ggttggaac atcagagaaa gtatga 36

<210> SEQ ID NO 362
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 362

gtggtgccgc ggacaaatta cagtctcctg ggaaag 36

<210> SEQ ID NO 363
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 363

gtggtgccgc ggcacttccc aagtgaggca at 32

<210> SEQ ID NO 364
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 364

gtggtgccgc ggctatactt ggggctgact tgetac 36

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<210> SEQ ID NO 365
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 365

gtggtgccgc ggttggtctt ttagcttggtg tttc 34

<210> SEQ ID NO 366
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 366

gtggtgccgc ggtgagactc ccaaaggcaa tc 32

<210> SEQ ID NO 367
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 367

gtggtgccgc ggttggtctt ttagcttggtg tttc 34

<210> SEQ ID NO 368
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 368

gtggtgccgc ggactgaggg gtgatcttgg tg 32

<210> SEQ ID NO 369
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 369

gtggtgccgc gggcagagaa agccagtcgg ta 32

<210> SEQ ID NO 370
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 370

gtggtgccgc ggtgagactc ccaaaggcaa tc 32

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<210> SEQ ID NO 371
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 371

gtggtgccgc gggcagagaa agccagtcgg ta 32

<210> SEQ ID NO 372
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 372

gtggtgccgc ggactgaggg gtgatcttgg tg 32

<210> SEQ ID NO 373
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 373

gtggtgccgc ggccagagtt cctagggcag ag 32

<210> SEQ ID NO 374
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 374

gtggtgccgc ggagctagtc cccacattcc ac 32

<210> SEQ ID NO 375
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 375

gtggtgccgc ggccagagtt cctagggcag ag 32

<210> SEQ ID NO 376
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 376

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gtggtgccgc ggggtggagg gaaacttttag gc 32

<210> SEQ ID NO 377
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 377

gtggtgccgc ggctcattct catgcctgga ca 32

<210> SEQ ID NO 378
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 378

gtggtgccgc ggagctagtc cccacattcc ac 32

<210> SEQ ID NO 379
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 379

gtggtgccgc ggtctcatgc ctggacaagt aact 34

<210> SEQ ID NO 380
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 380

gtggtgccgc ggggtggagg gaaacttttag gc 32

<210> SEQ ID NO 381
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 381

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<210> SEQ ID NO 382
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 382

gtggtgccgc ggcaccactg tctgcctaag ga 32

<210> SEQ ID NO 383

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 383

gtggtgccgc ggggcttggc cagaacttac cg 32

<210> SEQ ID NO 384

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 384

gtggtgccgc ggggtggagg gaaactttag gc 32

<210> SEQ ID NO 385

<211> LENGTH: 33

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 385

gtggtgccgc ggcgtagtgc caaaacaaac agt 33

<210> SEQ ID NO 386

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 386

gtggtgccgc ggcaccactg tctgcctaag ga 32

<210> SEQ ID NO 387

<211> LENGTH: 33

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 387

gtggtgccgc ggcgtagtgc caaaacaaac agt 33

<210> SEQ ID NO 388

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 388

gtggtgccgc ggggtggagg gaaactttag gc 32

<210> SEQ ID NO 389

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 389

gtggtgccgc gggcgagggc ctacttgata tg 32

<210> SEQ ID NO 390

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 390

gtggtgccgc ggcttcccaa gtgaggcaat gc 32

<210> SEQ ID NO 391

<211> LENGTH: 33

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 391

gtggtgccgc ggacgttttg tgctgctgta aca 33

<210> SEQ ID NO 392

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 392

gtggtgccgc ggctgcaggc acattctctt cc 32

<210> SEQ ID NO 393

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 393

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<210> SEQ ID NO 394

<211> LENGTH: 32

<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 394
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<210> SEQ ID NO 395
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<400> SEQUENCE: 395
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<210> SEQ ID NO 396
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 396
gtggtgccgc ggtctcttcc tcacacagct ga 32

<210> SEQ ID NO 397
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 397
gtggtgccgc ggggagcttg gaggaagag aa 32

<210> SEQ ID NO 398
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 398
gtggtgccgc ggcttcccaa gtgaggcaat gc 32

<210> SEQ ID NO 399
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 399
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<210> SEQ ID NO 400

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<211> LENGTH: 32
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<400> SEQUENCE: 400

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<400> SEQUENCE: 401

gtggtgccgc ggggatgaaa cagggcagga ac 32

<210> SEQ ID NO 402
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 402

gtggtgccgc ggttcccaag tgaggcaatg c 31

<210> SEQ ID NO 403
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 403

gtggtgccgc ggtttgcaga gccatgatga gg 32

<210> SEQ ID NO 404
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 404

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<210> SEQ ID NO 405
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 405

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<210> SEQ ID NO 406
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 406

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<210> SEQ ID NO 407
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 407

tagctcctac tcagactggt actctggtga cacaac 36

<210> SEQ ID NO 408
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 408

tagctcctac tcagactggt gacccaac 28

<210> SEQ ID NO 409
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 409

tagctcctac tctggtgaca caac 24

<210> SEQ ID NO 410
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 410

tagctcctac tcagactggt gacacaac 28

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 411

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tagctcctac tcagac 16

<210> SEQ ID NO 412
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 412

tagctcctac tcagactggt acacaac 27

<210> SEQ ID NO 413
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 413

tagctcctac tcagactgtg gtgaggtgac 30

<210> SEQ ID NO 414
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 414

tagctcctac tcagactctc tggtagacaca ac 32

<210> SEQ ID NO 415
<211> LENGTH: 31
<212> TYPE: DNA
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 415

tagctcctac tcagacctct ggtgacacaa c 31

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oligonucleotide

<400> SEQUENCE: 416

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<400> SEQUENCE: 417

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<210> SEQ ID NO 418

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 418

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 419

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<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 420

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<210> SEQ ID NO 421

<211> LENGTH: 33

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 421

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<210> SEQ ID NO 422

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 422

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<210> SEQ ID NO 423

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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oligonucleotide

<400> SEQUENCE: 423

cagaccacct gtggtctcct actggtgac 29

<210> SEQ ID NO 424
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 424

gctttgattt ccctaggg 18

<210> SEQ ID NO 425
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 425

gctttgattt ccagttctta ggcaa 25

<210> SEQ ID NO 426
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 426

gctttgattc ttaggcaa 18

<210> SEQ ID NO 427
<211> LENGTH: 62
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (13)..(53)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 427

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aa 62

<210> SEQ ID NO 428
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 428

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<210> SEQ ID NO 429
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 429

tcttaacccat taccatagag ttcttaggca ac 32

<210> SEQ ID NO 430
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 430

tcttaacccat taccaaagtt cttaggcaac 30

<210> SEQ ID NO 431
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 431

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<210> SEQ ID NO 432
<211> LENGTH: 14
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 432

gaaccaaacc cact 14

<210> SEQ ID NO 433
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 433

gaaccaaacc cacttagggg ataa 24

<210> SEQ ID NO 434
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polynucleotide

<400> SEQUENCE: 434

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 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 435

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Lys Val Gly Leu Glu Pro Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys
35     40     45

Gly Lys Ser Phe Ser Asp Pro Gly Ala Leu Val Arg His Gln Arg Thr
50     55     60

His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe
65     70     75     80

Ser Gln Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu
85     90     95

Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp
100    105    110

Asp Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys
115    120    125

Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp His Leu Thr Thr
130    135    140

His Gln Arg Thr His Thr Gly Ala Ala Ala Arg Ala Leu Val Lys Ser
145    150    155    160

Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg His Lys Leu Lys Tyr Val
165    170    175

Pro His Glu Tyr Ile Glu Leu Ile Glu Ile Ala Arg Asn Pro Thr Gln
180    185    190

Asp Arg Ile Leu Glu Met Lys Val Met Glu Phe Phe Met Lys Val Tyr
195    200    205

Gly Tyr Arg Gly Glu His Leu Gly Gly Ser Arg Lys Pro Asp Gly Ala
210    215    220

Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr Gly Val Ile Val Asp Thr
225    230    235    240

Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro Ile Gly Gln Ala Asp Glu
245    250    255

Met Gln Arg Tyr Val Lys Glu Asn Gln Thr Arg Asn Lys His Ile Asn
260    265    270

Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser Ser Val Thr Glu Phe Lys
275    280    285

Phe Leu Phe Val Ser Gly His Phe Lys Gly Asn Tyr Lys Ala Gln Leu
290    295    300

Thr Arg Leu Asn Arg Lys Thr Asn Cys Asn Gly Ala Val Leu Ser Val
305    310    315    320

Glu Glu Leu Leu Ile Gly Gly Glu Met Ile Lys Ala Gly Thr Leu Thr
325    330    335
  
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Gly	Arg	Gly	Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly
			355				360						365		
Pro	Arg	Ser	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp
			370				375						380		
Ile	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg
			385				390						395		
Lys	Val	Gly	Leu	Glu	Pro	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys
			405				410						415		
Gly	Lys	Ser	Phe	Ser	Gln	Ser	Gly	Asp	Leu	Arg	Arg	His	Gln	Arg	Thr
			420				425						430		
His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe
			435				440						445		
Ser	Thr	Ser	Gly	Ser	Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu
			450				455						460		
Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Gln	Arg	Ala
			465				470						475		
His	Leu	Glu	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys
			485				490						495		
Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Thr	His	Leu	Asp	Leu	Ile	Arg
			500				505						510		
His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys
			515				520						525		
Gly	Lys	Ser	Phe	Ser	Arg	Lys	Asp	Asn	Leu	Lys	Asn	His	Gln	Arg	Thr
			530				535						540		
His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe
			545				550						555		
Ser	Gln	Leu	Ala	His	Leu	Arg	Ala	His	Gln	Arg	Thr	His	Thr	Gly	Ala
			565				570						575		
Ala	Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu
			580				585						590		
Leu	Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile
			595				600						605		
Glu	Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val
			610				615						620		
Met	Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly
			625				630						635		
Gly	Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile
			645				650						655		
Asp	Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn
			660				665						670		
Leu	Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Glu	Arg	Tyr	Val	Glu	Glu	Asn
			675				680						685		
Gln	Thr	Arg	Asp	Lys	His	Leu	Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr
			690				695						700		
Pro	Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe
			705				710						715		
Lys	Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	His	Ile	Thr	Asn
			725				730						735		

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Cys Asn Gly Ala Val Leu Ser Val Glu Glu Leu Leu Ile Gly Gly Glu
740 745 750

Met Ile Lys Ala Gly Thr Leu Thr Leu Glu Glu Val Arg Arg Lys Phe
755 760 765

Asn Asn Gly Glu Ile Asn Phe
770 775

<210> SEQ ID NO 436

<211> LENGTH: 4725

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 436

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gacggacgtg ctttgcacgt ccggccccac cgagcgagcg agtgcgcata gagggagtgg	120
ccaactccat cactagaggt atggcagtga cgtaacgcga agcgcgcgaa gcgagaccac	180
gcctaccagc tgcgtcagca gtcagggtgac ccttttgcca cagtttgcca caccacgtgg	240
ccgctgaggg tatatattct cgagtgcgcg aaccaggagc tccattttga ccgcgaaatt	300
tgaacgagca gcagccatgc cgggggttcta cgagattgtc ctgaaggtcc cgagtgcct	360
ggacgagcac ctgccgggca tttctaactc gtttgtaaac tgggtggcgc agaaggaatg	420
ggagctgcgc ccggattctg acatggatcc gaatctgatt gagcaggcac ccctgaccgt	480
ggccgaaaag cttcagcgcg agttcctggt ggagtggcgc cgcgtgagta aggccccgga	540
ggccctcttt tttgtccagt tcgaaaaggg ggagacctac ttccacctgc acgtgctgat	600
tgagaccatc ggggtcaaat ccatggtggt cggccgctac gtgagccaga ttaaagagaa	660
gctggtgacc cgcactacc gcggggtcga gccgcagctt ccgaactggt tcgcggtgac	720
caaaacgcga aatggcgcgc gggggcggaa caaggtggtg gacgactgct acatcccca	780
ctacctgtc cccaagaccc agcccgagct ccagtgggcg tggactaaca tggaccagta	840
tttaagcgc tgtttgaatc tcgcggagcg taaacggctg gtggcgagc atctgacgca	900
cgtgtcgcag acgcaggagc agaacaaga gaatcagaac cccaattctg acgcgccggt	960
catcaggtca aaaacctcag ccaggtacat ggagctggtc ggggtggctgg tggaccgcgg	1020
gatcacgtca gaaaagcaat ggattcagga ggaccaggcc tcgtacatct cttcaacgc	1080
cgcctccaac tcgcggtccc agatcaaggc cgcgctggac aatgcctcca agatcatgag	1140
cctgacaaag acggctccgc actacctggt gggcagcaac ccgcgggagg acattaccaa	1200
aaatcgatc taccaaatcc tggagctgaa cgggtacgat ccgcagtacg cggcctccgt	1260
cttctgggc tgggcgcaaa agaagtccg gaagaggaa accatctggc tctttgggcc	1320
ggccacgacg ggtaaaacca acatcgcgga agccatcgcc cagcccgctc cttctacgg	1380
ctgcgtaaac tggaccaatg agaactttcc cttcaacgat tgcgtcgaca agatggtgat	1440
ctggtgggag gagggcaaga tgacggccaa ggtcgtggag agcgccaagg ccattctggg	1500
cggaaagcaag gtgcgcgtgg accaaaagt caagtcacgc gccagatcg aacccaactc	1560
cgtgatcgtc acctccaaca ccaacatgtg cgcggtgatt gacgggaaca gcaccacctt	1620
cgagcatcag cagccgctgc aggaccggat gtttaaattt gaacttacc gccgtttgga	1680

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cctgacttt	gggaaggtca	ccaaacagga	agtaaaggac	ttttccggt	gggttccga	1740
tcacgtgact	gacgtggctc	atgagttcta	cgtcagaaag	ggtggagcta	agaaacgccc	1800
cgcctccaat	gacgcggatg	taagcgagcc	aaaacggcag	tgcacgtcac	ttgcgcagcc	1860
gacaacgtca	gacgcggaag	caccggcgga	ctacgcggac	aggtaccaa	acaaatgttc	1920
tcgtcacgtg	ggcatgaatc	tgatgctttt	tccctgtaaa	acatgcgaga	gaatgaatca	1980
aatttccaat	gtctgtttta	cgcgtgttca	aagagactgt	ggggaatgct	tccttggaat	2040
gtcagaatct	caaccctgtt	ctgtcgtcaa	aaagaagact	tatcagaaac	tgtgtccaat	2100
tcacatatac	ctgggaaggg	caccgcgagat	tgctgttctg	gcctgcgatt	tggccaatgt	2160
ggacttggat	gactgtgttt	ctgagcaata	aatgacttaa	accaggtatg	gctgctgacg	2220
gttatcttcc	agattggctc	gaggacaacc	tttctgaagg	cattcgtgag	tggtagggctc	2280
tgaaacctgg	agtcctctca	cccaaagcga	accaacaaca	ccaggacaac	cgtcggggctc	2340
ttgtgcttcc	gggttacaaa	tacctcggac	cggtaacgg	actcgacaaa	ggagagccgg	2400
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cgggtgacaa	cccgtacctc	aagtacaacc	acgccgacgc	cgagtttcag	gagcgtcttc	2520
aagaagatac	gtcttttggg	ggcaaccttg	gcagagcagt	cttccaggcc	aaaaagagga	2580
tccttgagcc	tcttggcttg	gttgaggaag	cagctaaaac	ggctcctgga	aagaagaggc	2640
ctgtagatca	gtctcctcag	gaaccggact	catcatctgg	tgtaggcaaa	tcgggcaaac	2700
agcctgccag	aaaaagacta	aatttcggtc	agactggcga	ctcagagtca	gtccagacc	2760
ctcaacctct	cggagaacca	ccagcagccc	ccacaagttt	gggatcta	acaatggctt	2820
caggcgggtg	cgcaccaatg	gcagacaata	acgaggggtc	cgatggagtg	ggtaattcct	2880
caggaaattg	gcattgcgat	tcccaatggc	tgggcgacag	agtcacacc	accagacca	2940
gaacctgggc	cctgccact	tacaacaacc	atctctacaa	gcaaatctcc	agcgttcaa	3000
cgggagcttc	aaacgacaac	cactactttg	gtacagcac	ccctggggg	tattttgact	3060
ttaacagatt	ccactgccac	ttctcaccac	gtgactggca	gcgactcatt	aacaacaact	3120
ggggattccg	gccaagaaa	ctcagcttca	agctcttcaa	catccaagtt	aaagaggta	3180
cgcagaacga	tggcacgacg	actattgcca	ataaccttac	cagcacggtt	caagtgttta	3240
cggactcgga	gtatcagctc	ccgtacgtgc	tcgggtcggc	gcaccaaggc	tgtctccgc	3300
cgtttccagc	ggacgtcttc	atggtccctc	agtatggata	cctcaccctg	aacaacggaa	3360
gtcaagcggg	gggacgtcga	tccttttact	gcctggagta	cttccctctg	cagatgctaa	3420
ggactggaaa	taacttccaa	ttcagctata	ccttcgagga	tgtacctttt	cacagcagct	3480
acgtcacag	ccagagtgtg	gatcgcttga	tgaatcctct	tattgatcag	tatctgtact	3540
acctgaacag	aacgcaagga	acaacctctg	gaacaaccaa	ccaatcacgg	ctgcttttta	3600
gccaggctgg	gcctcagctc	atgtctttgc	aggccagaaa	ttggctacct	gggccctgct	3660
accggcaaca	gagactttca	aagactgcta	acgacaacaa	caacagtaac	tttccttgga	3720
cagcggccag	caaatactcat	ctcaatggcc	gcgactcgct	ggtgaatcca	ggaccagcta	3780
tggccagtc	caaggacgat	gaagaaaaat	ttttccctat	gcacggcaat	ctaataattg	3840
gcaaagaagg	gacaacggca	agtaacgcag	aattagataa	tgtaatgatt	acggatgaag	3900
aagagattcg	taccaccaat	cctgtggcaa	cagagcagta	tggaactgtg	gcaaataact	3960

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tgcagagctc aaatacagct cccacgacta gaactgtcaa tgatcagggg gccttacctg 4020
gcatgggtgtg gcaagatcgt gacgtgtacc ttcaaggacc tatctgggca aagattcctc 4080
acacggatgg acactttcat ccttctcctc tgatgggagg ctttggaactg aaacatccgc 4140
ctcctcaaat catgatcaaa aatactccgg taccggcaaa tcctccgacg actttcagcc 4200
eggccaagtt tgcttcattt atcactcagt actccactgg acagggtcagc gtggaaattg 4260
agtgggagct acagaaagaa aacagcaaac gttggaatcc agagattcag tacacttcca 4320
actacaacaa gtctgttaat gtggacttta ctgtagacac taatggtgtt tatagtgaac 4380
ctcgccctat tggaaccogg tatctcacac gaaacttgta atcctggta atcaataaac 4440
cgtttaattc gtttcagttg aactttggct cttgtgcact tcttatctta tcttgtttcc 4500
atggctactg cgtagataag cagcggcctg cggcgcttgc gcttcgcggt ttacaactgc 4560
tggttaatat ttaactctcg ccatacctct agtgatggag ttggccactc cctctatgcg 4620
cactcgctcg ctcgggtggg cgggacgtgc aaagcacgtc cgtctggcga cctttggtcg 4680
ccaggcccca ccgagcgagc gagtgcgcat agagggagtg gccaa 4725

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<210> SEQ ID NO 437

<211> LENGTH: 737

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 437

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Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1           5           10          15
Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Val Pro Gln Pro
20          25          30
Lys Ala Asn Gln Gln His Gln Asp Asn Arg Arg Gly Leu Val Leu Pro
35          40          45
Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
50          55          60
Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65          70          75          80
Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85          90          95
Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100         105         110
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Ile Leu Glu Pro
115        120        125
Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
130        135        140
Pro Val Asp Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Val Gly
145        150        155        160
Lys Ser Gly Lys Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
165        170        175
Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
180        185        190
Ala Ala Pro Thr Ser Leu Gly Ser Asn Thr Met Ala Ser Gly Gly Gly
195        200        205

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Ala	Pro	Met	Ala	Asp	Asn	Asn	Glu	Gly	Ala	Asp	Gly	Val	Gly	Asn	Ser
210					215						220				
Ser	Gly	Asn	Trp	His	Cys	Asp	Ser	Gln	Trp	Leu	Gly	Asp	Arg	Val	Ile
225				230						235					240
Thr	Thr	Ser	Thr	Arg	Thr	Trp	Ala	Leu	Pro	Thr	Tyr	Asn	Asn	His	Leu
				245					250					255	
Tyr	Lys	Gln	Ile	Ser	Ser	Ala	Ser	Thr	Gly	Ala	Ser	Asn	Asp	Asn	His
			260					265					270		
Tyr	Phe	Gly	Tyr	Ser	Thr	Pro	Trp	Gly	Tyr	Phe	Asp	Phe	Asn	Arg	Phe
		275					280					285			
His	Cys	His	Phe	Ser	Pro	Arg	Asp	Trp	Gln	Arg	Leu	Ile	Asn	Asn	Asn
290						295					300				
Trp	Gly	Phe	Arg	Pro	Lys	Lys	Leu	Ser	Phe	Lys	Leu	Phe	Asn	Ile	Gln
305					310					315					320
Val	Lys	Glu	Val	Thr	Gln	Asn	Asp	Gly	Thr	Thr	Thr	Ile	Ala	Asn	Asn
				325					330					335	
Leu	Thr	Ser	Thr	Val	Gln	Val	Phe	Thr	Asp	Ser	Glu	Tyr	Gln	Leu	Pro
			340					345					350		
Tyr	Val	Leu	Gly	Ser	Ala	His	Gln	Gly	Cys	Leu	Pro	Pro	Phe	Pro	Ala
		355					360					365			
Asp	Val	Phe	Met	Val	Pro	Gln	Tyr	Gly	Tyr	Leu	Thr	Leu	Asn	Asn	Gly
370						375					380				
Ser	Gln	Ala	Val	Gly	Arg	Ser	Ser	Phe	Tyr	Cys	Leu	Glu	Tyr	Phe	Pro
385					390					395					400
Ser	Gln	Met	Leu	Arg	Thr	Gly	Asn	Asn	Phe	Gln	Phe	Ser	Tyr	Thr	Phe
				405					410					415	
Glu	Asp	Val	Pro	Phe	His	Ser	Ser	Tyr	Ala	His	Ser	Gln	Ser	Leu	Asp
			420					425					430		
Arg	Leu	Met	Asn	Pro	Leu	Ile	Asp	Gln	Tyr	Leu	Tyr	Tyr	Leu	Asn	Arg
		435					440					445			
Thr	Gln	Gly	Thr	Thr	Ser	Gly	Thr	Thr	Asn	Gln	Ser	Arg	Leu	Leu	Phe
450						455					460				
Ser	Gln	Ala	Gly	Pro	Gln	Ser	Met	Ser	Leu	Gln	Ala	Arg	Asn	Trp	Leu
465					470					475					480
Pro	Gly	Pro	Cys	Tyr	Arg	Gln	Gln	Arg	Leu	Ser	Lys	Thr	Ala	Asn	Asp
				485					490					495	
Asn	Asn	Asn	Ser	Asn	Phe	Pro	Trp	Thr	Ala	Ala	Ser	Lys	Tyr	His	Leu
			500					505					510		
Asn	Gly	Arg	Asp	Ser	Leu	Val	Asn	Pro	Gly	Pro	Ala	Met	Ala	Ser	His
		515						520				525			
Lys	Asp	Asp	Glu	Glu	Lys	Phe	Phe	Pro	Met	His	Gly	Asn	Leu	Ile	Phe
530						535					540				
Gly	Lys	Glu	Gly	Thr	Thr	Ala	Ser	Asn	Ala	Glu	Leu	Asp	Asn	Val	Met
545					550					555					560
Ile	Thr	Asp	Glu	Glu	Glu	Ile	Arg	Thr	Thr	Asn	Pro	Val	Ala	Thr	Glu
				565				570						575	
Gln	Tyr	Gly	Thr	Val	Ala	Asn	Asn	Leu	Gln	Ser	Ser	Asn	Thr	Ala	Pro
			580					585					590		
Thr	Thr	Arg	Thr	Val	Asn	Asp	Gln	Gly	Ala	Leu	Pro	Gly	Met	Val	Trp
		595					600					605			
Gln	Asp	Arg	Asp	Val	Tyr	Leu	Gln	Gly	Pro	Ile	Trp	Ala	Lys	Ile	Pro

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610				615				620							
His	Thr	Asp	Gly	His	Phe	His	Pro	Ser	Pro	Leu	Met	Gly	Gly	Phe	Gly
625				630						635					640
Leu	Lys	His	Pro	Pro	Pro	Gln	Ile	Met	Ile	Lys	Asn	Thr	Pro	Val	Pro
			645						650					655	
Ala	Asn	Pro	Pro	Thr	Thr	Phe	Ser	Pro	Ala	Lys	Phe	Ala	Ser	Phe	Ile
		660						665					670		
Thr	Gln	Tyr	Ser	Thr	Gly	Gln	Val	Ser	Val	Glu	Ile	Glu	Trp	Glu	Leu
		675					680					685			
Gln	Lys	Glu	Asn	Ser	Lys	Arg	Trp	Asn	Pro	Glu	Ile	Gln	Tyr	Thr	Ser
	690					695				700					
Asn	Tyr	Asn	Lys	Ser	Val	Asn	Val	Asp	Phe	Thr	Val	Asp	Thr	Asn	Gly
	705				710					715					720
Val	Tyr	Ser	Glu	Pro	Arg	Pro	Ile	Gly	Thr	Arg	Tyr	Leu	Thr	Arg	Asn
			725							730				735	

Leu

<210> SEQ ID NO 438

<211> LENGTH: 406

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 438

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5					10					15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
		20						25					30		
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
		35					40					45			
Lys	Ser	Phe	Ser	Arg	Lys	Asp	Ala	Leu	Arg	Gly	His	Gln	Arg	Thr	His
	50					55					60				
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser
	65				70					75					80
His	Arg	Thr	Thr	Leu	Thr	Asn	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys
			85						90					95	
Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Gln	Arg	Asn	Ala
		100						105					110		
Leu	Ala	Gly	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys
		115					120					125			
Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	His	Lys	Asn	Ala	Leu	Gln	Asn	His
	130					135					140				
Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
	145				150					155					160
Lys	Ser	Phe	Ser	Asp	Pro	Gly	His	Leu	Val	Arg	His	Gln	Arg	Thr	His
			165						170					175	
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser
		180					185						190		
Thr	Ser	Gly	Asn	Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Ala	Ala
		195					200					205			
Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu

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210					215					220					
Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu
225					230					235					240
Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met
			245						250					255	
Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly
			260					265					270		
Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp
		275					280					285			
Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu
	290					295					300				
Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Glu	Arg	Tyr	Val	Glu	Glu	Asn	Gln
305						310					315				320
Thr	Arg	Asp	Lys	His	Leu	Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro
				325					330					335	
Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys
			340					345					350		
Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	His	Ile	Thr	Asn	Cys
		355						360				365			
Asn	Gly	Ala	Val	Leu	Ser	Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met
	370					375					380				
Ile	Lys	Ala	Gly	Thr	Leu	Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn
385						390					395				400
Asn	Gly	Glu	Ile	Asn	Phe										
			405												

<210> SEQ ID NO 439

<211> LENGTH: 406

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 439

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5					10					15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
		20						25					30		
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
		35				40					45				
Lys	Ser	Phe	Ser	Gln	Gln	Arg	Ser	Leu	Val	Gly	His	Gln	Arg	Thr	His
	50					55					60				
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser
65				70					75					80	
Asp	Lys	Lys	Asp	Leu	Thr	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys
			85					90						95	
Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Thr	Ser	Gly	His
			100					105						110	
Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys
			115				120					125			
Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Gln	Arg	Ala	His	Leu	Glu	Arg	His
	130						135					140			

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Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	145	150	155	160
Lys	Ser	Phe	Ser	Thr	Ser	Gly	Ser	Leu	Val	Arg	His	Gln	Arg	Thr	His	165	170	175	
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	180	185	190	
Thr	Ser	Gly	Asn	Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Ala	Ala	195	200	205	
Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu	210	215	220	
Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu	225	230	235	240
Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met	245	250	255	
Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly	260	265	270	
Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp	275	280	285	
Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu	290	295	300	
Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Gln	Arg	Tyr	Val	Lys	Glu	Asn	Gln	305	310	315	320
Thr	Arg	Asn	Lys	His	Ile	Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro	325	330	335	
Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys	340	345	350	
Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	Arg	Lys	Thr	Asn	Cys	355	360	365	
Asn	Gly	Ala	Val	Leu	Ser	Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met	370	375	380	
Ile	Lys	Ala	Gly	Thr	Leu	Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn	385	390	395	400
Asn	Gly	Glu	Ile	Asn	Phe											405			

<210> SEQ ID NO 440

<211> LENGTH: 320

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 440

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp	1	5	10	15
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val	20	25	30	
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Arg	Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met	35	40	45	
Arg	Asn	Phe	Ser	Ser	Lys	Gln	Ala	Leu	Ala	Val	His	Thr	Arg	Thr	His	50	55	60	
Thr	Gly	Glu	Lys	Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met	Arg	Asn	Phe	Ser	65	70	75	80

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Gln	Ser	Thr	Thr	Leu	Lys	Arg	His	Leu	Arg	Thr	His	Thr	Gly	Glu	Lys
				85					90					95	
Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met	Arg	Asn	Phe	Ser	Arg	Ser	Asp	His
			100					105					110		
Leu	Ser	Leu	His	Leu	Lys	Thr	His	Leu	Arg	Gly	Ser	Gln	Leu	Val	Lys
			115				120					125			
Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu	Arg	His	Lys	Leu	Lys	Tyr
			130			135					140				
Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu	Ile	Ala	Arg	Asn	Pro	Thr
145					150					155					160
Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met	Glu	Phe	Phe	Met	Lys	Val
				165					170					175	
Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly	Ser	Arg	Lys	Pro	Asp	Gly
			180					185					190		
Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp	Tyr	Gly	Val	Ile	Val	Asp
			195				200					205			
Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu	Pro	Ile	Gly	Gln	Ala	Asp
						215					220				
Glu	Met	Gln	Arg	Tyr	Val	Lys	Glu	Asn	Gln	Thr	Arg	Asn	Lys	His	Ile
225					230					235					240
Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro	Ser	Ser	Val	Thr	Glu	Phe
				245					250					255	
Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys	Gly	Asn	Tyr	Lys	Ala	Gln
			260					265					270		
Leu	Thr	Arg	Leu	Asn	Arg	Lys	Thr	Asn	Cys	Asn	Gly	Ala	Val	Leu	Ser
			275				280					285			
Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met	Ile	Lys	Ala	Gly	Thr	Leu
						295					300				
Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn	Asn	Gly	Glu	Ile	Asn	Phe
305					310					315					320

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<210> SEQ ID NO 441
<211> LENGTH: 320
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polypeptide
```

<400> SEQUENCE: 441

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5					10					15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
			20					25					30		
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Arg	Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met
		35					40					45			
Arg	Asn	Phe	Ser	Arg	Arg	Ala	His	Leu	Gln	Asn	His	Thr	Arg	Thr	His
	50					55					60				
Thr	Gly	Glu	Lys	Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met	Arg	Asn	Phe	Ser
65					70					75					80
Gln	Ser	Thr	Thr	Leu	Lys	Arg	His	Leu	Arg	Thr	His	Thr	Gly	Glu	Lys
				85					90					95	
Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met	Arg	Asn	Phe	Ser	Asp	Gly	Gly	His

-continued

100					105					110					
Leu	Thr	Arg	His	Leu	Lys	Thr	His	Leu	Arg	Gly	Ser	Gln	Leu	Val	Lys
		115					120				125				
Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu	Arg	His	Lys	Leu	Lys	Tyr
	130					135					140				
Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu	Ile	Ala	Arg	Asn	Pro	Thr
	145					150				155					160
Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met	Glu	Phe	Phe	Met	Lys	Val
			165							170					175
Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly	Ser	Arg	Lys	Pro	Asp	Gly
			180					185					190		
Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp	Tyr	Gly	Val	Ile	Val	Asp
		195					200					205			
Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu	Pro	Ile	Gly	Gln	Ala	Asp
	210					215					220				
Glu	Met	Glu	Arg	Tyr	Val	Glu	Glu	Asn	Gln	Thr	Arg	Asp	Lys	His	Leu
	225					230				235					240
Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro	Ser	Ser	Val	Thr	Glu	Phe
			245							250					255
Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys	Gly	Asn	Tyr	Lys	Ala	Gln
		260					265						270		
Leu	Thr	Arg	Leu	Asn	His	Ile	Thr	Asn	Cys	Asn	Gly	Ala	Val	Leu	Ser
		275					280					285			
Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met	Ile	Lys	Ala	Gly	Thr	Leu
	290					295					300				
Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn	Asn	Gly	Glu	Ile	Asn	Phe
	305					310				315					320

<210> SEQ ID NO 442
 <211> LENGTH: 42
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 oligonucleotide

<400> SEQUENCE: 442
 caaactagaa atgccatctt ccttgatggt ggaggtacct gc 42

<210> SEQ ID NO 443
 <211> LENGTH: 406
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polypeptide

<400> SEQUENCE: 443

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5					10					15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
		20					25						30		
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
		35				40					45				
Lys	Ser	Phe	Ser	Arg	Lys	Asp	Ala	Leu	Arg	Gly	His	Gln	Arg	Thr	His
	50					55					60				

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Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	65	70	75	80
His	Arg	Thr	Thr	Leu	Thr	Asn	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	85	90	95	
Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Gln	Arg	Asn	Ala	100	105	110	
Leu	Ala	Gly	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	115	120	125	
Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	His	Lys	Asn	Ala	Leu	Gln	Asn	His	130	135	140	
Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	145	150	155	160
Lys	Ser	Phe	Ser	Asp	Pro	Gly	His	Leu	Val	Arg	His	Gln	Arg	Thr	His	165	170	175	
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	180	185	190	
Thr	Ser	Gly	Asn	Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Ala	Ala	195	200	205	
Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu	210	215	220	
Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu	225	230	235	240
Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met	245	250	255	
Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly	260	265	270	
Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp	275	280	285	
Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu	290	295	300	
Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Glu	Arg	Tyr	Val	Glu	Glu	Asn	Gln	305	310	315	320
Thr	Arg	Asp	Lys	His	Leu	Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro	325	330	335	
Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys	340	345	350	
Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	His	Ile	Thr	Asn	Cys	355	360	365	
Asn	Gly	Ala	Val	Leu	Ser	Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met	370	375	380	
Ile	Lys	Ala	Gly	Thr	Leu	Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn	385	390	395	400
Asn	Gly	Glu	Ile	Asn	Phe											405			

<210> SEQ ID NO 444

<211> LENGTH: 406

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

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<400> SEQUENCE: 444

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5					10					15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
		20						25					30		
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
		35					40					45			
Lys	Ser	Phe	Ser	Gln	Gln	Arg	Ser	Leu	Val	Gly	His	Gln	Arg	Thr	His
	50					55					60				
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser
65					70					75				80	
Asp	Lys	Lys	Asp	Leu	Thr	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys
				85					90					95	
Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Thr	Ser	Gly	His
			100					105						110	
Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys
		115					120					125			
Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Gln	Arg	Ala	His	Leu	Glu	Arg	His
	130					135					140				
Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
145					150					155				160	
Lys	Ser	Phe	Ser	Thr	Ser	Gly	Ser	Leu	Val	Arg	His	Gln	Arg	Thr	His
				165					170					175	
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser
		180						185					190		
Thr	Ser	Gly	Asn	Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Ala	Ala
		195					200					205			
Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu
	210					215					220				
Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu
225				230						235				240	
Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met
				245				250						255	
Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly
		260						265					270		
Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp
		275					280					285			
Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu
	290					295					300				
Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Gln	Arg	Tyr	Val	Lys	Glu	Asn	Gln
305				310						315				320	
Thr	Arg	Asn	Lys	His	Ile	Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro
				325					330					335	
Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys
			340					345					350		
Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	Arg	Lys	Thr	Asn	Cys
		355					360					365			
Asn	Gly	Ala	Val	Leu	Ser	Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met
	370					375					380				
Ile	Lys	Ala	Gly	Thr	Leu	Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn
385					390					395					400

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Asn Gly Glu Ile Asn Phe
405

<210> SEQ ID NO 445
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: May or may not be present
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: May or may not be present

<400> SEQUENCE: 445

tatctgccca tgactggcgc agggg

25

<210> SEQ ID NO 446
<211> LENGTH: 320
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polypeptide

<400> SEQUENCE: 446

Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
1 5 10 15
Tyr Lys Asp Asp Asp Lys Met Ala Pro Lys Lys Lys Arg Lys Val
20 25 30
Gly Arg Leu Glu Pro Gly Glu Arg Pro Phe Gln Cys Arg Ile Cys Met
35 40 45
Arg Asn Phe Ser Ser Lys Gln Ala Leu Ala Val His Thr Arg Thr His
50 55 60
Thr Gly Glu Lys Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser
65 70 75 80
Gln Ser Thr Thr Leu Lys Arg His Leu Arg Thr His Thr Gly Glu Lys
85 90 95
Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp His
100 105 110
Leu Ser Leu His Leu Lys Thr His Leu Arg Gly Ser Gln Leu Val Lys
115 120 125
Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg His Lys Leu Lys Tyr
130 135 140
Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile Ala Arg Asn Pro Thr
145 150 155 160
Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu Phe Phe Met Lys Val
165 170 175
Tyr Gly Tyr Arg Gly Glu His Leu Gly Gly Ser Arg Lys Pro Asp Gly
180 185 190
Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr Gly Val Ile Val Asp
195 200 205
Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro Ile Gly Gln Ala Asp

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210					215					220						
Glu	Met	Gln	Arg	Tyr	Val	Lys	Glu	Asn	Gln	Thr	Arg	Asn	Lys	His	Ile	
225					230					235				240		
Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro	Ser	Ser	Val	Thr	Glu	Phe	
				245					250					255		
Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys	Gly	Asn	Tyr	Lys	Ala	Gln	
				260					265					270		
Leu	Thr	Arg	Leu	Asn	Arg	Lys	Thr	Asn	Cys	Asn	Gly	Ala	Val	Leu	Ser	
				275					280					285		
Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met	Ile	Lys	Ala	Gly	Thr	Leu	
				290					295					300		
Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn	Asn	Gly	Glu	Ile	Asn	Phe	
305					310					315				320		

<210> SEQ ID NO 447

<211> LENGTH: 320

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 447

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5				10						15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
			20					25					30		
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Arg	Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met
			35				40					45			
Arg	Asn	Phe	Ser	Arg	Arg	Ala	His	Leu	Gln	Asn	His	Thr	Arg	Thr	His
			50				55					60			
Thr	Gly	Glu	Lys	Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met	Arg	Asn	Phe	Ser
			65			70			75					80	
Gln	Ser	Thr	Thr	Leu	Lys	Arg	His	Leu	Arg	Thr	His	Thr	Gly	Glu	Lys
			85					90						95	
Pro	Phe	Gln	Cys	Arg	Ile	Cys	Met	Arg	Asn	Phe	Ser	Asp	Gly	Gly	His
			100				105						110		
Leu	Thr	Arg	His	Leu	Lys	Thr	His	Leu	Arg	Gly	Ser	Gln	Leu	Val	Lys
			115				120					125			
Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu	Arg	His	Lys	Leu	Lys	Tyr
			130			135			140						
Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu	Ile	Ala	Arg	Asn	Pro	Thr
			145			150			155					160	
Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met	Glu	Phe	Phe	Met	Lys	Val
			165					170						175	
Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly	Ser	Arg	Lys	Pro	Asp	Gly
			180					185					190		
Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp	Tyr	Gly	Val	Ile	Val	Asp
			195				200					205			
Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu	Pro	Ile	Gly	Gln	Ala	Asp
			210			215					220				
Glu	Met	Glu	Arg	Tyr	Val	Glu	Glu	Asn	Gln	Thr	Arg	Asp	Lys	His	Leu
225					230					235					240

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Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro	Ser	Ser	Val	Thr	Glu	Phe
				245					250					255	
Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys	Gly	Asn	Tyr	Lys	Ala	Gln
		260						265					270		
Leu	Thr	Arg	Leu	Asn	His	Ile	Thr	Asn	Cys	Asn	Gly	Ala	Val	Leu	Ser
		275				280						285			
Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met	Ile	Lys	Ala	Gly	Thr	Leu
	290				295						300				
Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn	Asn	Gly	Glu	Ile	Asn	Phe
305				310					315						320

<210> SEQ ID NO 448
 <211> LENGTH: 42
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 oligonucleotide

<400> SEQUENCE: 448

actagaaatg ccatcttctc tgatgttgga ggtacctgct ct 42

<210> SEQ ID NO 449
 <211> LENGTH: 406
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polypeptide

<400> SEQUENCE: 449

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

-continued

195					200					205					
Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu
210						215					220				
Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu
225						230					235				240
Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met
				245					250					255	
Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly
			260					265					270		
Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp
		275					280					285			
Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu
290						295					300				
Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Glu	Arg	Tyr	Val	Glu	Glu	Asn	Gln
305						310					315				320
Thr	Arg	Asp	Lys	His	Leu	Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro
				325					330					335	
Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys
			340					345					350		
Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	His	Ile	Thr	Asn	Cys
		355					360					365			
Asn	Gly	Ala	Val	Leu	Ser	Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met
370						375					380				
Ile	Lys	Ala	Gly	Thr	Leu	Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn
385						390					395				400
Asn	Gly	Glu	Ile	Asn	Phe										
			405												

<210> SEQ ID NO 450

<211> LENGTH: 406

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 450

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5					10					15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
			20					25					30		
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
		35				40					45				
Lys	Ser	Phe	Ser	Gln	Arg	Asn	Ala	Leu	Ala	Gly	His	Gln	Arg	Thr	His
	50					55					60				
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser
65					70						75				80
Gln	Gln	Arg	Ser	Leu	Val	Gly	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys
				85					90					95	
Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Asp	Lys	Lys	Asp
			100					105					110		
Leu	Thr	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys
						120							125		

-continued

Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Thr	Ser	Gly	His	Leu	Val	Arg	His
130						135					140				
Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
145					150					155					160
Lys	Ser	Phe	Ser	Gln	Arg	Ala	His	Leu	Glu	Arg	His	Gln	Arg	Thr	His
				165					170					175	
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser
			180					185					190		
Thr	Ser	Gly	Ser	Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Ala	Ala
		195					200					205			
Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys	Lys	Ser	Glu	Leu
		210				215					220				
Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile	Glu	Leu	Ile	Glu
225					230					235					240
Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu	Met	Lys	Val	Met
				245					250					255	
Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu	His	Leu	Gly	Gly
			260					265					270		
Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly	Ser	Pro	Ile	Asp
		275					280					285			
Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly	Gly	Tyr	Asn	Leu
	290					295					300				
Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Gln	Arg	Tyr	Val	Lys	Glu	Asn	Gln
305					310					315					320
Thr	Arg	Asn	Lys	His	Ile	Asn	Pro	Asn	Glu	Trp	Trp	Lys	Val	Tyr	Pro
				325					330					335	
Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser	Gly	His	Phe	Lys
			340					345					350		
Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	Arg	Lys	Thr	Asn	Cys
		355					360					365			
Asn	Gly	Ala	Val	Leu	Ser	Val	Glu	Glu	Leu	Leu	Ile	Gly	Gly	Glu	Met
	370					375					380				
Ile	Lys	Ala	Gly	Thr	Leu	Thr	Leu	Glu	Glu	Val	Arg	Arg	Lys	Phe	Asn
385					390					395					400
Asn	Gly	Glu	Ile	Asn	Phe										
				405											

<210> SEQ ID NO 451

<211> LENGTH: 378

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 451

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5					10					15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
			20					25					30		
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
		35				40					45				
Lys	Ser	Phe	Ser	Gln	Arg	Asn	Ala	Leu	Ala	Gly	His	Gln	Arg	Thr	His
	50					55					60				

-continued

Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	65	70	75	80
His	Lys	Asn	Ala	Leu	Gln	Asn	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	85	90	95	
Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Asp	Pro	Gly	His	100	105	110	
Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	115	120	125	
Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Thr	Ser	Gly	Asn	Leu	Val	Arg	His	130	135	140	
Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	145	150	155	160
Lys	Ser	Phe	Ser	Gln	Ser	Ser	Asn	Leu	Val	Arg	His	Gln	Arg	Thr	His	165	170	175	
Thr	Gly	Ala	Ala	Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys	180	185	190	
Lys	Ser	Glu	Leu	Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile	195	200	205	
Glu	Leu	Ile	Glu	Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu	210	215	220	
Met	Lys	Val	Met	Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu	225	230	235	240
His	Leu	Gly	Gly	Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly	245	250	255	
Ser	Pro	Ile	Asp	Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly	260	265	270	
Gly	Tyr	Asn	Leu	Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Glu	Arg	Tyr	Val	275	280	285	
Glu	Glu	Asn	Gln	Thr	Arg	Asp	Lys	His	Leu	Asn	Pro	Asn	Glu	Trp	Trp	290	295	300	
Lys	Val	Tyr	Pro	Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser	305	310	315	320
Gly	His	Phe	Lys	Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	His	325	330	335	
Ile	Thr	Asn	Cys	Asn	Gly	Ala	Val	Leu	Ser	Val	Glu	Glu	Leu	Leu	Ile	340	345	350	
Gly	Gly	Glu	Met	Ile	Lys	Ala	Gly	Thr	Leu	Thr	Leu	Glu	Glu	Val	Arg	355	360	365	
Arg	Lys	Phe	Asn	Asn	Gly	Glu	Ile	Asn	Phe							370	375		

<210> SEQ ID NO 452

<211> LENGTH: 378

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 452

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp	1	5	10	15

Tyr Lys Asp Asp Asp Lys Met Ala Pro Lys Lys Lys Arg Lys Val

-continued

20					25					30					
Gly	Arg	Leu	Glu	Pro	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
	35						40					45			
Lys	Ser	Phe	Ser	Gln	Gln	Arg	Ser	Leu	Val	Gly	His	Gln	Arg	Thr	His
	50					55					60				
Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser
	65					70					75				80
Asp	Lys	Lys	Asp	Leu	Thr	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys
			85						90					95	
Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Thr	Ser	Gly	His
			100					105						110	
Leu	Val	Arg	His	Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys
		115					120					125			
Pro	Glu	Cys	Gly	Lys	Ser	Phe	Ser	Gln	Arg	Ala	His	Leu	Glu	Arg	His
	130					135					140				
Gln	Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Lys	Cys	Pro	Glu	Cys	Gly
	145					150					155				160
Lys	Ser	Phe	Ser	Thr	Ser	Gly	Ser	Leu	Val	Arg	His	Gln	Arg	Thr	His
			165						170					175	
Thr	Gly	Ala	Ala	Ala	Arg	Ala	Leu	Val	Lys	Ser	Glu	Leu	Glu	Glu	Lys
		180					185						190		
Lys	Ser	Glu	Leu	Arg	His	Lys	Leu	Lys	Tyr	Val	Pro	His	Glu	Tyr	Ile
		195					200					205			
Glu	Leu	Ile	Glu	Ile	Ala	Arg	Asn	Pro	Thr	Gln	Asp	Arg	Ile	Leu	Glu
	210					215					220				
Met	Lys	Val	Met	Glu	Phe	Phe	Met	Lys	Val	Tyr	Gly	Tyr	Arg	Gly	Glu
	225					230					235				240
His	Leu	Gly	Gly	Ser	Arg	Lys	Pro	Asp	Gly	Ala	Ile	Tyr	Thr	Val	Gly
			245						250					255	
Ser	Pro	Ile	Asp	Tyr	Gly	Val	Ile	Val	Asp	Thr	Lys	Ala	Tyr	Ser	Gly
			260					265					270		
Gly	Tyr	Asn	Leu	Pro	Ile	Gly	Gln	Ala	Asp	Glu	Met	Gln	Arg	Tyr	Val
	275						280					285			
Lys	Glu	Asn	Gln	Thr	Arg	Asn	Lys	His	Ile	Asn	Pro	Asn	Glu	Trp	Trp
	290					295					300				
Lys	Val	Tyr	Pro	Ser	Ser	Val	Thr	Glu	Phe	Lys	Phe	Leu	Phe	Val	Ser
	305					310					315				320
Gly	His	Phe	Lys	Gly	Asn	Tyr	Lys	Ala	Gln	Leu	Thr	Arg	Leu	Asn	Arg
			325						330					335	
Lys	Thr	Asn	Cys	Asn	Gly	Ala	Val	Leu	Ser	Val	Glu	Glu	Leu	Leu	Ile
		340					345						350		
Gly	Gly	Glu	Met	Ile	Lys	Ala	Gly	Thr	Leu	Thr	Leu	Glu	Glu	Val	Arg
	355						360					365			
Arg	Lys	Phe	Asn	Asn	Gly	Glu	Ile	Asn	Phe						
	370						375								

<210> SEQ ID NO 453

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: May or may not be present
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (26)..(26)
<223> OTHER INFORMATION: May or may not be present

<400> SEQUENCE: 453

tgccatcttc cttgatgttg gaggtg

26

<210> SEQ ID NO 454
<211> LENGTH: 322
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 454

Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
1 5 10 15

Tyr Lys Asp Asp Asp Lys Met Ala Pro Lys Lys Lys Arg Lys Val
20 25 30

Gly Arg Leu Glu Pro Gly Glu Arg Pro Phe Gln Cys Arg Ile Cys Met
35 40 45

Arg Asn Phe Ser Ser Pro Ser Lys Leu Ala Arg His Thr Arg Thr His
50 55 60

Thr Gly Glu Lys Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser
65 70 75 80

Val Arg His Asn Leu Thr Arg His Leu Arg Thr His Thr Gly Glu Lys
85 90 95

Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Gln Arg Asn Asn
100 105 110

Leu Gly Arg His Leu Lys Thr His Thr Gly Ala Ala Ala Arg Ala Leu
115 120 125

Val Lys Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg His Lys Leu
130 135 140

Lys Tyr Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile Ala Arg Asn
145 150 155 160

Pro Thr Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu Phe Phe Met
165 170 175

Lys Val Tyr Gly Tyr Arg Gly Glu His Leu Gly Gly Ser Arg Lys Pro
180 185 190

Asp Gly Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr Gly Val Ile
195 200 205

Val Asp Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro Ile Gly Gln
210 215 220

Ala Asp Glu Met Gln Arg Tyr Val Lys Glu Asn Gln Thr Arg Asn Lys
225 230 235 240

His Ile Asn Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser Ser Val Thr
245 250 255

Glu Phe Lys Phe Leu Phe Val Ser Gly His Phe Lys Gly Asn Tyr Lys
260 265 270

Ala Gln Leu Thr Arg Leu Asn Arg Lys Thr Asn Cys Asn Gly Ala Val

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275	280	285
Leu Ser Val Glu Glu Leu	Leu Ile Gly Gly Glu Met	Ile Lys Ala Gly
290	295	300
Thr Leu Thr Leu Glu Glu	Val Arg Arg Lys Phe Asn Asn Gly Glu Ile	
305	310	315 320
Asn Phe		
<210> SEQ ID NO 455		
<211> LENGTH: 322		
<212> TYPE: PRT		
<213> ORGANISM: Artificial Sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide		
<400> SEQUENCE: 455		
Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp		
1	5	10 15
Tyr Lys Asp Asp Asp Asp Lys Met Ala Pro Lys Lys Lys Arg Lys Val		
	20	25 30
Gly Arg Leu Glu Pro Gly Glu Arg Pro Phe Gln Cys Arg Ile Cys Met		
	35	40 45
Arg Asn Phe Ser Ile Pro Asn His Leu Ala Arg His Thr Arg Thr His		
	50	55 60
Thr Gly Glu Lys Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser		
	65	70 75 80
Gln Ser Ala His Leu Lys Arg His Leu Arg Thr His Thr Gly Glu Lys		
	85	90 95
Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser His His Asn Ser		
	100	105 110
Leu Thr Arg His Leu Lys Thr His Thr Gly Ala Ala Ala Arg Ala Leu		
	115	120 125
Val Lys Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg His Lys Leu		
	130	135 140
Lys Tyr Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile Ala Arg Asn		
	145	150 155 160
Pro Thr Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu Phe Phe Met		
	165	170 175
Lys Val Tyr Gly Tyr Arg Gly Glu His Leu Gly Gly Ser Arg Lys Pro		
	180	185 190
Asp Gly Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr Gly Val Ile		
	195	200 205
Val Asp Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro Ile Gly Gln		
	210	215 220
Ala Asp Glu Met Glu Arg Tyr Val Glu Glu Asn Gln Thr Arg Asp Lys		
	225	230 235 240
His Leu Asn Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser Ser Val Thr		
	245	250 255
Glu Phe Lys Phe Leu Phe Val Ser Gly His Phe Lys Gly Asn Tyr Lys		
	260	265 270
Ala Gln Leu Thr Arg Leu Asn His Ile Thr Asn Cys Asn Gly Ala Val		
	275	280 285
Leu Ser Val Glu Glu Leu Leu Ile Gly Gly Glu Met Ile Lys Ala Gly		

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290	295	300
Thr Leu Thr Leu Glu Glu Val Arg Arg Lys Phe Asn Asn Gly Glu Ile		
305	310	315 320

Asn Phe

<210> SEQ ID NO 456
 <211> LENGTH: 6626
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 456

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gggggggggg gggggggggt ggccactccc tctctgcgcg ctgcctcgct cactgaggcc    60
gggcgaccaa aggtcgcccg acgcccgggc ttgcccggg cggcctcagt gagcgagcga    120
gcgcgagag agggagtggc caactccatc actaggggtt cctagatctg aattcggtac    180
ccgttacata acttacggta aatggccgcg ctggctgacc gcccaacgac ccccgcccat    240
tgacgtcaat aatgacgtat gttcccatag taacgccaat agggactttc cattgacgtc    300
aatgggtgga gtatttacgg taaactgccc acttggcagt acatcaagtg tatcatatgc    360
caagtacgcc ccctattgac gtcaatgacg gtaaatggcc cgcttggeat tatgcccagt    420
acatgacctt atgggacttt cctacttggc agtacatcta cgtattagtc atcgctatta    480
ccatggtgat gcggttttgg cagtacatca atgggcgtgg atagcggttt gactcacggg    540
gatttccaag tctccacccc attgacgtca atgggagttt gttttggcac caaatcaac    600
gggactttcc aaaatgtcgt aacaactccg ccccatgac gcaaatgggc ggtaggcggtg    660
tacggtggga ggtctatata agcagagctc gtttagtgaa cgcgcagatc gcctggagac    720
gccatccaag ctgttttgac ctccatagaa gacacgggga ccgatccagc ctccggactc    780
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gtggatgttg cctttacttc taggcctgta cggaagtgtt acttotgctc taaaagctgc    960
ggaattgtac ccgcggcccg ggatccaccg gtggctagcg tctataggcc ccccccttg    1020
gtggaattcg ccatgaggtc tgactacaaa gaccatgacg gtgattataa agatcatgac    1080
atcgattaca aggatgacga tgacaagatg gcccccaaga agaagaggaa ggtgggectc    1140
gagcccgag aaaaaccgta caagtgccct gagtgcggga aatcattctc cgaccctggg    1200
gcgctcgtec ggcaccaaag gacgcataca ggggaaaagc cgtataagtg ccccgagtgt    1260
ggaaagagct tctcgagag agcccacctt gaacgacacc aaagaacaca cactggtgag    1320
aaacctata agtggtccaga gtgcggcaaa tcgttttagca gatccgatga cttggtgcgc    1380
caccagcgga cacacggg tgaaaagccc tacaatgcc cgagtggtgg gaagtcgttt    1440
tcaaggtcgg atcatctgac taccatcag cgcaccata cgggagcggc cgcccgcgcc    1500
ctggtgaaga gcgagctgga ggagaagaag tccgagctgc ggcacaagct gaagtacgtg    1560
ccccacgagt acatcgagct gatcgagatc gccaggaacc ccaccagga ccgcctcctg    1620
gagatgaagg tgatggagtt cttcatgaag gtgtacggct acaggggaga gcacctgggc    1680
ggaagcagaa agcctgacgg cgccatctat acagtgggca gcccacga ttacggcggtg    1740

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atgcagagat	acgtgaagga	gaaccagacc	cgaataaagc	acatcaaccc	caacgagtgg	1860
tggaaggtgt	accctagcag	cgtgaccgag	ttcaagttcc	tggtcgtgag	cgccacttc	1920
aagggcaact	acaaggccca	gctgaccagg	ctgaaccgca	aaaccaactg	caatggcgcc	1980
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<210> SEQ ID NO 457
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 457

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<210> SEQ ID NO 458
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 458

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<210> SEQ ID NO 459
 <211> LENGTH: 3252
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 459

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<210> SEQ ID NO 460
<211> LENGTH: 83
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 460

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<210> SEQ ID NO 461
<211> LENGTH: 3333
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

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<400> SEQUENCE: 461

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<210> SEQ ID NO 462

<211> LENGTH: 120

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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polynucleotide

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<210> SEQ ID NO 463

<211> LENGTH: 144

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 463

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<210> SEQ ID NO 464

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 464

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<210> SEQ ID NO 465

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 465

tctaaggccg agtcttatga gcag 24

<210> SEQ ID NO 466

<211> LENGTH: 4564

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 466

atggactaca aagaccatga cgttgattat aaagatcatg acatcgatta caaggatgac 60

gatgacaaga tggcccccaa gaagaagagg aaggtgggcc gcggaatgga caagaagtac 120

tccattgggc tcgccatcgg cacaaacagc gtcggctggg ccgtcattac ggacgagtac 180

aaggtgccga gcaaaaaatt caaagttctg ggcaataccg atcgccacag cataaagaag 240

aacctcattg gcgccctcct gttcgactcc ggggaaaccg ccgaagccac gcggctcaaa 300

agaacagcac ggcgcagata taccgcgaga aagaatcgga tctgctacct gcaggagatc 360

tttagtaatg agatggctaa ggtggatgac tctttcttcc ataggctgga ggagtccttt 420

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ttggtggagg	aggataaaaa	gcacgagcgc	cacccaatct	ttggcaatat	cgtggacgag	480
gtggcgtacc	atgaaaagta	cccaaccata	tatcatctga	ggaagaagct	tgtagacagt	540
actgataagg	ctgacttgcg	gttgatctat	ctcgcgctgg	cgcataatgat	caaatctcgg	600
ggacacttcc	tcacgagagg	ggacctgaac	ccagacaaca	gcgatgtcga	caaactcttt	660
atccaactgg	ttcagactta	caatcagctt	ttcgaagaga	acccgatcaa	cgcacccgga	720
gttgacgcc	aagcaatcct	gagcgctagg	ctgtccaaat	cccggcggtc	cgaaaacctc	780
atcgcacagc	tccttgggga	gaagaagaac	ggcctgtttg	gtaatcttat	cgccctgtca	840
ctcggtctga	cccccaactt	taaatctaac	ttcgacctgg	ccgaagatgc	caagcttcaa	900
ctgagcaaag	acacctacga	tgatgatctc	gacaatctgc	tggcccagat	cgcgaccag	960
tacgcagacc	tttttttggc	ggcaaagaac	ctgtcagacg	ccattctgct	gagtgatatt	1020
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gatgagcacc	accaagactt	gactttgctg	aaggcccttg	tcagacagca	actgcctgag	1140
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ggagcaagcc	aggaggaatt	ttacaaattt	attaagccca	tcttgaaaaa	aatggacggc	1260
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caagaggatt	tctaccctct	tttgaaagat	aacagggaaa	agattgagaa	aatcctcaca	1440
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actcgcaaat	cagaagagac	catcactccc	tggaaacttcg	aggaagtcgt	ggataagggg	1560
gcctctgccc	agtccttcat	cgaaggatg	actaactttg	ataaaaatct	gcctaacgaa	1620
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gaagactatt	tcaaaaagat	tgaatgtttc	gactctgttg	aaatcagcgg	agtggaggat	1860
cgcttcaacg	catccctggg	aacgtatcac	gatctcctga	aaatcattaa	agacaaggac	1920
ttcctggaca	atgaggagaa	cgaggacatt	cttgaggaca	ttgtcctcac	ccttacgttg	1980
tttgaaagata	gggagatgat	tgaagaacgc	ttgaaaactt	acgctcatct	cttcgacgac	2040
aaagtcatga	aacagctcaa	gaggcgccga	tatacaggat	gggggcggct	gtcaagaaaa	2100
ctgatcaatg	ggatccgaga	caagcagagt	ggaaagacaa	tcctggattt	tcttaagtcc	2160
gatggatttg	ccaaccggaa	cttcatgcag	ttgatccatg	atgactctct	cacctttaag	2220
gaggacatcc	agaaagcaca	agtttctggc	cagggggaca	gtcttcacga	gcacatcgct	2280
aatcttgcat	gtagcccagc	tatcaaaaag	ggaataactgc	agaccgttaa	ggctgtggat	2340
gaactcgtca	aagtaatggg	aaggcataag	cccgagaata	tcgttatcga	gatggcccga	2400
gagaacccaa	ctaccagaa	gggacagaag	aacagtaggg	aaaggatgaa	gaggattgaa	2460
gagggataaa	aagaactggg	gtcccaaatc	cttaaggaac	acccagttga	aaacaccag	2520
cttcagaatg	agaagctcta	cctgtactac	ctgcagaacg	gcagggacat	gtacgtggat	2580
caggaactgg	acatcaatcg	gctctccgac	tacgacgtgg	atgccatcgt	gccccagtct	2640
tttctcaaag	atgattctat	tgataataaa	gtgttgacaa	gatccgataa	aaatagaggg	2700

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aagagtgata acgtccctc agaagaagtt gtcaagaaaa tgaaaaatta ttggcggcag	2760
ctgctgaacg ccaaaactgat cacacaacgg aagttcgata atctgactaa ggctgaacga	2820
ggcggcctgt ctgagttgga taaagccggc ttcacaaaa ggcagcttgt tgagacacgc	2880
cagatcacca agcacgtggc ccaaattctc gattcacgca tgaacaccaa gtacgatgaa	2940
aatgacaaac tgattcgaga ggtgaaagtt attactctga agtctaagct ggtctcagat	3000
ttcagaaaagg actttcagtt ttataagggt agagagatca acaattacca ccatgcgcgt	3060
gatgcctacc tgaatgcagt ggtaggcact gcacttatca aaaaatatcc caagcttgaa	3120
tctgaatttg ttacggaga ctataaagtg tacgatgta ggaaaatgat cgcaaagtct	3180
gagcaggaaa taggcaaggc caccgctaag tacttctttt acagcaatat tatgaatttt	3240
ttcaagacgg agattacact ggccaatgga gagattcgga agcgaccact tatcgaaaca	3300
aacggagaaa caggagaaat cgtgtgggac aagggtagggt atttcgcgac agtcgggaag	3360
gtcctgtcca tgccgcaggt gaacatcgtt aaaaagaccg aagtacagac cggaggcttc	3420
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tgggacccca agaaatacgg cggattcgat tctcctacag tcgcttacag tgtactggtt	3540
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cttgaataacg gccggaaaac aatgctcgct agtgcgggag agctgcagaa aggtaacgag	3780
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cttgatgaga tcacgagca aataagcgaa ttctccaaaa gagtgatect cgccgacgct	3960
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gcagaaaaca ttatccactt gtttactctg accaacttgg gcgcgcctgc agccttcaag	4080
tacttcgaca ccaccataga cagaaagcgg tacacctcta caaaggaggt cctggacgcc	4140
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ggcggagaca gcagggtgga ccccaagaag aagaggaagg tggctagcga tgctaagtca	4260
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gaggagtgga agctgctgga cactgctcag cagatcctgt acagaaatgt gatgctggag	4380
aactataaga acctgggttc cttgggttat cagcttacta agccagatgt gatcctccgg	4440
ttggagaagg gagaagagcc ctggctggtg gagagagaaa ttcaccaaga gaccatcct	4500
gattcagaga ctgcatttga aatcaaatca tcagttccga aaaagaaacg caaagttgct	4560
agcg	4564

<210> SEQ ID NO 467

<211> LENGTH: 388

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 467

taagcttcag ttttctcta gttcctgtta cattctgtg tgtctccatt agtgacctcc	60

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catagtccaa gcatgagcag ttctggccag gcccctgtcg gggtcagtgc cccacccccg	120
ccttctgggt ctgtgtaacc ttctaagcaa accttctggc tcaagcacag caatgctgag	180
tcatgatgag tcatgctgag gcttaggggtg tgtgcccaga tgttctcagc ctagagtgat	240
gactcctatc tgggtcccca gcaggatgct tacagggcag atggcaaaaa aaaggagaag	300
ctgaccacct gactaaaact ccacctcaaa cggcatcata aagaaaatgg atgctgaga	360
cagaatgtga catattctag aatatatt	388

<210> SEQ ID NO 468
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 468

cagccgctcg ctgcagcag	19

<210> SEQ ID NO 469
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 469

ctgctgcagc gacggctg	19

<210> SEQ ID NO 470
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 470

gctgggtgtc ccattgaaa	19

<210> SEQ ID NO 471
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 471

tttcaatggg acaccagc	19

<210> SEQ ID NO 472
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 472

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gtttattcag ccgggagtc 19

<210> SEQ ID NO 473
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 473

gactcccggc tgaataaac 19

<210> SEQ ID NO 474
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 474

tggagagttt gcaaggagc 19

<210> SEQ ID NO 475
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 475

gctccttgca aactctcca 19

<210> SEQ ID NO 476
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 476

ccctccagac tttccacct 19

<210> SEQ ID NO 477
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 477

aggtggaaag tctggaggg 19

<210> SEQ ID NO 478
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 478

aattttcttc caagttctc 19

<210> SEQ ID NO 479

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 479

gagaacttgg aagaaaatt 19

<210> SEQ ID NO 480

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 480

ctgctggagag aagaaaggg 19

<210> SEQ ID NO 481

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 481

ccctttcttc tctccgcag 19

<210> SEQ ID NO 482

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 482

agagccaccc cctggctcc 19

<210> SEQ ID NO 483

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 483

ggagccaggg ggtggctct 19

<210> SEQ ID NO 484

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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oligonucleotide

<400> SEQUENCE: 484

cgaagccaac cgcggcggg 19

<210> SEQ ID NO 485
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 485

cccgcgcgg ttggcttcg 19

<210> SEQ ID NO 486
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 486

agagggaaga cgatcgccc 19

<210> SEQ ID NO 487
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 487

gggcgatcgt cttccctct 19

<210> SEQ ID NO 488
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 488

cccctttaac tttcctccg 19

<210> SEQ ID NO 489
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 489

cggaggaaag ttaaagggg 19

<210> SEQ ID NO 490
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 490

gcagccccgc ttccttcaa 19

<210> SEQ ID NO 491
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 491

ttgaaggaag cggggctgc 19

<210> SEQ ID NO 492
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 492

caccgcagcc gctcgctgca gcag 24

<210> SEQ ID NO 493
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 493

aaacctgctg cagcgagcgg ctgc 24

<210> SEQ ID NO 494
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 494

caccggctgg gtgtccatt gaaa 24

<210> SEQ ID NO 495
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 495

aaactttcaa tgggacacc agcc 24

<210> SEQ ID NO 496
<211> LENGTH: 24

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 496

caccggttta ttcagccggg agtc 24

<210> SEQ ID NO 497
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 497

aaacgactcc cggctgaata aacc 24

<210> SEQ ID NO 498
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 498

caccgtggag agtttgcaag gagc 24

<210> SEQ ID NO 499
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 499

aaacgctcct tgcaaaactct ccac 24

<210> SEQ ID NO 500
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 500

caccgccctc cagactttcc acct 24

<210> SEQ ID NO 501
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 501

aaacaggtgg aaagtctgga gggc 24

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<210> SEQ ID NO 502
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 502

caccgaattt tcttccaagt tctc 24

<210> SEQ ID NO 503
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 503

aaacgagaac ttggaagaaa attc 24

<210> SEQ ID NO 504
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 504

caccgctgcg gagagaagaa aggg 24

<210> SEQ ID NO 505
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 505

aaaccccttt cttctctcgc cagc 24

<210> SEQ ID NO 506
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 506

caccgagagc cacccttg ctcc 24

<210> SEQ ID NO 507
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 507

aaacggagcc agggggtggc tctc 24

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<210> SEQ ID NO 508
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 508

caccgcgaag ccaaccgcgg cggg 24

<210> SEQ ID NO 509
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 509

aaaccccgcc gcggttggtc tcgc 24

<210> SEQ ID NO 510
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 510

caccgagagg gaagacgatc gccc 24

<210> SEQ ID NO 511
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 511

aaacgggcga tcgtcttccc tctc 24

<210> SEQ ID NO 512
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 512

caccgccctt ttaactttcc tccg 24

<210> SEQ ID NO 513
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 513

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aaaccggagg aaagttaaag gggc 24

<210> SEQ ID NO 514
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 514

caccggcagc cccgcttctc tcaa 24

<210> SEQ ID NO 515
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 515

aaacttgaag gaagcggggc tgcc 24

<210> SEQ ID NO 516
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 516

cgagagcgag aggagggag 19

<210> SEQ ID NO 517
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 517

ctccctctctc tcgctctcg 19

<210> SEQ ID NO 518
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 518

gagagagctt gagagcgcg 19

<210> SEQ ID NO 519
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 519

cgcgctctca agctctctc

19

<210> SEQ ID NO 520

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 520

ggtggagggg gcggggccc

19

<210> SEQ ID NO 521

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 521

gggccccgcc ccctccacc

19

<210> SEQ ID NO 522

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 522

ggtatccacg taaatcaaa

19

<210> SEQ ID NO 523

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 523

tttgatttac gtggatacc

19

<210> SEQ ID NO 524

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 524

ccaatcactg gctccggtc

19

<210> SEQ ID NO 525

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 525

gaccggagcc agtgattgg 19

<210> SEQ ID NO 526
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 526

ggcgcccgag ggaagaaga 19

<210> SEQ ID NO 527
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 527

tcttcttccc tcgggcgcc 19

<210> SEQ ID NO 528
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 528

gggtgggggt accagagga 19

<210> SEQ ID NO 529
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 529

tcctctggta cccccaccc 19

<210> SEQ ID NO 530
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 530

ccggggacag aagagaggg 19

<210> SEQ ID NO 531
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<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 531

ccctctcttc tgtcccg 19

<210> SEQ ID NO 532
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 532

gagagagagt gggagaagc 19

<210> SEQ ID NO 533
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 533

gcttctccca ctctctctc 19

<210> SEQ ID NO 534
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 534

aaagtaactg tcaaatgcg 19

<210> SEQ ID NO 535
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 535

cgcatTTgac agttacttt 19

<210> SEQ ID NO 536
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 536

ttaaccagag cgcccagtc 19

<210> SEQ ID NO 537

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<211> LENGTH: 19
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 537

gactggg'gcgc tctgg’ttaa 19

<210> SEQ ID NO 538
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 538

cgtcggagct gcccgctag 19

<210> SEQ ID NO 539
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 539

ctagcgggca gctccgacg 19

<210> SEQ ID NO 540
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 540

caccgcgaga gcgagaggag ggag 24

<210> SEQ ID NO 541
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 541

aaacctccct cctctcgctc tcgc 24

<210> SEQ ID NO 542
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 542

caccggagag agcttgagag cgcg 24

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<210> SEQ ID NO 543
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 543

aaaccgcgct ctcaagctct ctcc 24

<210> SEQ ID NO 544
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 544

caccgggtgg agggggcggg gcc 24

<210> SEQ ID NO 545
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 545

aaacggggccc cgccccctcc accc 24

<210> SEQ ID NO 546
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 546

caccgggtat ccacgtaaat caaa 24

<210> SEQ ID NO 547
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 547

aaactttgat ttacgtggat accc 24

<210> SEQ ID NO 548
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 548

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caccgccaat cactggctcc ggtc 24

<210> SEQ ID NO 549
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 549

aaacgaccgg agccagtgat tggc 24

<210> SEQ ID NO 550
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 550

caccggg'gcgc ccgagggaag aaga 24

<210> SEQ ID NO 551
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 551

aaactcttct tccctcgggc gccc 24

<210> SEQ ID NO 552
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 552

caccgggggtg ggggtaccag agga 24

<210> SEQ ID NO 553
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 553

aaactcctct ggtaccccca cccc 24

<210> SEQ ID NO 554
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 554

caccgccggg gacagaagag aggg 24

<210> SEQ ID NO 555

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 555

aaacccctct cttctgtccc cggc 24

<210> SEQ ID NO 556

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 556

caccggagag agagtgggag aagc 24

<210> SEQ ID NO 557

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 557

aaacgcttct cccactctct ctcc 24

<210> SEQ ID NO 558

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 558

caccgaaagt aactgtcaaa tgcg 24

<210> SEQ ID NO 559

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 559

aaaccgcatt tgacagttac ttcc 24

<210> SEQ ID NO 560

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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oligonucleotide

<400> SEQUENCE: 560

caccgttaac cagagcgccc agtc 24

<210> SEQ ID NO 561
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 561

aaacgactgg gcgctctggt taac 24

<210> SEQ ID NO 562
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 562

caccgcgtcg gagctgcccc ctag 24

<210> SEQ ID NO 563
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 563

aaacctagcg ggcagctccg acgc 24

<210> SEQ ID NO 564
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 564

gagacacaca gaaatgtaac 20

<210> SEQ ID NO 565
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 565

ggtggggcac tgaccccgac 20

<210> SEQ ID NO 566
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 566

ctagagtgat gactcctatc 20

<210> SEQ ID NO 567
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 567

gactaaaact ccacctcaaa 20

<210> SEQ ID NO 568
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 568

aatatgtcac attctgtctc 20

<210> SEQ ID NO 569
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 569

ggactatggg aggtcactaa 20

<210> SEQ ID NO 570
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 570

gctcatgctt ggactatggg 20

<210> SEQ ID NO 571
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 571

gttctggcca ggcccctgtc 20

<210> SEQ ID NO 572
<211> LENGTH: 20

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 572

agtgccccac cccgccttc 20

<210> SEQ ID NO 573
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 573

gtggggcact gaccccgaca 20

<210> SEQ ID NO 574
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 574

aaccttctaa gcaaaccttc 20

<210> SEQ ID NO 575
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 575

gttacacaga accagaaggc 20

<210> SEQ ID NO 576
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 576

gaaggttaca cagaaccaga 20

<210> SEQ ID NO 577
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 577

agtcatgatg agtcatgctg 20

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<210> SEQ ID NO 578
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 578

gatgagtcac gctgaggctt 20

<210> SEQ ID NO 579
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 579

actctaggct gagaacatct 20

<210> SEQ ID NO 580
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 580

gtccccagca ggatgcttac 20

<210> SEQ ID NO 581
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 581

gccctgtaag catcctgctg 20

<210> SEQ ID NO 582
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 582

cagggcagat ggcaaaaaaa 20

<210> SEQ ID NO 583
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 583

gaggtggagt tttagtcagg 20

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<210> SEQ ID NO 584
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 584

aaacggcatc ataaagaaaa 20

<210> SEQ ID NO 585
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polynucleotide

<400> SEQUENCE: 585

gagacacaca gaaatgtaac gttttagagc tagaaatagc aagttaaaat aaggctagtc 60

cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 586
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polynucleotide

<400> SEQUENCE: 586

ggtggggcac tgaccccgac gttttagagc tagaaatagc aagttaaaat aaggctagtc 60

cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 587
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polynucleotide

<400> SEQUENCE: 587

ctagagtgat gactcctatc gttttagagc tagaaatagc aagttaaaat aaggctagtc 60

cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 588
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polynucleotide

<400> SEQUENCE: 588

gactaaaact ccacctcaaa gttttagagc tagaaatagc aagttaaaat aaggctagtc 60

cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 589

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<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 589

aatatgtcac attctgtctc gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 590
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 590

ggactatggg aggtcactaa gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 591
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 591

gctcatgctt ggactatggg gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 592
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 592

gttctggcca ggcccctgtc gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 593
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 593

agtgccccac ccccgcttc gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 594
<211> LENGTH: 105

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 594

gtggggcact gaccccgaca gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt ttccc 105

<210> SEQ ID NO 595
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 595

aaccttctaa gcaaaccttc gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt ttccc 105

<210> SEQ ID NO 596
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 596

gttacacaga accagaaggc gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt ttccc 105

<210> SEQ ID NO 597
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 597

gaagggttaca cagaaccaga gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt ttccc 105

<210> SEQ ID NO 598
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 598

agtcacgatg agtcacgctg gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt ttccc 105

<210> SEQ ID NO 599
<211> LENGTH: 105
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 599

gatgagtcac gctgaggctt gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 600
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 600

actctaggct gagaacatct gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 601
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 601

gtccccgaca ggatgcttac gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 602
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 602

gccctgtaag catcctgctg gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 603
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 603

cagggcagat ggcaaaaaaa gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tttcc 105

<210> SEQ ID NO 604
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 604

gagggtggagt tttagtcagg gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt ttcc 105

<210> SEQ ID NO 605
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 605

aaacggcatc ataagaaaa gttttagagc tagaaatagc aagttaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt ttcc 105

<210> SEQ ID NO 606
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 606

tgtactctct gaggtgctc 19

<210> SEQ ID NO 607
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 607

acgcagataa gaaccagtt 19

<210> SEQ ID NO 608
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 608

catcaagtca gccatcagc 19

<210> SEQ ID NO 609
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 609

gagtcaccct cctggaaac 19

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<210> SEQ ID NO 610
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 610

cctgggctcc ggggcgttt 19

<210> SEQ ID NO 611
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 611

ggccctgcg gccaccgcg 19

<210> SEQ ID NO 612
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 612

ctccctcct gcccgtag 19

<210> SEQ ID NO 613
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 613

aggtttgaa agggcgtgc 19

<210> SEQ ID NO 614
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 614

actccactgc actccagtct 20

<210> SEQ ID NO 615
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 615

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tctgtggggg acctgcactg 20

<210> SEQ ID NO 616
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 616

ggggcgccag ttgtgtctcc 20

<210> SEQ ID NO 617
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 617

acaccattgc caccaccatt 20

<210> SEQ ID NO 618
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 618

caatgacccc ttcattgacc 20

<210> SEQ ID NO 619
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 619

ttgattttgg agggatctcg 20

<210> SEQ ID NO 620
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 620

ggaatccatg gagggaagat 20

<210> SEQ ID NO 621
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

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<400> SEQUENCE: 621

tggtctcgct caggtcagtg

20

<210> SEQ ID NO 622

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 622

ctctctgctc ctttgccaca

20

<210> SEQ ID NO 623

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 623

gtgctcttcg gggttcagga

20

<210> SEQ ID NO 624

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 624

cgaaagagaa agcgaaccag tatcgagaac

30

<210> SEQ ID NO 625

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 625

cgttgtgcat agtcgctgct tgatcgc

27

<210> SEQ ID NO 626

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 626

gagtttggtc caaattgtta ctctt

25

<210> SEQ ID NO 627

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 627

gggaaatggt ctaggagagt aaagt 25

<210> SEQ ID NO 628
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 628

gattggcttt gatttccta ggg 23

<210> SEQ ID NO 629
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 629

gcctactcag actgttactc tgg 23

<210> SEQ ID NO 630
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 630

gcagttgcct aagaactggt ggg 23

<210> SEQ ID NO 631
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 631

ggggctccac cctcacgagt ggg 23

<210> SEQ ID NO 632
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 632

gccttcttta tcccctatcg agg 23

<210> SEQ ID NO 633
<211> LENGTH: 48
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(45)

<400> SEQUENCE: 633

gaa gga cca ttt gac gtt cag ctc cta ctc aga ctg tta ctc tgg tga 48
Glu Gly Pro Phe Asp Val Gln Leu Leu Leu Arg Leu Leu Leu Trp
1 5 10 15

<210> SEQ ID NO 634
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 634

Glu Gly Pro Phe Asp Val Gln Leu Leu Leu Arg Leu Leu Leu Trp
1 5 10 15

<210> SEQ ID NO 635
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(42)

<400> SEQUENCE: 635

gaa gga cca ttt gac gtt cag ctc cta ctc aga ctg tct ctg gtga 46
Glu Gly Pro Phe Asp Val Gln Leu Leu Leu Arg Leu Ser Leu
1 5 10

<210> SEQ ID NO 636
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
peptide

<400> SEQUENCE: 636

Glu Gly Pro Phe Asp Val Gln Leu Leu Leu Arg Leu Ser Leu
1 5 10

<210> SEQ ID NO 637
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 637

gccuacucag acuguuacuc 20

<210> SEQ ID NO 638
<211> LENGTH: 19

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 638

cccaccagtt cttaggcaa 19

<210> SEQ ID NO 639
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 639

cccaccagtt cttaggcaac 20

<210> SEQ ID NO 640
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 640

tttgatttcc agttcttagg 20

<210> SEQ ID NO 641
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 641

attaccatag agttcttag 19

<210> SEQ ID NO 642
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 642

gtggataaag gcaacaatg 19

<210> SEQ ID NO 643
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 643

cctcgatagg ggataa 16

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<210> SEQ ID NO 644
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 644

accaaaccga cttaggggat aaa 23

<210> SEQ ID NO 645
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 645

ggtatcttaa ggacctccaa g 21

<210> SEQ ID NO 646
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 646

taggaagtaa attaatgtga agcttgccaa ttaatccaaa tcttacc 47

<210> SEQ ID NO 647
<211> LENGTH: 83
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 647

tttccttttt gcaaaaccga aaatatttta gctcctactc agactgttag agtaggagga 60

gaaggagggc tgtggctctt gtg 83

<210> SEQ ID NO 648
<211> LENGTH: 83
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (31)..(31)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(69)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 648

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```
aaaacccccca aaccctcccn ctttttcttc ntactctaac agtgtgagta ggaggccaca      60
cttagctgng gctctagtaa gtc                                              83
```

```
<210> SEQ ID NO 649
<211> LENGTH: 94
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (50)..(50)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
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<400> SEQUENCE: 649

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cttctttttt cttttttgca aaaacccaaa atatatttagc tcctactcan accgttttcg      60
gggggtatatt tgtatttgga tgaaacactt gatg                                  94
```

```
<210> SEQ ID NO 650
<211> LENGTH: 93
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 650

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tcttcttttt tccttccgcg aaaaacccaa aatattttcg ctctactca ggacctggtc      60
gtggggtatt ttgtatttgg atgaaacact tga                                  93
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```
<210> SEQ ID NO 651
<211> LENGTH: 104
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (21)..(22)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
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<400> SEQUENCE: 651

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tcaaggaaga tggcatttct nntttggaga tggcagtttc cttagtaacc acaggttgtg      60
tcaccatggc cagtaagaag gatgccacac ttgtgtgtgt cgct                      104
```

```
<210> SEQ ID NO 652
<211> LENGTH: 86
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (35)..(36)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (42)..(44)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
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<400> SEQUENCE: 652

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ttggagatag cagtttcctt ggtaagtaca ggtnntcac cnnnacagtg tgagtaggag	60
gccacactta gctgtggctc tagtaa	86

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<210> SEQ ID NO 653
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (54)..(54)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (61)..(63)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(69)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

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<400> SEQUENCE: 653

tttctagttt ggagatggca gtttccttag taaccacagg ttgtgtcacc aggnctcgag	60
nnntgggtnt g	71

```

<210> SEQ ID NO 654
<211> LENGTH: 87
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

```

<400> SEQUENCE: 654

cagttgccat agtcttcact aaccgcgtca ccagagctca ggacctggtc gtgggggtatt	60
ttgtatttgg atgaacact tgatggt	87

```

<210> SEQ ID NO 655
<211> LENGTH: 92
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (52)..(52)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (59)..(59)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (78)..(78)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

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<400> SEQUENCE: 655

tattgtatct tggtttttct tcaactgctgg ccagtttact aacaatctga antaagccna	60
ttaatccaaa tcttacnnga cgaaaaatca aa	92

```

<210> SEQ ID NO 656
<211> LENGTH: 89

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: a, c, t, g, unknown or other
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (57)..(57)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 656

atggtntttt gtcattgcng gccagttttn taacaatgtg atcaatgcc aattaatncaa      60
atcttaccag aggaaaaatc aagccacag                                     89

<210> SEQ ID NO 657
<211> LENGTH: 94
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (93)..(94)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 657

aataaaaaga aaaaaatatg ataaactgct cccagtataa aatacagagc taagacaaga      60
acgtttcatt ggctgttaaa agtcggaaat cgnn                                     94

<210> SEQ ID NO 658
<211> LENGTH: 94
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer

<400> SEQUENCE: 658

ttctgattta aaattccctt gaataggaag taaattaatt tgaagcttgc caattaatcc      60
aaatcttacc agaggaaaaa tcaagccaca gata                                     94

<210> SEQ ID NO 659
<211> LENGTH: 90
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer

<400> SEQUENCE: 659
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caaaacaaga tgggataaaa tcttctttct gatttaaaat tcccttgaat aggaagtaaa 60

ttactagagc tgttcaaggc ctgaactcta 90

<210> SEQ ID NO 660

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (49)..(49)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (51)..(52)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (54)..(54)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 660

ctgatttaaa attccttga ataggaagta aattactaga gctgttcang nncngaactc 60

taggctagca tttccagccc aaaacaaaca cagttc 96

<210> SEQ ID NO 661

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (8)..(26)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 661

ttgtttgnnn nnnnnnnnnn nnnnnn 26

<210> SEQ ID NO 662

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (6)..(24)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 662

caccgnnnnn nnnnnnnnnn nnnn 24

<210> SEQ ID NO 663

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<220> FEATURE:

<221> NAME/KEY: modified_base

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<222> LOCATION: (6)..(24)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 663

tcccannnnn nnnnnnnnnn nnnn 24

<210> SEQ ID NO 664
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (6)..(24)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 664

cctcgnnnnn nnnnnnnnnn nnnn 24

<210> SEQ ID NO 665
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (5)..(23)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 665

aaacnnnnnn nnnnnnnnnn nnncaa 26

<210> SEQ ID NO 666
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (5)..(23)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 666

aaacnnnnnn nnnnnnnnnn nnnn 24

<210> SEQ ID NO 667
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (5)..(23)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 667

aaacnnnnnn nnnnnnnnnn nnnt 24

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<210> SEQ ID NO 668
<211> LENGTH: 24
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide
<220> FEATURE:
<221> NAME/KEY: modified base
<222> LOCATION: (5)..(23)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 668

aaacnnnnnn nnnnnnnnnn nnnnc 24

<210> SEQ ID NO 669
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 669

ttgtttgtct tcgagaagac ctgttt 26

<210> SEQ ID NO 670
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 670

caccgggtct tcgagaagac ctgttt 26

<210> SEQ ID NO 671
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 671

tcccaggtct tcgagaagac ctgttt 26

<210> SEQ ID NO 672
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 672

cctcgggtct tcgagaagac ctgttt 26

<210> SEQ ID NO 673
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 673

ggcctaccgc taaggctagc ctagt

25

<210> SEQ ID NO 674

<211> LENGTH: 1411

<212> TYPE: PRT

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 674

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

-continued

Tyr	Ala	Asp	Leu	Phe	Leu	Ala	Ala	Lys	Asn	Leu	Ser	Asp	Ala	Ile	Leu	325	330	335	
Leu	Ser	Asp	Ile	Leu	Arg	Val	Asn	Thr	Glu	Ile	Thr	Lys	Ala	Pro	Leu	340	345	350	
Ser	Ala	Ser	Met	Ile	Lys	Arg	Tyr	Asp	Glu	His	His	Gln	Asp	Leu	Thr	355	360	365	
Leu	Leu	Lys	Ala	Leu	Val	Arg	Gln	Gln	Leu	Pro	Glu	Lys	Tyr	Lys	Glu	370	375	380	
Ile	Phe	Phe	Asp	Gln	Ser	Lys	Asn	Gly	Tyr	Ala	Gly	Tyr	Ile	Asp	Gly	385	390	395	400
Gly	Ala	Ser	Gln	Glu	Glu	Phe	Tyr	Lys	Phe	Ile	Lys	Pro	Ile	Leu	Glu	405	410	415	
Lys	Met	Asp	Gly	Thr	Glu	Glu	Leu	Leu	Val	Lys	Leu	Asn	Arg	Glu	Asp	420	425	430	
Leu	Leu	Arg	Lys	Gln	Arg	Thr	Phe	Asp	Asn	Gly	Ser	Ile	Pro	His	Gln	435	440	445	
Ile	His	Leu	Gly	Glu	Leu	His	Ala	Ile	Leu	Arg	Arg	Gln	Glu	Asp	Phe	450	455	460	
Tyr	Pro	Phe	Leu	Lys	Asp	Asn	Arg	Glu	Lys	Ile	Glu	Lys	Ile	Leu	Thr	465	470	475	480
Phe	Arg	Ile	Pro	Tyr	Tyr	Val	Gly	Pro	Leu	Ala	Arg	Gly	Asn	Ser	Arg	485	490	495	
Phe	Ala	Trp	Met	Thr	Arg	Lys	Ser	Glu	Glu	Thr	Ile	Thr	Pro	Trp	Asn	500	505	510	
Phe	Glu	Glu	Val	Val	Asp	Lys	Gly	Ala	Ser	Ala	Gln	Ser	Phe	Ile	Glu	515	520	525	
Arg	Met	Thr	Asn	Phe	Asp	Lys	Asn	Leu	Pro	Asn	Glu	Lys	Val	Leu	Pro	530	535	540	
Lys	His	Ser	Leu	Leu	Tyr	Glu	Tyr	Phe	Thr	Val	Tyr	Asn	Glu	Leu	Thr	545	550	555	560
Lys	Val	Lys	Tyr	Val	Thr	Glu	Gly	Met	Arg	Lys	Pro	Ala	Phe	Leu	Ser	565	570	575	
Gly	Glu	Gln	Lys	Lys	Ala	Ile	Val	Asp	Leu	Leu	Phe	Lys	Thr	Asn	Arg	580	585	590	
Lys	Val	Thr	Val	Lys	Gln	Leu	Lys	Glu	Asp	Tyr	Phe	Lys	Lys	Ile	Glu	595	600	605	
Cys	Phe	Asp	Ser	Val	Glu	Ile	Ser	Gly	Val	Glu	Asp	Arg	Phe	Asn	Ala	610	615	620	
Ser	Leu	Gly	Thr	Tyr	His	Asp	Leu	Leu	Lys	Ile	Ile	Lys	Asp	Lys	Asp	625	630	635	640
Phe	Leu	Asp	Asn	Glu	Glu	Asn	Glu	Asp	Ile	Leu	Glu	Asp	Ile	Val	Leu	645	650	655	
Thr	Leu	Thr	Leu	Phe	Glu	Asp	Arg	Glu	Met	Ile	Glu	Glu	Arg	Leu	Lys	660	665	670	
Thr	Tyr	Ala	His	Leu	Phe	Asp	Asp	Lys	Val	Met	Lys	Gln	Leu	Lys	Arg	675	680	685	
Arg	Arg	Tyr	Thr	Gly	Trp	Gly	Arg	Leu	Ser	Arg	Lys	Leu	Ile	Asn	Gly	690	695	700	
Ile	Arg	Asp	Lys	Gln	Ser	Gly	Lys	Thr	Ile	Leu	Asp	Phe	Leu	Lys	Ser	705	710	715	720
Asp	Gly	Phe	Ala	Asn	Arg	Asn	Phe	Met	Gln	Leu	Ile	His	Asp	Asp	Ser				

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725						730						735					
Leu	Thr	Phe	Lys	Glu	Asp	Ile	Gln	Lys	Ala	Gln	Val	Ser	Gly	Gln	Gly		
			740						745			750					
Asp	Ser	Leu	His	Glu	His	Ile	Ala	Asn	Leu	Ala	Gly	Ser	Pro	Ala	Ile		
			755						760			765					
Lys	Lys	Gly	Ile	Leu	Gln	Thr	Val	Lys	Val	Val	Asp	Glu	Leu	Val	Lys		
			770						775			780					
Val	Met	Gly	Arg	His	Lys	Pro	Glu	Asn	Ile	Val	Ile	Glu	Met	Ala	Arg		
			785						790			795			800		
Glu	Asn	Gln	Thr	Thr	Gln	Lys	Gly	Gln	Lys	Asn	Ser	Arg	Glu	Arg	Met		
			805						810			815					
Lys	Arg	Ile	Glu	Glu	Gly	Ile	Lys	Glu	Leu	Gly	Ser	Gln	Ile	Leu	Lys		
			820						825			830					
Glu	His	Pro	Val	Glu	Asn	Thr	Gln	Leu	Gln	Asn	Glu	Lys	Leu	Tyr	Leu		
			835						840			845					
Tyr	Tyr	Leu	Gln	Asn	Gly	Arg	Asp	Met	Tyr	Val	Asp	Gln	Glu	Leu	Asp		
			850						855			860					
Ile	Asn	Arg	Leu	Ser	Asp	Tyr	Asp	Val	Asp	His	Ile	Val	Pro	Gln	Ser		
			865						870			875			880		
Phe	Leu	Lys	Asp	Asp	Ser	Ile	Asp	Asn	Lys	Val	Leu	Thr	Arg	Ser	Asp		
			885						890			895					
Lys	Asn	Arg	Gly	Lys	Ser	Asp	Asn	Val	Pro	Ser	Glu	Glu	Val	Val	Lys		
			900						905			910					
Lys	Met	Lys	Asn	Tyr	Trp	Arg	Gln	Leu	Leu	Asn	Ala	Lys	Leu	Ile	Thr		
			915						920			925					
Gln	Arg	Lys	Phe	Asp	Asn	Leu	Thr	Lys	Ala	Glu	Arg	Gly	Gly	Leu	Ser		
			930						935			940					
Glu	Leu	Asp	Lys	Ala	Gly	Phe	Ile	Lys	Arg	Gln	Leu	Val	Glu	Thr	Arg		
			945						950			955			960		
Gln	Ile	Thr	Lys	His	Val	Ala	Gln	Ile	Leu	Asp	Ser	Arg	Met	Asn	Thr		
			965						970			975					
Lys	Tyr	Asp	Glu	Asn	Asp	Lys	Leu	Ile	Arg	Glu	Val	Lys	Val	Ile	Thr		
			980						985			990					
Leu	Lys	Ser	Lys	Leu	Val	Ser	Asp	Phe	Arg	Lys	Asp	Phe	Gln	Phe	Tyr		
			995						1000			1005					
Lys	Val	Arg	Glu	Ile	Asn	Asn	Tyr	His	His	Ala	His	Asp	Ala	Tyr			
			1010						1015			1020					
Leu	Asn	Ala	Val	Val	Gly	Thr	Ala	Leu	Ile	Lys	Lys	Tyr	Pro	Lys			
			1025						1030			1035					
Leu	Glu	Ser	Glu	Phe	Val	Tyr	Gly	Asp	Tyr	Lys	Val	Tyr	Asp	Val			
			1040						1045			1050					
Arg	Lys	Met	Ile	Ala	Lys	Ser	Glu	Gln	Glu	Ile	Gly	Lys	Ala	Thr			
			1055						1060			1065					
Ala	Lys	Tyr	Phe	Phe	Tyr	Ser	Asn	Ile	Met	Asn	Phe	Phe	Lys	Thr			
			1070						1075			1080					
Glu	Ile	Thr	Leu	Ala	Asn	Gly	Glu	Ile	Arg	Lys	Arg	Pro	Leu	Ile			
			1085						1090			1095					
Glu	Thr	Asn	Gly	Glu	Thr	Gly	Glu	Ile	Val	Trp	Asp	Lys	Gly	Arg			
			1100						1105			1110					
Asp	Phe	Ala	Thr	Val	Arg	Lys	Val	Leu	Ser	Met	Pro	Gln	Val	Asn			
			1115						1120			1125					

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<210> SEQ ID NO 675
<211> LENGTH: 1483
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 675
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1				5					10					15	
	Tyr	Lys	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
			20					25					30		
Gly	Arg	Gly	Met	Asp	Lys	Lys	Tyr	Ser	Ile	Gly	Leu	Ala	Ile	Gly	Thr

-continued

35					40					45					
Asn	Ser	Val	Gly	Trp	Ala	Val	Ile	Thr	Asp	Glu	Tyr	Lys	Val	Pro	Ser
50						55					60				
Lys	Lys	Phe	Lys	Val	Leu	Gly	Asn	Thr	Asp	Arg	His	Ser	Ile	Lys	Lys
65					70					75					80
Asn	Leu	Ile	Gly	Ala	Leu	Leu	Phe	Asp	Ser	Gly	Glu	Thr	Ala	Glu	Ala
				85					90					95	
Thr	Arg	Leu	Lys	Arg	Thr	Ala	Arg	Arg	Arg	Tyr	Thr	Arg	Arg	Lys	Asn
			100					105					110		
Arg	Ile	Cys	Tyr	Leu	Gln	Glu	Ile	Phe	Ser	Asn	Glu	Met	Ala	Lys	Val
			115					120					125		
Asp	Asp	Ser	Phe	Phe	His	Arg	Leu	Glu	Glu	Ser	Phe	Leu	Val	Glu	Glu
	130					135					140				
Asp	Lys	Lys	His	Glu	Arg	His	Pro	Ile	Phe	Gly	Asn	Ile	Val	Asp	Glu
145					150					155					160
Val	Ala	Tyr	His	Glu	Lys	Tyr	Pro	Thr	Ile	Tyr	His	Leu	Arg	Lys	Lys
				165					170					175	
Leu	Val	Asp	Ser	Thr	Asp	Lys	Ala	Asp	Leu	Arg	Leu	Ile	Tyr	Leu	Ala
			180					185					190		
Leu	Ala	His	Met	Ile	Lys	Phe	Arg	Gly	His	Phe	Leu	Ile	Glu	Gly	Asp
			195				200						205		
Leu	Asn	Pro	Asp	Asn	Ser	Asp	Val	Asp	Lys	Leu	Phe	Ile	Gln	Leu	Val
	210					215					220				
Gln	Thr	Tyr	Asn	Gln	Leu	Phe	Glu	Glu	Asn	Pro	Ile	Asn	Ala	Ser	Gly
225					230					235					240
Val	Asp	Ala	Lys	Ala	Ile	Leu	Ser	Ala	Arg	Leu	Ser	Lys	Ser	Arg	Arg
				245					250					255	
Leu	Glu	Asn	Leu	Ile	Ala	Gln	Leu	Pro	Gly	Glu	Lys	Lys	Asn	Gly	Leu
			260					265					270		
Phe	Gly	Asn	Leu	Ile	Ala	Leu	Ser	Leu	Gly	Leu	Thr	Pro	Asn	Phe	Lys
			275				280					285			
Ser	Asn	Phe	Asp	Leu	Ala	Glu	Asp	Ala	Lys	Leu	Gln	Leu	Ser	Lys	Asp
	290					295					300				
Thr	Tyr	Asp	Asp	Asp	Leu	Asp	Asn	Leu	Leu	Ala	Gln	Ile	Gly	Asp	Gln
305					310					315					320
Tyr	Ala	Asp	Leu	Phe	Leu	Ala	Ala	Lys	Asn	Leu	Ser	Asp	Ala	Ile	Leu
				325					330					335	
Leu	Ser	Asp	Ile	Leu	Arg	Val	Asn	Thr	Glu	Ile	Thr	Lys	Ala	Pro	Leu
			340					345					350		
Ser	Ala	Ser	Met	Ile	Lys	Arg	Tyr	Asp	Glu	His	His	Gln	Asp	Leu	Thr
		355					360					365			
Leu	Leu	Lys	Ala	Leu	Val	Arg	Gln	Gln	Leu	Pro	Glu	Lys	Tyr	Lys	Glu
	370					375					380				
Ile	Phe	Phe	Asp	Gln	Ser	Lys	Asn	Gly	Tyr	Ala	Gly	Tyr	Ile	Asp	Gly
385					390					395					400
Gly	Ala	Ser	Gln	Glu	Glu	Phe	Tyr	Lys	Phe	Ile	Lys	Pro	Ile	Leu	Glu
				405					410					415	
Lys	Met	Asp	Gly	Thr	Glu	Glu	Leu	Leu	Val	Lys	Leu	Asn	Arg	Glu	Asp
			420					425					430		
Leu	Leu	Arg	Lys	Gln	Arg	Thr	Phe	Asp	Asn	Gly	Ser	Ile	Pro	His	Gln
			435					440					445		

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Ile	His	Leu	Gly	Glu	Leu	His	Ala	Ile	Leu	Arg	Arg	Gln	Glu	Asp	Phe
450						455					460				
Tyr	Pro	Phe	Leu	Lys	Asp	Asn	Arg	Glu	Lys	Ile	Glu	Lys	Ile	Leu	Thr
465					470					475					480
Phe	Arg	Ile	Pro	Tyr	Tyr	Val	Gly	Pro	Leu	Ala	Arg	Gly	Asn	Ser	Arg
				485					490					495	
Phe	Ala	Trp	Met	Thr	Arg	Lys	Ser	Glu	Glu	Thr	Ile	Thr	Pro	Trp	Asn
			500					505					510		
Phe	Glu	Glu	Val	Val	Asp	Lys	Gly	Ala	Ser	Ala	Gln	Ser	Phe	Ile	Glu
			515				520					525			
Arg	Met	Thr	Asn	Phe	Asp	Lys	Asn	Leu	Pro	Asn	Glu	Lys	Val	Leu	Pro
	530					535					540				
Lys	His	Ser	Leu	Leu	Tyr	Glu	Tyr	Phe	Thr	Val	Tyr	Asn	Glu	Leu	Thr
545					550					555					560
Lys	Val	Lys	Tyr	Val	Thr	Glu	Gly	Met	Arg	Lys	Pro	Ala	Phe	Leu	Ser
				565					570					575	
Gly	Glu	Gln	Lys	Lys	Ala	Ile	Val	Asp	Leu	Leu	Phe	Lys	Thr	Asn	Arg
			580					585					590		
Lys	Val	Thr	Val	Lys	Gln	Leu	Lys	Glu	Asp	Tyr	Phe	Lys	Lys	Ile	Glu
			595				600					605			
Cys	Phe	Asp	Ser	Val	Glu	Ile	Ser	Gly	Val	Glu	Asp	Arg	Phe	Asn	Ala
	610					615					620				
Ser	Leu	Gly	Thr	Tyr	His	Asp	Leu	Leu	Lys	Ile	Ile	Lys	Asp	Lys	Asp
625					630					635					640
Phe	Leu	Asp	Asn	Glu	Glu	Asn	Glu	Asp	Ile	Leu	Glu	Asp	Ile	Val	Leu
				645					650					655	
Thr	Leu	Thr	Leu	Phe	Glu	Asp	Arg	Glu	Met	Ile	Glu	Glu	Arg	Leu	Lys
			660					665					670		
Thr	Tyr	Ala	His	Leu	Phe	Asp	Asp	Lys	Val	Met	Lys	Gln	Leu	Lys	Arg
		675					680					685			
Arg	Arg	Tyr	Thr	Gly	Trp	Gly	Arg	Leu	Ser	Arg	Lys	Leu	Ile	Asn	Gly
	690					695					700				
Ile	Arg	Asp	Lys	Gln	Ser	Gly	Lys	Thr	Ile	Leu	Asp	Phe	Leu	Lys	Ser
705					710					715					720
Asp	Gly	Phe	Ala	Asn	Arg	Asn	Phe	Met	Gln	Leu	Ile	His	Asp	Asp	Ser
				725					730					735	
Leu	Thr	Phe	Lys	Glu	Asp	Ile	Gln	Lys	Ala	Gln	Val	Ser	Gly	Gln	Gly
			740					745					750		
Asp	Ser	Leu	His	Glu	His	Ile	Ala	Asn	Leu	Ala	Gly	Ser	Pro	Ala	Ile
		755					760					765			
Lys	Lys	Gly	Ile	Leu	Gln	Thr	Val	Lys	Val	Val	Asp	Glu	Leu	Val	Lys
	770					775					780				
Val	Met	Gly	Arg	His	Lys	Pro	Glu	Asn	Ile	Val	Ile	Glu	Met	Ala	Arg
785					790					795					800
Glu	Asn	Gln	Thr	Thr	Gln	Lys	Gly	Gln	Lys	Asn	Ser	Arg	Glu	Arg	Met
				805					810					815	
Lys	Arg	Ile	Glu	Glu	Gly	Ile	Lys	Glu	Leu	Gly	Ser	Gln	Ile	Leu	Lys
			820					825					830		
Glu	His	Pro	Val	Glu	Asn	Thr	Gln	Leu	Gln	Asn	Glu	Lys	Leu	Tyr	Leu
			835				840						845		

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Tyr	Tyr	Leu	Gln	Asn	Gly	Arg	Asp	Met	Tyr	Val	Asp	Gln	Glu	Leu	Asp
850						855					860				
Ile	Asn	Arg	Leu	Ser	Asp	Tyr	Asp	Val	Asp	Ala	Ile	Val	Pro	Gln	Ser
865					870					875					880
Phe	Leu	Lys	Asp	Asp	Ser	Ile	Asp	Asn	Lys	Val	Leu	Thr	Arg	Ser	Asp
				885					890					895	
Lys	Asn	Arg	Gly	Lys	Ser	Asp	Asn	Val	Pro	Ser	Glu	Glu	Val	Val	Lys
			900					905					910		
Lys	Met	Lys	Asn	Tyr	Trp	Arg	Gln	Leu	Leu	Asn	Ala	Lys	Leu	Ile	Thr
		915					920					925			
Gln	Arg	Lys	Phe	Asp	Asn	Leu	Thr	Lys	Ala	Glu	Arg	Gly	Gly	Leu	Ser
	930					935						940			
Glu	Leu	Asp	Lys	Ala	Gly	Phe	Ile	Lys	Arg	Gln	Leu	Val	Glu	Thr	Arg
945					950					955					960
Gln	Ile	Thr	Lys	His	Val	Ala	Gln	Ile	Leu	Asp	Ser	Arg	Met	Asn	Thr
			965					970						975	
Lys	Tyr	Asp	Glu	Asn	Asp	Lys	Leu	Ile	Arg	Glu	Val	Lys	Val	Ile	Thr
		980						985					990		
Leu	Lys	Ser	Lys	Leu	Val	Ser	Asp	Phe	Arg	Lys	Asp	Phe	Gln	Phe	Tyr
		995					1000					1005			
Lys	Val	Arg	Glu	Ile	Asn	Asn	Tyr	His	His	Ala	His	Asp	Ala	Tyr	
	1010					1015						1020			
Leu	Asn	Ala	Val	Val	Gly	Thr	Ala	Leu	Ile	Lys	Lys	Tyr	Pro	Lys	
	1025					1030						1035			
Leu	Glu	Ser	Glu	Phe	Val	Tyr	Gly	Asp	Tyr	Lys	Val	Tyr	Asp	Val	
	1040					1045						1050			
Arg	Lys	Met	Ile	Ala	Lys	Ser	Glu	Gln	Glu	Ile	Gly	Lys	Ala	Thr	
	1055					1060						1065			
Ala	Lys	Tyr	Phe	Phe	Tyr	Ser	Asn	Ile	Met	Asn	Phe	Phe	Lys	Thr	
	1070					1075						1080			
Glu	Ile	Thr	Leu	Ala	Asn	Gly	Glu	Ile	Arg	Lys	Arg	Pro	Leu	Ile	
	1085					1090						1095			
Glu	Thr	Asn	Gly	Glu	Thr	Gly	Glu	Ile	Val	Trp	Asp	Lys	Gly	Arg	
	1100					1105						1110			
Asp	Phe	Ala	Thr	Val	Arg	Lys	Val	Leu	Ser	Met	Pro	Gln	Val	Asn	
	1115					1120						1125			
Ile	Val	Lys	Lys	Thr	Glu	Val	Gln	Thr	Gly	Gly	Phe	Ser	Lys	Glu	
	1130					1135						1140			
Ser	Ile	Leu	Pro	Lys	Arg	Asn	Ser	Asp	Lys	Leu	Ile	Ala	Arg	Lys	
	1145					1150						1155			
Lys	Asp	Trp	Asp	Pro	Lys	Lys	Tyr	Gly	Gly	Phe	Asp	Ser	Pro	Thr	
	1160					1165						1170			
Val	Ala	Tyr	Ser	Val	Leu	Val	Val	Ala	Lys	Val	Glu	Lys	Gly	Lys	
	1175					1180						1185			
Ser	Lys	Lys	Leu	Lys	Ser	Val	Lys	Glu	Leu	Leu	Gly	Ile	Thr	Ile	
	1190					1195						1200			
Met	Glu	Arg	Ser	Ser	Phe	Glu	Lys	Asn	Pro	Ile	Asp	Phe	Leu	Glu	
	1205					1210						1215			
Ala	Lys	Gly	Tyr	Lys	Glu	Val	Lys	Lys	Asp	Leu	Ile	Ile	Lys	Leu	
	1220					1225						1230			
Pro	Lys	Tyr	Ser	Leu	Phe	Glu	Leu	Glu	Asn	Gly	Arg	Lys	Arg	Met	

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1235	1240	1245
Leu Ala Ser Ala Gly Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu		
1250	1255	1260
Pro Ser Lys Tyr Val Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu		
1265	1270	1275
Lys Leu Lys Gly Ser Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe		
1280	1285	1290
Val Glu Gln His Lys His Tyr Leu Asp Glu Ile Ile Glu Gln Ile		
1295	1300	1305
Ser Glu Phe Ser Lys Arg Val Ile Leu Ala Asp Ala Asn Leu Asp		
1310	1315	1320
Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp Lys Pro Ile Arg		
1325	1330	1335
Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu Thr Asn Leu		
1340	1345	1350
Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg		
1355	1360	1365
Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp Ala Thr Leu Ile		
1370	1375	1380
His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser		
1385	1390	1395
Gln Leu Gly Gly Asp Pro Ile Ala Gly Ser Lys Ala Ser Pro Lys		
1400	1405	1410
Lys Lys Arg Lys Val Gly Arg Ala Asp Ala Leu Asp Asp Phe Asp		
1415	1420	1425
Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp		
1430	1435	1440
Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu		
1445	1450	1455
Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Ile Asn		
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Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser		
1475	1480	

<210> SEQ ID NO 676

<211> LENGTH: 2048

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 676

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Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
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Tyr Lys Asp Asp Asp Asp Lys Met Ala Pro Lys Lys Lys Arg Lys Val
20          25          30

Gly Arg Gly Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr
35          40          45

Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser
50          55          60

Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys
65          70          75          80

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Asn	Leu	Ile	Gly	Ala	Leu	Leu	Phe	Asp	Ser	Gly	Glu	Thr	Ala	Glu	Ala	
			85						90					95		
Thr	Arg	Leu	Lys	Arg	Thr	Ala	Arg	Arg	Arg	Tyr	Thr	Arg	Arg	Lys	Asn	
			100					105						110		
Arg	Ile	Cys	Tyr	Leu	Gln	Glu	Ile	Phe	Ser	Asn	Glu	Met	Ala	Lys	Val	
		115					120					125				
Asp	Asp	Ser	Phe	Phe	His	Arg	Leu	Glu	Glu	Ser	Phe	Leu	Val	Glu	Glu	
	130					135					140					
Asp	Lys	Lys	His	Glu	Arg	His	Pro	Ile	Phe	Gly	Asn	Ile	Val	Asp	Glu	
	145				150					155					160	
Val	Ala	Tyr	His	Glu	Lys	Tyr	Pro	Thr	Ile	Tyr	His	Leu	Arg	Lys	Lys	
			165						170					175		
Leu	Val	Asp	Ser	Thr	Asp	Lys	Ala	Asp	Leu	Arg	Leu	Ile	Tyr	Leu	Ala	
		180						185					190			
Leu	Ala	His	Met	Ile	Lys	Phe	Arg	Gly	His	Phe	Leu	Ile	Glu	Gly	Asp	
		195					200					205				
Leu	Asn	Pro	Asp	Asn	Ser	Asp	Val	Asp	Lys	Leu	Phe	Ile	Gln	Leu	Val	
	210					215					220					
Gln	Thr	Tyr	Asn	Gln	Leu	Phe	Glu	Glu	Asn	Pro	Ile	Asn	Ala	Ser	Gly	
	225				230					235					240	
Val	Asp	Ala	Lys	Ala	Ile	Leu	Ser	Ala	Arg	Leu	Ser	Lys	Ser	Arg	Arg	
			245						250					255		
Leu	Glu	Asn	Leu	Ile	Ala	Gln	Leu	Pro	Gly	Glu	Lys	Lys	Asn	Gly	Leu	
		260						265					270			
Phe	Gly	Asn	Leu	Ile	Ala	Leu	Ser	Leu	Gly	Leu	Thr	Pro	Asn	Phe	Lys	
		275					280					285				
Ser	Asn	Phe	Asp	Leu	Ala	Glu	Asp	Ala	Lys	Leu	Gln	Leu	Ser	Lys	Asp	
	290					295					300					
Thr	Tyr	Asp	Asp	Asp	Leu	Asp	Asn	Leu	Leu	Ala	Gln	Ile	Gly	Asp	Gln	
	305				310					315					320	
Tyr	Ala	Asp	Leu	Phe	Leu	Ala	Ala	Lys	Asn	Leu	Ser	Asp	Ala	Ile	Leu	
			325						330					335		
Leu	Ser	Asp	Ile	Leu	Arg	Val	Asn	Thr	Glu	Ile	Thr	Lys	Ala	Pro	Leu	
		340						345					350			
Ser	Ala	Ser	Met	Ile	Lys	Arg	Tyr	Asp	Glu	His	His	Gln	Asp	Leu	Thr	
		355					360					365				
Leu	Leu	Lys	Ala	Leu	Val	Arg	Gln	Gln	Leu	Pro	Glu	Lys	Tyr	Lys	Glu	
	370					375					380					
Ile	Phe	Phe	Asp	Gln	Ser	Lys	Asn	Gly	Tyr	Ala	Gly	Tyr	Ile	Asp	Gly	
	385				390					395					400	
Gly	Ala	Ser	Gln	Glu	Glu	Phe	Tyr	Lys	Phe	Ile	Lys	Pro	Ile	Leu	Glu	
			405						410					415		
Lys	Met	Asp	Gly	Thr	Glu	Glu	Leu	Leu	Val	Lys	Leu	Asn	Arg	Glu	Asp	
		420						425					430			
Leu	Leu	Arg	Lys	Gln	Arg	Thr	Phe	Asp	Asn	Gly	Ser	Ile	Pro	His	Gln	
		435					440					445				
Ile	His	Leu	Gly	Glu	Leu	His	Ala	Ile	Leu	Arg	Arg	Gln	Glu	Asp	Phe	
	450					455					460					
Tyr	Pro	Phe	Leu	Lys	Asp	Asn	Arg	Glu	Lys	Ile	Glu	Lys	Ile	Leu	Thr	
	465				470					475				480		
Phe	Arg	Ile	Pro	Tyr	Tyr	Val	Gly	Pro	Leu	Ala	Arg	Gly	Asn	Ser	Arg	

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485								490					495				
Phe	Ala	Trp	Met	Thr	Arg	Lys	Ser	Glu	Glu	Thr	Ile	Thr	Pro	Trp	Asn		
			500					505					510				
Phe	Glu	Glu	Val	Val	Asp	Lys	Gly	Ala	Ser	Ala	Gln	Ser	Phe	Ile	Glu		
			515				520					525					
Arg	Met	Thr	Asn	Phe	Asp	Lys	Asn	Leu	Pro	Asn	Glu	Lys	Val	Leu	Pro		
	530					535					540						
Lys	His	Ser	Leu	Leu	Tyr	Glu	Tyr	Phe	Thr	Val	Tyr	Asn	Glu	Leu	Thr		
545					550					555					560		
Lys	Val	Lys	Tyr	Val	Thr	Glu	Gly	Met	Arg	Lys	Pro	Ala	Phe	Leu	Ser		
				565					570					575			
Gly	Glu	Gln	Lys	Lys	Ala	Ile	Val	Asp	Leu	Leu	Phe	Lys	Thr	Asn	Arg		
			580					585					590				
Lys	Val	Thr	Val	Lys	Gln	Leu	Lys	Glu	Asp	Tyr	Phe	Lys	Lys	Ile	Glu		
		595					600					605					
Cys	Phe	Asp	Ser	Val	Glu	Ile	Ser	Gly	Val	Glu	Asp	Arg	Phe	Asn	Ala		
	610					615					620						
Ser	Leu	Gly	Thr	Tyr	His	Asp	Leu	Leu	Lys	Ile	Ile	Lys	Asp	Lys	Asp		
625					630					635					640		
Phe	Leu	Asp	Asn	Glu	Glu	Asn	Glu	Asp	Ile	Leu	Glu	Asp	Ile	Val	Leu		
				645					650					655			
Thr	Leu	Thr	Leu	Phe	Glu	Asp	Arg	Glu	Met	Ile	Glu	Glu	Arg	Leu	Lys		
			660					665					670				
Thr	Tyr	Ala	His	Leu	Phe	Asp	Asp	Lys	Val	Met	Lys	Gln	Leu	Lys	Arg		
		675					680					685					
Arg	Arg	Tyr	Thr	Gly	Trp	Gly	Arg	Leu	Ser	Arg	Lys	Leu	Ile	Asn	Gly		
	690					695					700						
Ile	Arg	Asp	Lys	Gln	Ser	Gly	Lys	Thr	Ile	Leu	Asp	Phe	Leu	Lys	Ser		
705				710					715						720		
Asp	Gly	Phe	Ala	Asn	Arg	Asn	Phe	Met	Gln	Leu	Ile	His	Asp	Asp	Ser		
				725					730					735			
Leu	Thr	Phe	Lys	Glu	Asp	Ile	Gln	Lys	Ala	Gln	Val	Ser	Gly	Gln	Gly		
			740					745					750				
Asp	Ser	Leu	His	Glu	His	Ile	Ala	Asn	Leu	Ala	Gly	Ser	Pro	Ala	Ile		
		755					760					765					
Lys	Lys	Gly	Ile	Leu	Gln	Thr	Val	Lys	Val	Val	Asp	Glu	Leu	Val	Lys		
	770					775					780						
Val	Met	Gly	Arg	His	Lys	Pro	Glu	Asn	Ile	Val	Ile	Glu	Met	Ala	Arg		
785					790					795					800		
Glu	Asn	Gln	Thr	Thr	Gln	Lys	Gly	Gln	Lys	Asn	Ser	Arg	Glu	Arg	Met		
				805					810					815			
Lys	Arg	Ile	Glu	Glu	Gly	Ile	Lys	Glu	Leu	Gly	Ser	Gln	Ile	Leu	Lys		
			820					825					830				
Glu	His	Pro	Val	Glu	Asn	Thr	Gln	Leu	Gln	Asn	Glu	Lys	Leu	Tyr	Leu		
		835					840					845					
Tyr	Tyr	Leu	Gln	Asn	Gly	Arg	Asp	Met	Tyr	Val	Asp	Gln	Glu	Leu	Asp		
	850					855					860						
Ile	Asn	Arg	Leu	Ser	Asp	Tyr	Asp	Val	Asp	Ala	Ile	Val	Pro	Gln	Ser		
865					870					875					880		
Phe	Leu	Lys	Asp	Asp	Ser	Ile	Asp	Asn	Lys	Val	Leu	Thr	Arg	Ser	Asp		
				885					890					895			

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Lys	Asn	Arg	Gly	Lys	Ser	Asp	Asn	Val	Pro	Ser	Glu	Glu	Val	Val	Lys	900	905	910	
Lys	Met	Lys	Asn	Tyr	Trp	Arg	Gln	Leu	Leu	Asn	Ala	Lys	Leu	Ile	Thr	915	920	925	
Gln	Arg	Lys	Phe	Asp	Asn	Leu	Thr	Lys	Ala	Glu	Arg	Gly	Gly	Leu	Ser	930	935	940	
Glu	Leu	Asp	Lys	Ala	Gly	Phe	Ile	Lys	Arg	Gln	Leu	Val	Glu	Thr	Arg	945	950	955	960
Gln	Ile	Thr	Lys	His	Val	Ala	Gln	Ile	Leu	Asp	Ser	Arg	Met	Asn	Thr	965	970	975	
Lys	Tyr	Asp	Glu	Asn	Asp	Lys	Leu	Ile	Arg	Glu	Val	Lys	Val	Ile	Thr	980	985	990	
Leu	Lys	Ser	Lys	Leu	Val	Ser	Asp	Phe	Arg	Lys	Asp	Phe	Gln	Phe	Tyr	995	1000	1005	
Lys	Val	Arg	Glu	Ile	Asn	Asn	Tyr	His	His	Ala	His	Asp	Ala	Tyr		1010	1015	1020	
Leu	Asn	Ala	Val	Val	Gly	Thr	Ala	Leu	Ile	Lys	Lys	Tyr	Pro	Lys		1025	1030	1035	
Leu	Glu	Ser	Glu	Phe	Val	Tyr	Gly	Asp	Tyr	Lys	Val	Tyr	Asp	Val		1040	1045	1050	
Arg	Lys	Met	Ile	Ala	Lys	Ser	Glu	Gln	Glu	Ile	Gly	Lys	Ala	Thr		1055	1060	1065	
Ala	Lys	Tyr	Phe	Phe	Tyr	Ser	Asn	Ile	Met	Asn	Phe	Phe	Lys	Thr		1070	1075	1080	
Glu	Ile	Thr	Leu	Ala	Asn	Gly	Glu	Ile	Arg	Lys	Arg	Pro	Leu	Ile		1085	1090	1095	
Glu	Thr	Asn	Gly	Glu	Thr	Gly	Glu	Ile	Val	Trp	Asp	Lys	Gly	Arg		1100	1105	1110	
Asp	Phe	Ala	Thr	Val	Arg	Lys	Val	Leu	Ser	Met	Pro	Gln	Val	Asn		1115	1120	1125	
Ile	Val	Lys	Lys	Thr	Glu	Val	Gln	Thr	Gly	Gly	Phe	Ser	Lys	Glu		1130	1135	1140	
Ser	Ile	Leu	Pro	Lys	Arg	Asn	Ser	Asp	Lys	Leu	Ile	Ala	Arg	Lys		1145	1150	1155	
Lys	Asp	Trp	Asp	Pro	Lys	Lys	Tyr	Gly	Gly	Phe	Asp	Ser	Pro	Thr		1160	1165	1170	
Val	Ala	Tyr	Ser	Val	Leu	Val	Val	Ala	Lys	Val	Glu	Lys	Gly	Lys		1175	1180	1185	
Ser	Lys	Lys	Leu	Lys	Ser	Val	Lys	Glu	Leu	Leu	Gly	Ile	Thr	Ile		1190	1195	1200	
Met	Glu	Arg	Ser	Ser	Phe	Glu	Lys	Asn	Pro	Ile	Asp	Phe	Leu	Glu		1205	1210	1215	
Ala	Lys	Gly	Tyr	Lys	Glu	Val	Lys	Lys	Asp	Leu	Ile	Ile	Lys	Leu		1220	1225	1230	
Pro	Lys	Tyr	Ser	Leu	Phe	Glu	Leu	Glu	Asn	Gly	Arg	Lys	Arg	Met		1235	1240	1245	
Leu	Ala	Ser	Ala	Gly	Glu	Leu	Gln	Lys	Gly	Asn	Glu	Leu	Ala	Leu		1250	1255	1260	
Pro	Ser	Lys	Tyr	Val	Asn	Phe	Leu	Tyr	Leu	Ala	Ser	His	Tyr	Glu		1265	1270	1275	

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Lys 1280	Leu	Lys	Gly	Ser	Pro	Glu 1285	Asp	Asn	Glu	Gln	Lys 1290	Gln	Leu	Phe
Val 1295	Glu	Gln	His	Lys	His	Tyr 1300	Leu	Asp	Glu	Ile	Ile 1305	Glu	Gln	Ile
Ser 1310	Glu	Phe	Ser	Lys	Arg	Val 1315	Ile	Leu	Ala	Asp	Ala 1320	Asn	Leu	Asp
Lys 1325	Val	Leu	Ser	Ala	Tyr	Asn 1330	Lys	His	Arg	Asp	Lys 1335	Pro	Ile	Arg
Glu 1340	Gln	Ala	Glu	Asn	Ile	Ile 1345	His	Leu	Phe	Thr	Leu 1350	Thr	Asn	Leu
Gly 1355	Ala	Pro	Ala	Ala	Phe	Lys 1360	Tyr	Phe	Asp	Thr	Thr 1365	Ile	Asp	Arg
Lys 1370	Arg	Tyr	Thr	Ser	Thr	Lys 1375	Glu	Val	Leu	Asp	Ala 1380	Thr	Leu	Ile
His 1385	Gln	Ser	Ile	Thr	Gly	Leu 1390	Tyr	Glu	Thr	Arg	Ile 1395	Asp	Leu	Ser
Gln 1400	Leu	Gly	Gly	Asp	Pro	Ile 1405	Ala	Gly	Ser	Lys	Ala 1410	Ser	Pro	Lys
Lys 1415	Lys	Arg	Lys	Val	Gly	Arg 1420	Ala	Ile	Phe	Lys	Pro 1425	Glu	Glu	Leu
Arg 1430	Gln	Ala	Leu	Met	Pro	Thr 1435	Leu	Glu	Ala	Leu	Tyr 1440	Arg	Gln	Asp
Pro 1445	Glu	Ser	Leu	Pro	Phe	Arg 1450	Gln	Pro	Val	Asp	Pro 1455	Gln	Leu	Leu
Gly 1460	Ile	Pro	Asp	Tyr	Phe	Asp 1465	Ile	Val	Lys	Ser	Pro 1470	Met	Asp	Leu
Ser 1475	Thr	Ile	Lys	Arg	Lys	Leu 1480	Asp	Thr	Gly	Gln	Tyr 1485	Gln	Glu	Pro
Trp 1490	Gln	Tyr	Val	Asp	Asp	Ile 1495	Trp	Leu	Met	Phe	Asn 1500	Asn	Ala	Trp
Leu 1505	Tyr	Asn	Arg	Lys	Thr	Ser 1510	Arg	Val	Tyr	Lys	Tyr 1515	Cys	Ser	Lys
Leu 1520	Ser	Glu	Val	Phe	Glu	Gln 1525	Glu	Ile	Asp	Pro	Val 1530	Met	Gln	Ser
Leu 1535	Gly	Tyr	Cys	Cys	Gly	Arg 1540	Lys	Leu	Glu	Phe	Ser 1545	Pro	Gln	Thr
Leu 1550	Cys	Cys	Tyr	Gly	Lys	Gln 1555	Leu	Cys	Thr	Ile	Pro 1560	Arg	Asp	Ala
Thr 1565	Tyr	Tyr	Ser	Tyr	Gln	Asn 1570	Arg	Tyr	His	Phe	Cys 1575	Glu	Lys	Cys
Phe 1580	Asn	Glu	Ile	Gln	Gly	Glu 1585	Ser	Val	Ser	Leu	Gly 1590	Asp	Asp	Pro
Ser 1595	Gln	Pro	Gln	Thr	Thr	Ile 1600	Asn	Lys	Glu	Gln	Phe 1605	Ser	Lys	Arg
Lys 1610	Asn	Asp	Thr	Leu	Asp	Pro 1615	Glu	Leu	Phe	Val	Glu 1620	Cys	Thr	Glu
Cys 1625	Gly	Arg	Lys	Met	His	Gln 1630	Ile	Cys	Val	Leu	His 1635	His	Glu	Ile
Ile 1640	Trp	Pro	Ala	Gly	Phe	Val 1645	Cys	Asp	Gly	Cys	Leu 1650	Lys	Lys	Ser
Ala	Arg	Thr	Arg	Lys	Glu	Asn	Lys	Phe	Ser	Ala	Lys	Arg	Leu	Pro

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1655	1660	1665
Ser Thr Arg Leu Gly Thr Phe	Leu Glu Asn Arg Val	Asn Asp Phe
1670	1675	1680
Leu Arg Arg Gln Asn His Pro	Glu Ser Gly Glu Val	Thr Val Arg
1685	1690	1695
Val Val His Ala Ser Asp Lys	Thr Val Glu Val Lys	Pro Gly Met
1700	1705	1710
Lys Ala Arg Phe Val Asp Ser	Gly Glu Met Ala Glu	Ser Phe Pro
1715	1720	1725
Tyr Arg Thr Lys Ala Leu Phe	Ala Phe Glu Glu Ile	Asp Gly Val
1730	1735	1740
Asp Leu Cys Phe Phe Gly Met	His Val Gln Glu Tyr	Gly Ser Asp
1745	1750	1755
Cys Pro Pro Pro Asn Gln Arg	Arg Val Tyr Ile Ser	Tyr Leu Asp
1760	1765	1770
Ser Val His Phe Phe Arg Pro	Lys Cys Leu Arg Thr	Ala Val Tyr
1775	1780	1785
His Glu Ile Leu Ile Gly Tyr	Leu Glu Tyr Val Lys	Lys Leu Gly
1790	1795	1800
Tyr Thr Thr Gly His Ile Trp	Ala Cys Pro Pro Ser	Glu Gly Asp
1805	1810	1815
Asp Tyr Ile Phe His Cys His	Pro Pro Asp Gln Lys	Ile Pro Lys
1820	1825	1830
Pro Lys Arg Leu Gln Glu Trp	Tyr Lys Lys Met Leu	Asp Lys Ala
1835	1840	1845
Val Ser Glu Arg Ile Val His	Asp Tyr Lys Asp Ile	Phe Lys Gln
1850	1855	1860
Ala Thr Glu Asp Arg Leu Thr	Ser Ala Lys Glu Leu	Pro Tyr Phe
1865	1870	1875
Glu Gly Asp Phe Trp Pro Asn	Val Leu Glu Glu Ser	Ile Lys Glu
1880	1885	1890
Leu Glu Gln Glu Glu Glu Glu	Arg Lys Arg Glu Glu	Asn Thr Ser
1895	1900	1905
Asn Glu Ser Thr Asp Val Thr	Lys Gly Asp Ser Lys	Asn Ala Lys
1910	1915	1920
Lys Lys Asn Asn Lys Lys Thr	Ser Lys Asn Lys Ser	Ser Leu Ser
1925	1930	1935
Arg Gly Asn Lys Lys Lys Pro	Gly Met Pro Asn Val	Ser Asn Asp
1940	1945	1950
Leu Ser Gln Lys Leu Tyr Ala	Thr Met Glu Lys His	Lys Glu Val
1955	1960	1965
Phe Phe Val Ile Arg Leu Ile	Ala Gly Pro Ala Ala	Asn Ser Leu
1970	1975	1980
Pro Pro Ile Val Asp Pro Asp	Pro Leu Ile Pro Cys	Asp Leu Met
1985	1990	1995
Asp Gly Arg Asp Ala Phe Leu	Thr Leu Ala Arg Asp	Lys His Leu
2000	2005	2010
Glu Phe Ser Ser Leu Arg Arg	Ala Gln Trp Ser Thr	Met Cys Met
2015	2020	2025
Leu Val Glu Leu His Thr Gln	Ser Gln Asp Tyr Pro	Tyr Asp Val
2030	2035	2040

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Pro Asp Tyr Ala Ser
2045

<210> SEQ ID NO 677
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<223> OTHER INFORMATION: a, c, t, g, unknown or other

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nnnnnnnnnn nnnnnnnnnn ngg 23

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<222> LOCATION: (2)..(21)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

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gnnnnnnnnn nnnnnnnnnn ngg 23

<210> SEQ ID NO 679
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gtgtagagta agtcagccta 20

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oligonucleotide

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gttggacaga acttaccgac 20

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oligonucleotide

<400> SEQUENCE: 681

gtttgcttcg ctataaaacg 20

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<210> SEQ ID NO 682
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oligonucleotide

<400> SEQUENCE: 682

gtctgaggat ggggccgcaa 20

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oligonucleotide

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ggatctgtca aatcgctgc 20

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oligonucleotide

<400> SEQUENCE: 684

gccaggatgg cattgggcag 20

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<400> SEQUENCE: 685

gctgaatctg cggtaggcag 20

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 686

gttcttttgt tcttctagcc 20

<210> SEQ ID NO 687
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oligonucleotide

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ggaaaagctt gagcaagtca 20

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<400> SEQUENCE: 688

ggaagagttg cccctgcgcc 20

<210> SEQ ID NO 689
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gacaaatctc cagtggataa 20

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oligonucleotide

<400> SEQUENCE: 690

gtgtttctca ggtaaagctc 20

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ggaaggacca tttgacgtta 20

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gaactgctat ttcagtttcc 20

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oligonucleotide

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<400> SEQUENCE: 693

gccagccact cagccagtga

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<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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ggtatgcttt tctgttaaag

20

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<211> LENGTH: 20

<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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gctcctggac tgaccactat

20

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<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 696

ggaacagagg cgtccccagt tgg

23

<210> SEQ ID NO 697

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 697

ggaggctaga acaatcatta

20

<210> SEQ ID NO 698

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 698

gacaagaaca ccttcagaac

20

<210> SEQ ID NO 699

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 699

gggtttctgt gattttcttt 20

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<400> SEQUENCE: 700

gggcaaaga cctccgccag 20

<210> SEQ ID NO 701
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<212> TYPE: DNA
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<400> SEQUENCE: 701

gttgagaag cattcataaa 20

<210> SEQ ID NO 702
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 702

gtcgctcact caccctgcaa 20

<210> SEQ ID NO 703
<211> LENGTH: 20
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oligonucleotide

<400> SEQUENCE: 703

gaaaagagct gatgaaacaa 20

<210> SEQ ID NO 704
<211> LENGTH: 20
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 704

gtacactttt caaaatgctt 20

<210> SEQ ID NO 705
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 705

ggagatgatc atcaagcaga 20

<210> SEQ ID NO 706
<211> LENGTH: 20
<212> TYPE: DNA
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<400> SEQUENCE: 706

gctttgaaag agcaataaaa 20

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 707

gcacaaaagt caaatcggaa 20

<210> SEQ ID NO 708
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 708

gatttcaata taagattcgg 20

<210> SEQ ID NO 709
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 709

gcttaagcaa tcccgaactc 20

<210> SEQ ID NO 710
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 710

gaggccaaac ctcggcttac 20

<210> SEQ ID NO 711

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 711

gttcgaaaat ttcaggtaag 20

<210> SEQ ID NO 712
<211> LENGTH: 20
<212> TYPE: DNA
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<400> SEQUENCE: 712

ggcagaacag gagataacag 20

<210> SEQ ID NO 713
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 713

ggcggccctc gcccttctct 20

<210> SEQ ID NO 714
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 714

gtagtgatcg tggatacgag 20

<210> SEQ ID NO 715
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<220> FEATURE:
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oligonucleotide

<400> SEQUENCE: 715

gtacagccct cgggttatat 20

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<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 716

gggaaggaat taagcccgaa 20

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<210> SEQ ID NO 717
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<212> TYPE: DNA
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oligonucleotide

<400> SEQUENCE: 717

gggaacagct ttcgtagttg 20

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oligonucleotide

<400> SEQUENCE: 718

gataaagtc agtgctgatc 20

<210> SEQ ID NO 719
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 719

gaaaaccaga gcttcggtca 20

<210> SEQ ID NO 720
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 720

ggagtcttct gggcaggctt aaaggctaac c 31

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<211> LENGTH: 31
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 721

gtcgggtgag catgtcttta atctacctcg a 31

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 722

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ggtgtcacca gagtaacagt 20

<210> SEQ ID NO 723
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oligonucleotide

<400> SEQUENCE: 723

gtgatcatca agcagaaggt 20

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oligonucleotide

<400> SEQUENCE: 724

gaacttcgaa aatttcaggt a 21

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<212> TYPE: DNA
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<220> FEATURE:
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oligonucleotide

<400> SEQUENCE: 725

ggaaactcat caaatatgcg t 21

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<220> FEATURE:
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oligonucleotide

<400> SEQUENCE: 726

gtcatttaca ctaacacgca t 21

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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oligonucleotide

<400> SEQUENCE: 727

ggaatgaaac tcatcaaata t 21

<210> SEQ ID NO 728
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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oligonucleotide

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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oligonucleotide

<400> SEQUENCE: 729

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<210> SEQ ID NO 730

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 730

gaattggaaa atgtgatggg a 21

<210> SEQ ID NO 731

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 731

gatgatcatc aagcagaagg t 21

<210> SEQ ID NO 732

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 732

gagatgatca tcaagcagaa g 21

<210> SEQ ID NO 733

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 733

gcattttttc tcataccttc t 21

<210> SEQ ID NO 734

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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oligonucleotide

<400> SEQUENCE: 734

gtcctactca gactgttact c 21

<210> SEQ ID NO 735
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 735

gacaggttgt gtcaccagag t 21

<210> SEQ ID NO 736
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oligonucleotide

<400> SEQUENCE: 736

gttatcattt tttctcatc c 21

<210> SEQ ID NO 737
<211> LENGTH: 21
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 737

gttgccctaag aactggtggg a 21

<210> SEQ ID NO 738
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 738

gaaacagttg cctaagaact g 21

<210> SEQ ID NO 739
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 739

gtttcccacc agttcttagg c 21

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<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 740

gtggctttga tttccctagg g 21

<210> SEQ ID NO 741
<211> LENGTH: 21
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 741

gtagggaat caaagccaat g 21

<210> SEQ ID NO 742
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 742

ggaccctagg gaaatcaaag c 21

<210> SEQ ID NO 743
<211> LENGTH: 21
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 743

gtgagggtc caccctcacg a 21

<210> SEQ ID NO 744
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 744

gaaggattga gggctccacc c 21

<210> SEQ ID NO 745
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 745

ggctccacc tcacgagtg g 21

<210> SEQ ID NO 746
<211> LENGTH: 21

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        oligonucleotide

<400> SEQUENCE: 746

gtatccccta tcgaggaaac c                               21

<210> SEQ ID NO 747
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        oligonucleotide

<400> SEQUENCE: 747

ggataaagaa ggcctatttc a                               21

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        oligonucleotide

<400> SEQUENCE: 748

gaggccttct ttatccccta t                               21

<210> SEQ ID NO 749
<211> LENGTH: 21
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        oligonucleotide

<400> SEQUENCE: 749

gtgagggtc caccctcacg a                               21

<210> SEQ ID NO 750
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        oligonucleotide

<400> SEQUENCE: 750

ggataaagaa ggcctatttc a                               21

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What is claimed is:

1. A DNA targeting system for deleting exon 51 of a dystrophin gene, the system comprising Cas9 and at least one guide RNA (gRNA), wherein the at least one gRNA binds and targets a polynucleotide sequence comprising SEQ ID NO: 67, SEQ ID NO: 70, SEQ ID NO: 100, or SEQ ID NO: 680.
2. An isolated polynucleotide encoding the DNA targeting system of claim 1.
3. A vector comprising the isolated polynucleotide of claim 2.

4. A cell comprising the isolated polynucleotide of claim 2.
5. A method of treating a subject in need thereof having a mutant dystrophin gene, the method comprising administering to the subject the DNA targeting system of claim 1.
6. The method of claim 5, wherein the subject is suffering from Duchenne muscular dystrophy.
7. A method of correcting a mutant dystrophin gene in a cell, the method comprising administering to a cell containing a mutant dystrophin gene the DNA targeting system of claim 1.

8. A composition for genome editing in a muscle of a subject, the composition comprising a modified adeno-associated virus (AAV) vector and a nucleotide sequence encoding the DNA targeting system of claim **1**, wherein the muscle is skeletal muscle or cardiac muscle.

9. The composition of claim **8**, wherein the modified AAV vector has enhanced cardiac and skeletal muscle tissue tropism.

* * * * *