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(54) **METHODS AND MATERIALS FOR GENE EDITING**

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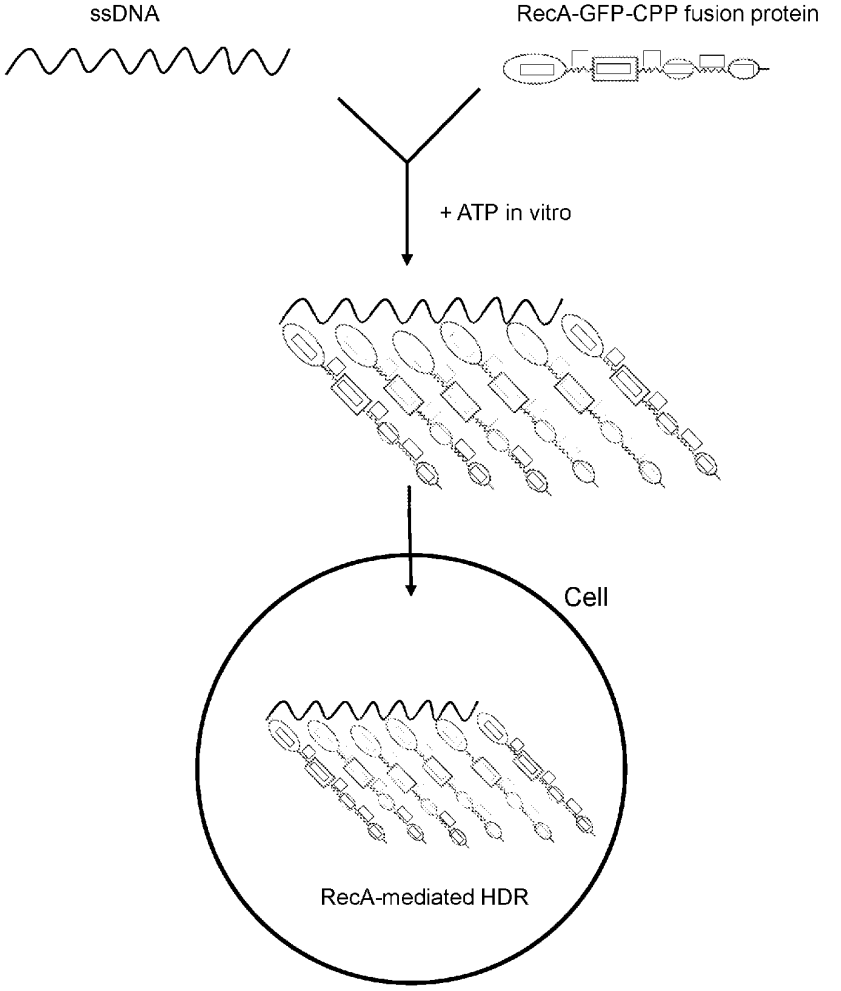
(57) **ABSTRACT**

This document relates to methods and materials for gene editing. For example, methods and materials for using a RecA polypeptide fused to a cell penetrating peptide to edit (e.g., correct) a gene are provided.

Specification includes a Sequence Listing.



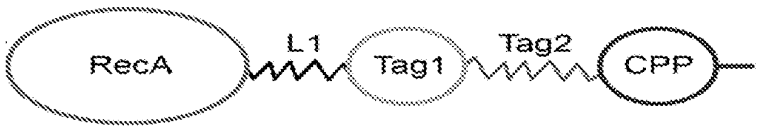


FIG. 1A

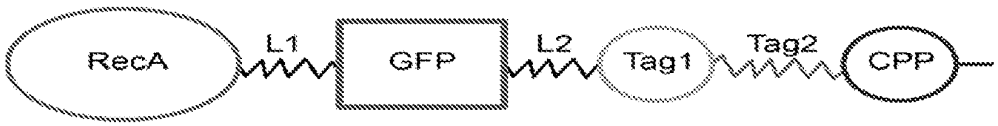


FIG. 1B

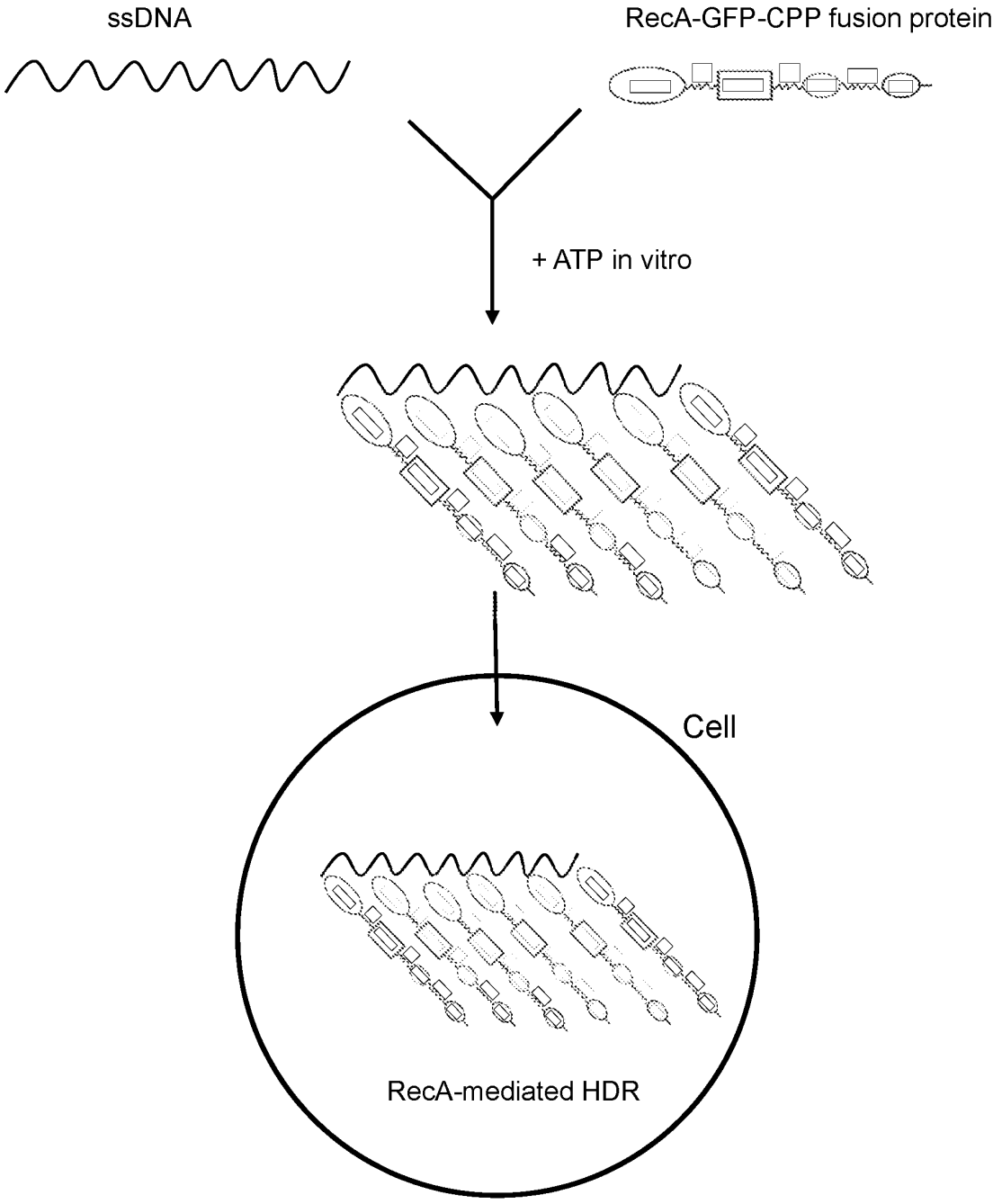

ssDNA
RecA-GFP-CPP fusion protein
+ ATP in vitro
Cell
RecA-mediated HDR

FIG. 2

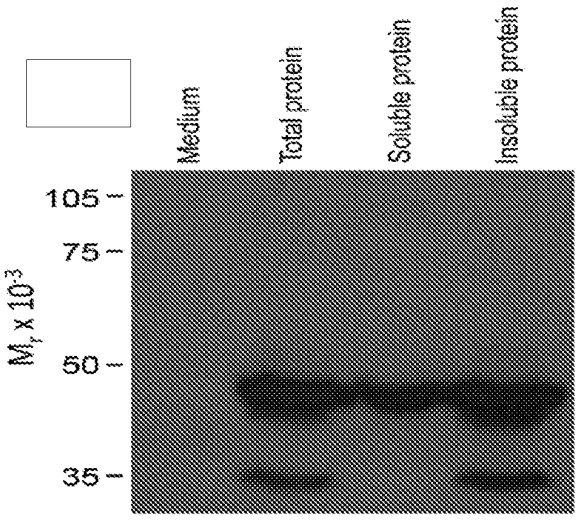

Medium
Total protein
Soluble protein
Insoluble protein
105
75
50
35
$M_r \times 10^{-3}$

FIG. 3A

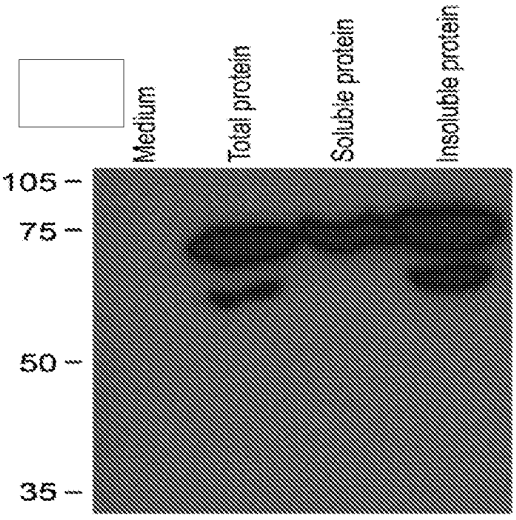

Medium
Total protein
Soluble protein
Insoluble protein
105
75
50
35

FIG. 3B

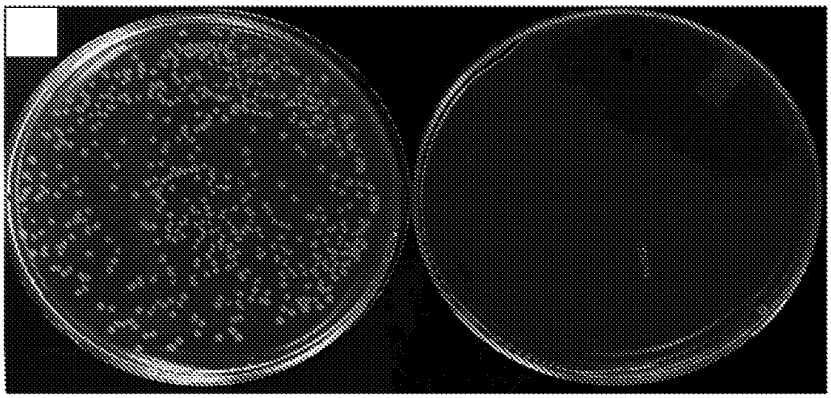


FIG. 4A

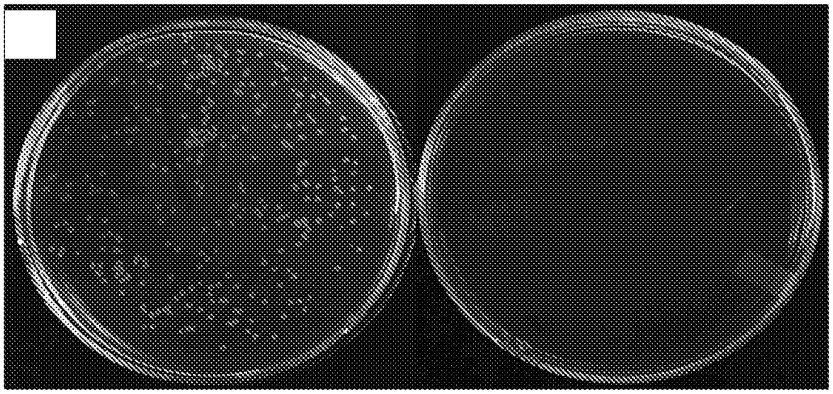


FIG. 4B

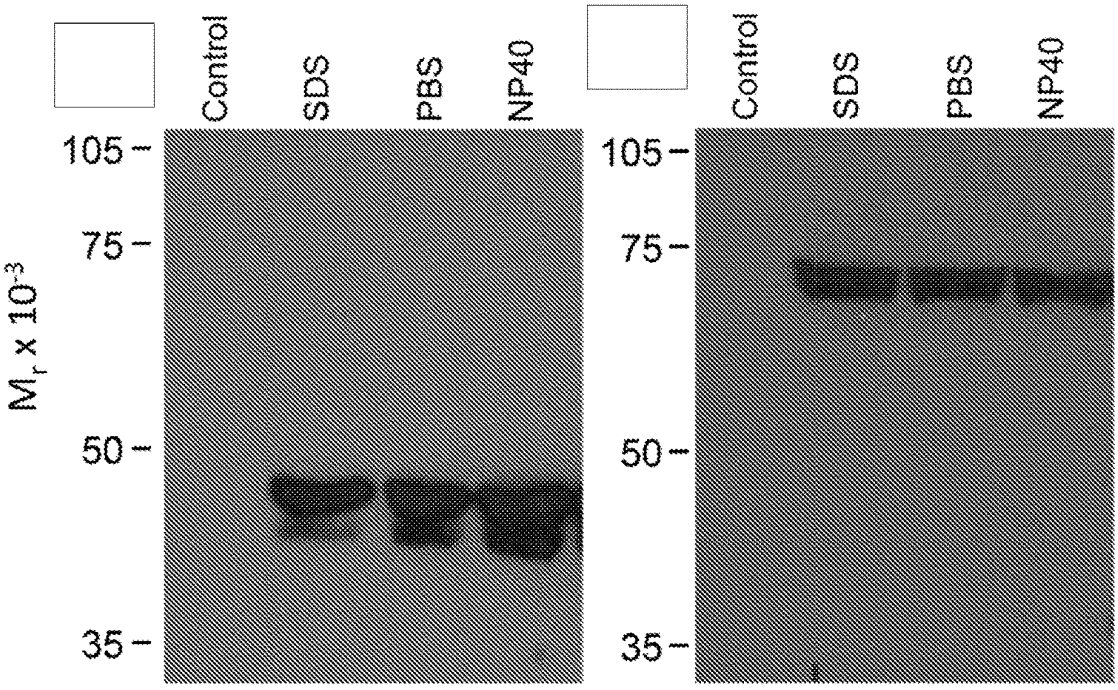

Control
SDS
PBS
NP40
105
75
50
35
$M_r \times 10^{-3}$
Control
SDS
PBS
NP40
105
75
50
35

FIG. 5A

FIG. 5B

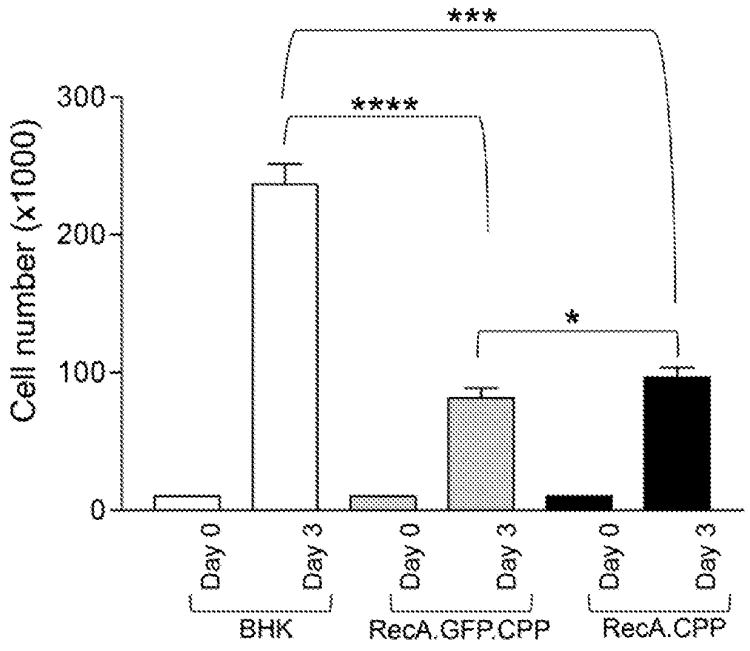

***
****
*
300
200
100
0
Cell number (x1000)
Day 0
Day 3
Day 0
Day 3
Day 0
Day 3
BHK
RecA.GFP.CPP
RecA.CPP

FIG. 6

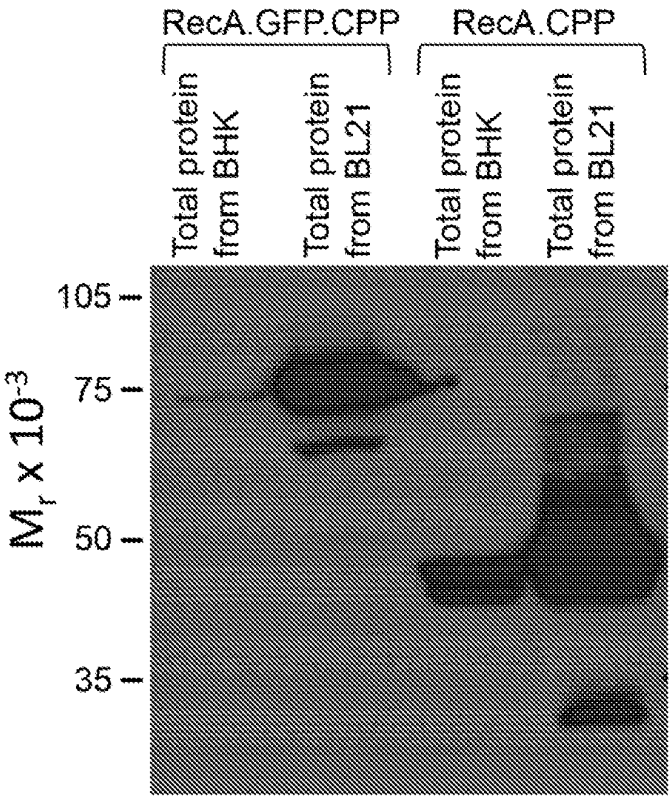

RecA.GFP.CPP
RecA.CPP
Total protein from BHK
Total protein from BL21
Total protein from BHK
Total protein from BL21
105
75
50
35
$M_r \times 10^{-3}$

FIG. 7

GenBank Accession No. AML00775 (SEQ ID NO:1)

maidenkqka laaalgqiek qfgkgsimrl gedrsmdvet istgslsldi algagglpmg
riveiygpes sgkttltlqv iaaaqregkt cafidaehal dpiyarklgv didnllcsqp
dtgeqaleic dalarsgavd vivvdsvaal tpkaeiegei gdshmglaar mmsqamrkla
gnlkqsntll ifinqirmki gvmfgnpett tggnalkfya svrldirrig avkegenvvg
setrvkvvkn kiaapfkqae fqilygegin fygelvdlgv kekliekaga wysykgekig
qgkanatawl kdnpetakei ekkvrellls npnstpdfsv ddsegvaetn edf

FIG. 8A

GenBank Accession No. CAA41395 (SEQ ID NO:2)

mtqtpdreka lelavaqiek sygkgsvmrl gdearqpisv iptgsialdv algigglprg
rvieiygpes sgkttvalha vanaqaaggv aafidaehal dpdyakklgv dtdsllvsqp
dtgeqaleia dmlirsgald ivvidsvaal vpraelegem gdshvglqar lmsqalrkmt
galnnsgtta ifinqlrdki gvmfgspett tggkalkfya svrmdvrrve tlkdgtnavg
nrtrvkvvkn kclaegtrif dpvtgtthri edvvdgrkpi hvvaaakdgt lharpvvswf
dqgtrdvigl riaggaivwa tpdhkvltey gwraagelrk gdrvaqprrf dgfgdsapip
adharllgyl igdgrdgwvg gktpinfinv qraliddvtr iaatlgcaah pqgrislaia
hrpgerngva dlcqqagiyg klawektipn wffepdiaad ivgnllfglf esdgwvsreq
tgalrvgytt tseqlahqih wlllrfgvgs tvrdydptqk rpsivngrri qskrqvfevr
isgmdnvtaf aesvpmwgpr gaaliqaipe atqgrrrgsq atylaaemtd avlnylderg
vtaqeaaami gvasgdprgg mkqvlgasrl rrdrvqalad alddkflhdm laeelrysvi
revlptrrar tfdleveelh tlvaegvvvh ncsppfkqae fdilygkgis regslidmgv
dqglirksga wftyegeqlg qgkenarnfl venadvadei ekkikeklgi gavvtddpsn
dgvlpapvdf

FIG. 8B

GenBank Accession No. NP_389576 (SEQ ID NO:3)

msdrqaaldm alkqiekqfg kgsimklgek tdtristvps gslaldtalg iggyprgrii
evygpessgk ttvalhaiae vqqqggqaaf idaehaldpv yaqklgvnie elllsqpdtg
eqaleiaeal vrsgavdivv vdsvaalvpk aeiegdmgds hvglqarlms qalrklsgai
nksktiaifi nqirekvgvm fgnpettpgg ralkfyssvr levrraeqlk qgndvmgnkt
kikvvknkva ppfrtaevdi mygegiskeg eiidlgteld ivqksgswys yeeerlgqgr
enakqflken kdimlmiqeq irehygldnn gvvqqqaeet qeelefee

FIG. 8C

GenBank Accession No. WP_002209446 (SEQ ID NO:4)

MAIDENKQKA LAAALGQIEK QFGKGSIMRL GEDRSMDVET ISTGSLSLDI ALGAGGLPMG
RIVEIYGPES SGKTTLTLQV IAAAQREGKT CAFIDAEHAL DPIYAKKLGV DIDNLLCSQP
DTGEQALEIC DALTRSGAVD VIIVDSVAAL TPKAEIEGEI GDSHMGLAAR MMSQAMRKLA
GNLKNANTLL IFINQIRMKI GVMFGNPETT TGGNALKFYA SVRLDIRRIG AVKDGDVVVG
SETRVKVVKN KIAAPFKQAE FQILYGEGIN INGELVDLGV KHKLIEKAGA WYSYNGDKIG
QGKANASNYL KENPAIAAEL DKKLREMLLN GGNGEQPVAA ATAEFADGAD ETNEEF

FIG. 8D

GenBank Accession No. NC_000913.3 (SEQ ID NO:9)

ttaaaaatct tcgttagttt ctgctacgcc ttcgctatca tctacagaga aatccggcgt
tgagttcggg ttgctcagca gcaactcacg tactttcttc tcgatctctt tcgcggtttc
cgggttatct ttcagccagg cagtcgcatt cgctttaccc tgaccgatct tctcaccttt
gtagctgtac cacgcgcctg ctttctcgat cagcttctct tttacgccca ggtcaaccag
ttcgccgtag aagttgatac cttcgccgta gaggatctgg aattcagcct gtttaaacgg
cgcagcgatt ttgttcttca ccactttcac gcgggtttcg ctacccacca cgttttcgcc
ctctttcacc gcgccgatac gacggatgtc gagacgaaca gaggcgtaga atttcagcgc
gttaccaccg gtagtggttt ccgggttacc gaacatcaca ccaattttca tacggatctg
gttgatgaag atcagcagcg tgttggactg cttcaggtta cccgccagct tacgcatcgc
ctggctcatc atacgtgccg caaggcccat gtgagagtcg ccgatttcgc cttcgatttc
cgctttcggc gtcagtgccg ccacggagtc aacgacgata acgtctactg cgccagaacg
cgccagggcg tcacagattt ccagtgcctg ctcgccggtg tccggctggg agcacagcag
gttgtcgata tcgacgccca gtttacgtgc gtagattggg tccagcgcgt gttcagcatc
gataaacgca caggttttac cttcacgctg cgctgcggcg atcacctgca gcgtcagcgt
ggttttaccg gaagattccg gtccgtagat ttcgacgata cggcccatcg gcagaccacc
tgccccaagc gcgatatcca gtgaaagcga accggtagag atggtttcca catccatgga
acggtcttca cccaggcgca tgatggagcc tttaccaaat tgtttctcaa tctggcccag
tgctgccgcc aacgctttct gtttgttttc gtcgatagcc at

FIG. 9A

GenBank Accession No. NC_000962.3 (SEQ ID NO:10)

tcagaagtcg acgggggcgg gcaggacacc gtcatttgag ggatcatcgg tcaccacggc
accaatgcca agcttttcct tgatcttctt ctcgatctcg tcagccacgt cggcgttctc
caccaagaag ttgcgggcat tctccttgcc ctggccgagc tgctcgccct cgtaggtgaa
ccaggcaccc gacttgcgga tgaggccctg atccacaccc atgtcgatca gcgagccctc
cctgctgatt cccttgccgt agaggatgtc gaactcggcc tgcttgaagg ggggcgaaca
gttgtgcacg acaacccctt cggcgacgag ggtgtgcagt tcctcgacct cgaggtcgaa
cgttcgtgcc cgccgcgttg gcagcacttc tcggatcacg gaatagcgga gttcttccgc
cagcatgtcg tgcaggaatt tgtcatccag ggcatccgcg agcgcctgca cgcgatcccg
acgaaggcgg ctggcaccta agacctgctt cattccaccg cgggggtccc cggaagctac
accgatcatg gccgcggcct cctgcgcggt cacgccgcgc tcgtccagat aattcagcac
ggcatcggtc atctctgcag ccagatatgt cgcttgcgat ccacgacgcc gcccctgcgt
ggcttctgga atcgcctgga taagcgcggc accgcgcggc ccccacatgg gaactgactc
cgcgaatgcc gtgacgttat ccatacccga gatccggacc tcgaacactt gacgtttgct
ctggatccgt cgaccgttga cgatgctcgg ccgcttctgg gtcggatcgt aatctcgaac
ggtgctcccg acaccgaacc gcagcagcag ccaatgaatc tgatgcgcga gttgttcaga
ggtcgtcgtg taaccgaccc gaagtgcccc ggtctgttcc cggctcaccc acccgtcgct
ttcgaacagg ccgaagagca gattgccgac aatgtcggcc gcgatgtccg gctcgaagaa
ccaattcgga atcgtcttct cccacgcgag cttgccgtag ataccggcct gctgacaaag
gtctgccaca ccgttgcgct caccgggtcg atgagcgatc gcgagtgaga tacgcccctg
cggatgggcc gcgcaaccga gcgtcgcagc gattcgcgtc acgtcgtcaa tgagcgcccg
ctgaacattg atgaagttga tcggagtctt gccccccacc caaccatccc tgccatctcc
gatcaggtag ccaagcagcc gggcatgatc cgccggaatc ggcgcactgt caccgaatcc
atcgaagcgt cgcggttgcg ccaccctgtc tcccttgcgg agttccccgg cggcacgcca
gccgtactct gtcagcacct tgtgatcggg tgtcgcccac acgatggcgc caccggcgat
ccgcaacccg atcacatccc gcgttccctg gtcgaaccag gacaccacgg gccgcgcatg
cagcgttccg tccttggcag cagccacgac atgaataggc ttgcgcccat cgacaacatc
ctcgatgcga tgcgttgtac cggtgaccgg atcgaagatc cgagtgccct ctgcgaggca
cttgttcttg acgaccttga cccgggtgcg gttgccgacc gcgttggtac cgtccttgag
cgtctcgact cgccgcacgt ccatgcgcac cgacgcgtag aacttcaacg cctttccgcc
cgttgtcgtc tcgggcgacc cgaacatcac tccgatcttg tcgcggagct ggttgatgaa
gatcgccgtg gtgcccgaat tattcagcgc gccggtcatt ttccgcagcg cctggctcat
cagccgggcc tgcagcccga cgtggctgtc gcccatctcg ccttcgagct ccgcgcgcgg
caccagcgcc gccaccgagt cgatcaccac gatgtcaagc gcacccgagc ggatcagcat
gtcggcgatc tcgagtgcct gttccccggt gtccggctgg ctgaccagca gcgaatcggt
gtcgacaccg agcttcttgg catagtccgg atccagcgcg tgctcggcgt cgatgaacgc
cgcaacacca ccggcggcct gagcgttggc caccgcgtgc agcgccacgg tggtcttacc
cgacgactcc gggccgtata tctctatcac ccggccacgc ggcaggccgc caatgcccag
ggccacgtct agtgcgatgg atccggtcgg aatgaccgaa atcggctgac gcgcctcgtc
gccgaggcgc atcaccgaac ctttgccgta actcttctcg atctgggcca ctgccagctc
gagcgccttt tcccgatcgg gggtctgcgt cat

FIG. 9B

GenBank Accession No. NC_000964.3 (SEQ ID NO:11)

atgagtgatc gtcaggcagc cttagatatg gctcttaaac aaatagaaaa acagttcggc
aaaggttcca ttatgaaact gggagaaaag acagatacaa gaatttctac tgtaccaagc
ggctccctcg ctcttgatac agcactggga attggcggat atcctcgcgg acggattatt
gaagtatacg gtcctgaaag ctcaggtaaa acaactgtgg cgcttcatgc gattgctgaa
gttcagcagc agggcggaca agccgcgttt atcgatgcgg agcatgcgtt agatccggta
tacgcgcaaa agctcggtgt taacatcgaa gagcttttac tgtctcagcc tgacacaggc
gagcaggcgc ttgaaattgc ggaagcattg gttcgaagcg gggcagttga cattgtcgtt
gtcgactctg tagccgctct cgttccgaaa gcggaaattg aaggcgacat gggagattcg
catgtcggtt tacaagcacg cttaatgtct caagcgcttc gtaagctttc aggggccatt
aacaaatcga agacaatcgc gattttcatt aaccaaattc gtgaaaaagt cggtgttatg
ttcgggaacc cggaaacaac acctggcggc cgtgcgttga aattctattc ttccgtgcgt
cttgaagtgc gccgtgctga acagctgaaa caaggcaacg acgtaatggg gaacaaaacg
aaaatcaaag tcgtgaaaaa caaggtggct ccgccgttcc gtacagccga ggttgacatt
atgtacggag aaggcatttc aaaagaaggc gaaatcattg atctaggaac tgaacttgat
atcgtgcaaa aaagcggttc atggtactct tatgaagaag agcgtcttgg ccaaggccgt
gaaaatgcaa aacaattcct gaaagaaaat aaagatatca tgctgatgat ccaggagcaa
attcgcgaac attacggctt ggataataac ggagtagtgc agcagcaagc tgaagagaca
caagaagaac tcgaatttga agaataa

FIG. 9C

GenBank Accession No. DQ769876.1 (SEQ ID NO:12)

atggctattg atgagaataa acaaaaggcg ttagcagcag cactgggcca aattgaaaaa
caattcggta aaggctctat tatgcgcctt ggcgaagacc gctcaatgga tgttgaaacc
atctctaccg gctccctttc ccttgatatt gcactggggg ctggtggctt accaatgggg
cgtatcgttg agatttatgg cccagaatca tcaggtaaga cgacactgac attacaggtt
atcgccgccg cacagcgtga aggcaaaacg tgtgcattta tcgatgccga acatgccctt
gacccaatct atgccaagaa attgggtgta gatattgata acctattgtg ttctcagcca
gatactggcg agcaggcact ggaaatttgt gatgcgctga ctcgctctgg tgcggttgac
gttatcatcg ttgactccgt agcggcattg acaccaaaag ctgaaattga aggtgaaatt
ggcgattctc atatgggcct tgccgcgcgt atgatgagcc aggctatgcg taagctggcg
ggtaacctga agaatgcgaa taccttactg atttttatca accaaatccg catgaaaatt
ggcgtgatgt ttggtaaccc agaaaccact accggtggca acgctcttaa attttacgct
tctgtacgtt tggatatccg ccgtattggt gcagtaaaag atggtgatgt ggtcgtgggg
agtgaaaccc gcgttaaagt cgttaaaaac aagattgctg cgccattcaa acaagctgaa
ttccagatcc tctacggtga aggcattaat atcaacggtg aactggttga cttaggtgtt
aaacacaaac tgattgagaa agctggcgca tggtatagct ataacggtga taaaattggt
cagggtaaag ccaatgccag caactattta aaagaaaacc cagccattgc tgctgagtta
gataaaaaac tgcgtgaaat gctacttaat ggcggcaatg gtgaacaacc tgttgctgcg
gcaacagcag aattcgccga tggtgcagat gaaaccaacg aagaattttag

FIG. 9D

cDNA encoding a WT GFP (SEQ ID NO:30)

ATGGAGAGCGACGAGAGCGGCCTGCCCGCCATGGAGATCGAGTGCCGCATCACCGGCACCCTG
AACGGCGTGGAGTTCGAGCTGGTGGGCGGCGGAGAGGGCACCCCCAAGCAGGGCCGCATGACC
AACAAGATGAAGAGCACCAAAGGCGCCCTGACCTTCAGCCCCTACCTGCTGAGCCACGTGATG
GGCTACGGCTTCTACCACTTCGGCACCTACCCCAGCGGCTACGAGAACCCCTTCCTGCACGCC
ATCAACAACGGCGGCTACACCAACACCCGCATCGAGAAGTACGAGGACGGCGGCGTGCTGCAC
GTGAGCTTCAGCTACCGCTACGAGGCCGGCCGCGTGATCGGCGACTTCAAGGTGGTGGGCACC
GGCTTCCCCGAGGACAGCGTGATCTTCACCGACAAGATCATCCGCAGCAACGCCACCGTGGAG
CACCTGCACCCCATGGGCGATAACGTGCTGGTGGGCAGCTTCGCCCGCACCTTCAGCCTGCGC
GACGGCGGCTACTACAGCTTCGTGGTGGACAGCCACATGCACTTCAAGAGCGCCATCCACCCC
AGCATCCTGCAGAACGGGGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAGCTGCACAGCAAC
ACCGAGCTGGGCATCGTGGAGTACCAGCACGCCTTCAAGACCCCCATTGCCTTCGCCAGATCC
CGCGCTCAGTCGTCCAATTCTGCCGTGGACGGCACCGCCGGACCCGGCTCCACCGGATCTCGC
TAAGAATTCGTCGACAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTT
AACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATT
GCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAg
GAgTTGTGGCCCGTTGTCAGGCAACGTGGCgTGGTGTGCACTGTGTTTGCTGACgCAaCCCCC
ACTGGTTGGGGCATTGCCACCACcTGTCAgCTCcTTTcCGGGAcTTT

FIG. 10A

cDNA having a frame shift deletion encoding a mutated GFP (SEQ ID NO:31)

ATGGAGAGCGACGAGAGCGGCCTGCCCGCCATGGAGATCGAGTGCCGCATCACCGGCACCCTG
AACGGCGTGGAGTTCGAGCTGGTGGGCGGCGGAGAGGGCACCCCCAAGCAGGGCCGCATGACC
AACAAGATGAAGAGCACCAAAGGCGCCCTGACCTTCAGCCCCTACCTGCTGAGCCACG**ΔΔΔΔ**G
GGCTACGGCTTCTACCACTTCGGCACCTACCCCAGCGGCTACGAGAACCCCTTCCTGCACGCC
ATCAACAACGGCGGCTACACCAACACCCGCATCGAGAAGTACGAGGACGGCGGCGTGCTGCAC
GTGAGCTTCAGCTACCGCTACGAGGCCGGCCGCGTGATCGGCGACTTCAAGGTGGTGGGCACC
GGCTTCCCCGAGGACAGCGTGATCTTCACCGACAAGATCATCCGCAGCAACGCCACCGTGGAG
CACCTGCACCCCATGGGCGATAACGTGCTGGTGGGCAGCTTCGCCCGCACCTTCAGCCTGCGC
GACGGCGGCTACTACAGCTTCGTGGTGGACAGCCACATGCACTTCAAGAGCGCCATCCACCCC
AGCATCCTGCAGAACGGGGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAGCTGCACAGCAAC
ACCGAGCTGGGCATCGTGGAGTACCAGCACGCCTTCAAGACCCCCATTGCCTTCGCCAGATCC
CGCGCTCAGTCGTCCAATTCTGCCGTGGACGGCACCGCCGGACCCGGCTCCACCGGATCTCGC
TAAGAATTCGTCGACAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTT
AACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATT
GCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAg
GAgTTGTGGCCCGTTGTCAGGCAACGTGGCgTGGTGTGCACTGTGTTTGCTGACgCAaCCCCC
ACTGGTTGGGGCATTGCCACCACcTGTCAgCTCcTTTcCGGGAcTTT

FIG. 10B

cDNA encoding a WT DHFR (SEQ ID NO:38)

aagctttatc cccgctgcca tcatggttcg accattgaac tgcatcgtcg ccgtgtccca
agatatgggg attggcaaga acggagacct accctggcct ccgctcagga acgagtggaa
gtacttccaa agaatgacca caacctcttc agtggaaggt aaacagaatc tggtgattat
gggtaggaaa acctggttct ccattcctga gaagaatcga cctttaaagg acagaattaa
tatagttctc agtagagaac tcaaagaacc accacgagga gctcattttc ttgccaaaag
tttggatgat gccttaagac ttattgaaca accggaattg gcaagtaaag tagacatggt
ttggatagtc ggaggcagtt ctgtttacca ggaagccatg aatcaaccag gccacctcag
actctttgtg acaaggatca tgcaggaatt tgaaagtgac acgtttttcc cagaaattga
tttggggaaa tataaacttc tcccagaata cccaggcgtc ctctctgagg tccaggagga
aaaaggcatc aagtataagt ttgaagtcta cgagaagaaa gactaacagg aagatgcttt
caagttctct gctcccctcc taaagctatg catttttata agaccatggg actttgctg
gctttagatc t

FIG. 11A

cDNA having a frame shift deletion encoding a mutated DHFR (SEQ ID NO:39)

aagctttatc cccgctgcca tcatggttcg accattgaac tgcatcgtcg ccgtgtccca
agatatgggg attggcaaga acggagacct accctggcct ccgctcagga acgagtggaa
gtacttccaa agaa**ΔΔ**acca caacctcttc agtggaaggt aaacagaatc tggtgattat
gggtaggaaa acctggttct ccattcctga gaagaatcga cctttaaagg acagaattaa
tatagttctc agtagagaac tcaaagaacc accacgagga gctcattttc ttgccaaaag
tttggatgat gccttaagac ttattgaaca accggaattg gcaagtaaag tagacatggt
ttggatagtc ggaggcagtt ctgtttacca ggaagccatg aatcaaccag gccacctcag
actctttgtg acaaggatca tgcaggaatt tgaaagtgac acgtttttcc cagaaattga
tttggggaaa tataaacttc tcccagaata cccaggcgtc ctctctgagg tccaggagga
aaaaggcatc aagtataagt ttgaagtcta cgagaagaaa gactaacagg aagatgcttt
caagttctct gctcccctcc taaagctatg cattttata agaccatggg acttttgctg
gctttagatc t

FIG. 11B

METHODS AND MATERIALS FOR GENE EDITING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Patent Application Ser. No. 62/571,457, filed on Oct. 12, 2017, and U.S. Patent Application Ser. No. 62/627,729, filed on Feb. 7, 2018. The entire contents of which are hereby incorporated by reference.

BACKGROUND

1. Technical Field

[0002] This document relates to methods and materials involved in gene editing. For example, this document provides methods and materials for using a RecA polypeptide fused to a cell penetrating peptide (CPP) to edit (e.g., correct) a gene.

2. Background Information

[0003] Many genetic disorders, such as color blindness (see, e.g., Nathans et al., 1989 *Science* 245:831-838; Weitz et al., 1992 *Am J Hum Genet* 50:498-507; Winderickx et al., 1992 *Nat Genet* 1:251-256; and Mackey, 1994 *Eye* (*Lond*) 8(Pt 4):431-436); cystic fibrosis (see, e.g., Kerem et al., 1989 *Science* 245:1073-1080; and Bobadilla et al., 2002 *Hum Mutat* 19:575-606); haemochromatosis (see, e.g., Feder et al., 1996 *Nat Genet* 13:399-408; and Pietrangelo et al., 1999 *N Engl J Med* 341:725-732); haemophilia (see, e.g., Gitschier et al., 1985 *Nature* 315:427-430; Rees et al., 1985 *Nature* 316:643-645; Bentley et al., 1986 *Cell* 45:343-348; Davis et al., 1987 *Blood* 69:140-143; Youssoufian et al., 1986 *Nature* 324:380-382; Diuguid et al., 1986 *Proc Natl Acad Sci USA* 83:5803-5807; and Gitschier et al., 1986 *Science* 232:1415-1416); phenylketonuria (see, e.g., DiLella et al., 1987 *Nature* 327:333-336; and Lyonnet et al., 1989 *Am J Hum Genet* 44:511-517); polycystic kidney disease (see, e.g., Bisceglia et al., 2006 *Adv Anat Pathol* 13:26-56; and Audrezet et al., 2012 *Hum Mutat* 33:1239-1250); sickle-cell disease (see, e.g., ghr.nim.nih.gov/condition/sickle-cell-disease); and some of the duchenne muscular dystrophy (see, e.g., Aartsma-Rus et al., 2006 *Muscle Nerve* 34:135-144), are caused by small deletion/insertion or simple point mutations. For example, a deletion of three nucleotide (nt) coding for phenylalanine at position of 508 (ΔF508) in the cystic fibrosis transmembrane conductance regulator (CFTR) or ATP-binding cassette transporter C7 (ABCC7) gene, the most common mutation in cystic fibrosis, results in thermolability and mis-folding of the CFTR/ABCC7 ion channel protein on the apical membrane of epithelial cells (see, e.g., Cheng et al., 1990 *Cell* 63:827-834; and Denning et al., 1992 *Nature* 358:761-764) and causes cystic fibrosis. Such disease-causing mutations can potentially be corrected by homology-directed recombination (HDR).

[0004] However, HDR is a complex processing of orchestrated reactions involving multiple factors. In addition, presynaptic single stranded DNA (ssDNA) invasion (searching for homologous sequences) plays a crucial role for initiation of the HDR. The greatest challenge in HDR-mediated gene correction is the creation of recombinogenic DNA ends near the mutation site. Development of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system provides a mean to cut the DNA (e.g., by making a double strand DNA (dsDNA) break) near the mutation site (see, e.g., Ramirez et al., 2008 *Nat Methods* 5:374-375; Maeder et al., 2008 *Mol Cell* 31:294-301; Boch, 2011 *Nat Biotechnol* 29:135-136; Jinek et al., 2012 *Science* 337:816-821; Pennisi, 2013 *Science* 341:833-836; Ran et al., 2013 *Nature protocols* 8:2281-2308; Ran et al., 2013 *Cell* 154:1380-1389; and Cong et al., 2013 *Science* 339:819-823). Unfortunately, non-homologous end-joining (NHEJ), albeit without ensuring restoration of the DNA sequence around the break site, plays a dominant role over HDR for any dsDNA break repair in mammalian cells (see, e.g., Fu et al., 2013 *Nat Biotechnol* 31:822-826; Mali et al., 2013 *Nat Biotechnol* 31:833-838; and Hsu et al., 2013 *Nat Biotechnol* 31:827-832), meaning that the efficiency of the HDR-mediated repair of the mutation near the CRISPR/Cas9-gRNA cutting site could be low. In addition, the modifications at the break site, including a few nucleotides insertion (see, e.g., Roth et al., 1989 *Mol Cell Biol* 9(7):3049-3057; and Chang et al., 1987 *Proc Natl Acad Sci USA* 84:4959-4963) and/or deletion (see, e.g., Smithies et al., 1985 *Nature* 317:230-234), may cause deleterious mutations, suggesting that mutations introduced by CRISPR/Cas9 system may dominate the HDR of the disease-causing mutations. In fact, the frequency of mutations introduced by guideRNA complementary to the target DNA is significantly higher than the gene-correction mediated by HDR (see, e.g., Thomas et al., 1986 *Cell* 44:419-428; and Xu et al., 2017 *Mol Ther Nucleic Acids* 16:429-438). In addition, the random dsDNA break insertions, such as CRISPR/Cas9 DNA or donor DNA insertion into chromosomes, and/or off-target modifications may also cause mutations that affect normal cell functions. Furthermore, it has been reported that unexpected mutations occurred after CRISPR-Cas9-mediated genome editing in vivo (see, e.g., Roth et al., 1989 *Mol Cell Biol* 9:3049-3057; and Schaefer et al., 2017 *Nat Methods* 14(6):547-548), suggesting that safety is a very important issue in CRISPR/Cas9 mediated gene correction. Thus, a safer technology is critically needed in the design of strategies to correct mutations in genetic disease.

SUMMARY

[0005] This document relates to methods and materials for gene editing. For example, this document provides methods and materials for using a RecA polypeptide fused to a cell penetrating peptide (CPP) to edit (e.g., correct) a nucleic acid sequence (e.g., a coding sequence such as a gene) within a cell. In some cases, the methods and materials provided herein can be used to correct a nucleic acid sequence containing one or more mutations such as deletions/insertions and/or point mutations. For example, a RecA polypeptide fused to a CPP can be used insert/delete a nucleic acid sequence (e.g., a coding sequence such as a gene) within a cell to correct a nucleic acid sequence containing one or more mutations such as deletions/insertions and/or point mutations. In some cases, the methods and materials provided herein can be used to treat a mammal having a genetic disease or genetic condition (e.g., a monogenetic disease or a monogenetic condition) caused, at least in part, by one or more mutations such as a deletion/insertion and/or a point mutation in a nucleic acid sequence (e.g., a coding sequence such as a gene) within a cell. For example, a RecA polypeptide fused to a CPP can be used insert a

nucleic acid sequence (e.g., a coding sequence such as a gene) within a cell of a mammal to correct a nucleic acid sequence containing one or more mutations such as deletions/insertions and/or point mutations in the cell to treat the mammal.

[0006] In general, one aspect of this document features fusion proteins including a RecA polypeptide and CPP. The RecA polypeptide can include an amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4. The RecA polypeptide can be at the N-terminus of the fusion protein. The CPP can be a trans-activating transcriptional activator (TAT) peptide sequence, a Pep-1 peptide sequence, or a MPG peptide sequence. When the CPP is a TAT peptide, the TAT peptide sequence can include the amino acid sequence YGRKKRRQRRR (SEQ ID NO:5). When the CPP is a Pep-1 peptide, the Pep-1 peptide sequence can include the amino acid sequence KETWWETWWTEWSQPKKKRKV (SEQ ID NO:6). When the CPP is a MPG peptide, the MPG peptide sequence can include the amino acid sequence SVVDRVAEQDTQA (SEQ ID NO:7). The CPP can be at the C-terminus of the fusion protein. The fusion protein further also can include a peptide linker (e.g., present between the RecA polypeptide and the CPP). The peptide linker can be a peptide sequence including SGLRSRAAANT (SEQ ID NO:8), one or more alanine residues, one or more glycine residues, or combinations thereof. The fusion protein also can include a peptide tag. The peptide tag can include an antibody epitope (e.g., a multidrug resistance protein 1 (MRP1) antibody epitope). The peptide tag can include a fluorescent protein (e.g., a green fluorescent protein GFP)). The fusion protein can include both a MRP1 antibody epitope and a GFP.

[0007] In another aspect, this document features fusion proteins including, from N-terminus to C-terminus, a RecA polypeptide, a linker, a first tag, a second tag, and a CPP. For example, the fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide including the amino acid sequence set forth in SEQ ID NO:4, an L1 linker, a MRP1 antibody epitope for a first tag, ten histidine residues for a second tag, and a TAT peptide including the amino acid sequence YGRKKRRQRRR (SEQ ID NO:5) for a CPP.

[0008] In another aspect, this document features fusion proteins including, from N-terminus to C-terminus, a RecA polypeptide, a first linker, a green fluorescent protein, a second linker, a first tag, a second tag, and a CPP. For example, the fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide including the amino acid sequence set forth in SEQ ID NO:4, an L1 linker as a first linker, 2 alanine residues as a second linker, a MRP1 antibody epitope as a first tag, ten histidine residues as a second tag, and a TAT peptide including the amino acid sequence YGRKKRRQRRR (SEQ ID NO:5) for a CPP.

[0009] In another aspect, this document features nucleic acid constructs encoding a fusion protein including a RecA polypeptide and CPP. The nucleic acid construct can include a nucleic acid sequence encoding a RecA polypeptide. The nucleic acid sequence encoding a RecA polypeptide can include a nucleic acid sequence set forth in SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12. The nucleic acid construct can include a nucleic acid sequence encoding a CPP. The nucleic acid sequence encoding said CPP can include a nucleic acid sequence set forth in SEQ ID NO:13

[0010] In another aspect, this document features nucleoprotein filaments including one or more fusion proteins including a RecA polypeptide and CPP, and a single stranded oligonucleotide, where the single stranded oligonucleotide can hybridize to a target sequence. The target sequence can have one or more mutations, and the single stranded oligonucleotide can include a corrected nucleic acid sequence.

[0011] In another aspect, this document features methods for editing the genome of a cell. The methods can include, or consist essentially of, contacting a cell with a fusion protein including a RecA polypeptide and CPP; and a single stranded oligonucleotide, where the single stranded oligonucleotide can hybridize to a target sequence within the cell having one or more mutations, and where the single stranded oligonucleotide includes a corrected nucleic acid sequence. The cell can be a prokaryotic cell. The cell can be a eukaryotic cell. The eukaryotic cell can be a mammalian cell.

[0012] In another aspect, this document features methods for treating a mammal having a monogenetic disease. The methods can include, or consist essentially of, contacting a cell in a mammal having a monogenetic disease with a fusion protein including a RecA polypeptide and CPP and a single stranded oligonucleotide, where the single stranded oligonucleotide can hybridize to a target sequence in a genome within the cell, where the target sequence includes a nucleic acid sequence having one or more disease-causing mutations, and where the single stranded oligonucleotide includes a corrected nucleic sequence. The mammal can be a human. The monogenetic disease can be color blindness, cystic fibrosis, haemochromatosis, haemophilia, phenylketonuria, polycystic kidney disease, Tay-Sachs disease, Huntington's disease, Marfan syndrome, sickle-cell disease, duchenne muscular dystrophy, or cancer. The fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide, a linker, a first tag, a second tag, and a CPP. For example, the fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide including the amino acid sequence set forth in SEQ ID NO:4, an L1 linker, a MRP1 antibody epitope as a first tag, ten histidine residues as a second tag, and a TAT peptide including the amino acid sequence YGRKKRRQRRR (SEQ ID NO:5) as a CPP. The fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide, a first linker, a green fluorescent protein, a second linker, a first tag, a second tag, and a CPP. For example, the fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide including the amino acid sequence set forth in SEQ ID NO:4, an L1 linker as a first linker, GFP, 2 alanine residues as a second linker, a MRP1 antibody epitope as a first tag, ten histidine residues as a second tag, and a TAT peptide including the amino acid sequence YGRKKRRQRRR (SEQ ID NO:5) as a CPP.

[0013] In another aspect, this document features methods for detecting HDR mediated gene correction in a cell having a modified nucleic acid sequence, where the modified nucleic acid sequence can encode a polypeptide having a loss-of-function mutation. The methods can include, or consist essentially of, contacting a cell having a modified nucleic acid sequence with a fusion protein including a RecA polypeptide and CPP, and a single stranded oligonucleotide, where the single stranded oligonucleotide can hybridize to the modified nucleic acid sequence, where the single stranded oligonucleotide includes a corrected nucleic acid sequence, and where the corrected nucleic acid, in the

presence of HDR, can replace the modified nucleic acid sequence and can encode a functional polypeptide; such that detection of the functional polypeptide indicates the present of HDR in the cell. The cell can be a eukaryotic cell. The cell can be a human cell. The modified nucleic acid sequence can encode a reporter polypeptide having a loss-of-function mutation, and detection of the reporter function can indicate the present of HDR in the cell. The reporter polypeptide can be GFP, the modified nucleic acid sequence encoding a GFP having a loss-of-function mutation can include the sequence set forth in SEQ ID NO:31, and the single stranded oligonucleotide including an insertion can include a sequence set forth in SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, or SEQ ID NO:37. The reporter polypeptide can be a dihydrofolate reductase (DHFR) polypeptide, the modified nucleic acid sequence encoding a DHFR having a loss-of-function mutation can include the sequence set forth in SEQ ID NO:39, and the single stranded oligonucleotide including an insertion can include a sequence set forth in SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, or SEQ ID NO:45.

[0014] 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 to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0015] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

[0016] FIGS. **1**A and **1**B are diagrams showing exemplary RecA-CPP fusion proteins. FIG. **1**A shows the design of a shorter version of a RecA-CPP fusion protein (RecA-CPP) containing a RecA polypeptide, a linker (L1), a first tag (Tag1), a second tag (Tag2), and a CPP. FIG. **1**B shows the design of a longer version of a RecA-CPP fusion protein (RecA-GFP-CPP) containing a RecA polypeptide, a linker (L1), green fluorescent protein (GFP), a second linker (L2), a first tag (Tag1), a second tag (Tag2), and a CPP.

[0017] FIG. **2** is a schematic diagram showing an exemplary ssDNA:RecA-GFP-CPP fusion protein mediated transfection to correct disease-causing gene mutations.

[0018] FIGS. **3**A and **3**B show that RecA-CPP fusion protein expressed in bacteria is in the soluble fraction. FIG. **3**A is a representative western blot (100 μg protein per lane) of the shorter RecA-CPP fusion protein probed with multidrug resistance protein 1 (MRP1) mAb 42.4. FIG. **3**B is a representative western blot (100 μg protein per lane) of the longer RecA-GFP-CPP fusion protein probed with MRP1 mAb 42.4.

[0019] FIGS. **4**A and **4**B show that bacterial growth is completely inhibited by the addition of IPTG at 37° C. FIG. **4**A shows that, after transformation of the DL21 competent cells with the shorter version of the RecA-CPP fusion construct in pET32a vector, the cells were plated out on plates with 100 μg/mL ampicillin (the plate on the left) or with 100 μg/mL ampicillin and 0.25 mM IPTG (the plate on the right). FIG. **4**B shows that, after transformation of the DL21 competent cells with the longer version of the RecA-GFP-CPP fusion construct in pET32a vector, the cells were plated out on plates with 100 μg/mL ampicillin (the plate on the left) or with 100 μg/mL ampicillin and 0.25 mM IPTG (the plate on the right).

[0020] FIGS. **5**A and **5**B show the expression of RecA-CPP fusion proteins in BHK cells. FIG. **5**A is a representative western blot (100 μg protein per lane) showed that majority of the shorter RecA-CPP fusion protein expressed in BHK cells is in soluble fraction. FIG. **5**B is a representative western blot (100 μg protein per lane) showed that majority of the longer RecA-GFP-CPP fusion protein expressed in BHK cells is also in soluble fraction.

[0021] FIG. **6** contains a graph showing that expression of RecA-CPP fusion protein in BHK cells significantly inhibited cell growth. 10,000 cells were plated out on day 0 and counted after 3 days incubation at 37° C. The numbers of cells, after 3 days incubation, were: 236,667±25,403 (BHK); 81,500±12,817 (RecA-GFP-CPP); and 96,300±12,817 (RecA-CPP). * indicates that the P value is 0.2302; ***, 0.0010; ****, 0.0007.

[0022] FIG. **7** contains an image of a western blot showing a comparison of the fusion proteins expressed in bacteria and in BHK cells. The representative western blot (100 μg protein per lane), probed with MRP1 mAb 42.4, showed that RecA-GFP-CPP or RecA-CPP expressed in BHK cells is significantly less than in DL21 bacteria cells.

[0023] FIGS. **8**A-**8**D contain amino acid sequences of exemplary RecA polypeptides. FIG. **8**A contains SEQ ID NO:1. FIG. **8**B contains SEQ ID NO:2. FIG. **8**C contains SEQ ID NO:3. FIG. **8**D contains SEQ ID NO:4.

[0024] FIGS. **9**A-**9**D contain nucleic acid sequences encoding exemplary RecA polypeptides. FIG. **9**A contains SEQ ID NO:9. FIG. **9**B contains SEQ ID NO:10. FIG. **9**C contains SEQ ID NO:11. FIG. **9**D contains SEQ ID NO:12.

[0025] FIGS. **10**A-**10**B contains nucleic acid sequences encoding GFP polypeptides. FIG. **10**A contains a nucleic acid sequence (SEQ ID NO:30) encoding a wild type GFP. FIG. **10**B contains a nucleic acid sequence having a deletion of 4 nucleotides from 185 to 188 (TGAT) of a GFP coding sequence (SEQ ID NO:31) such that the nucleic acid sequence encodes a non-functional GFP. The Δ symbols indicate the deleted nucleotides.

[0026] FIGS. **11**A-**11**B contains nucleic acid sequences encoding mouse dihydrofolate reductase (DHFR) polypeptides. FIG. **11**A contains a nucleic acid sequence (SEQ ID NO:38) encoding a wild type DHFR. FIG. **11**B contains a nucleic acid sequence having a deletion of 2 nucleotides from 135 to 136 (TG) of a DHFR coding sequence (SEQ ID NO:39) such that the nucleic acid sequence encodes a non-functional DHFR. The Δ symbols indicate the deleted nucleotides.

DETAILED DESCRIPTION

[0027] This document provides methods and materials for gene editing. For example, this document provides methods and materials for using a RecA polypeptide fused to a cell penetrating peptide (CPP) to edit (e.g., correct) a gene. In some cases, the methods and materials provided herein can

be used to correct a nucleic acid sequence (e.g., a coding sequence such as a gene) containing one or more mutations such as small deletions/insertions and/or point mutations. In some cases, the methods and materials provided herein can be used to treat a mammal having a genetic disease or genetic condition (e.g., a monogenetic disease or monogenetic condition) caused, at least in part, by one or more mutations in a nucleic acid sequence (e.g., a coding sequence such as a gene) within one or more cells in the mammal. Also provided herein are fusion proteins containing a RecA polypeptide and a CPP, nucleic acid constructs encoding a fusion protein comprising a RecA polypeptide and a CPP, and nucleoprotein filaments containing one or more (e.g., one, two, three, four, five, six, seven, eight, nine, or more) fusion proteins described herein (e.g., fusion proteins including a RecA polypeptide and a CPP) and a single stranded oligonucleotide (e.g., a ssDNA).

[0028] In some cases, the methods and materials provided herein do not cause additional mutations (e.g., mutations caused by dsDNA break-mediated insertion). For example, in some cases, the methods and materials provided herein do not include any nuclease (e.g., any sequence-specific nuclease) and/or capable of introducing a dsDNA break. Examples of nucleases capable of introducing a dsDNA break include, without limitation, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR associated proteins (Cas enzymes such as Cas9). For example, in some cases, the methods and materials provided herein do not include any gene editing systems that include one or more nucleases capable of introducing a dsDNA break. Examples of gene editing systems that include one or more nucleases capable of introducing a dsDNA break include, without limitation, CRISPR/Cas systems such as a CRISPR/Cas9 system.

[0029] In some cases, the methods and materials provided herein can include HDR. For example, the methods and materials provided herein can include HDR in the absence of any dsDNA break. For example, as described herein an ABCC1/MRP1/ΔF728 model system (see, e.g., Xu et al., 2017 *Mol Ther Nucleic Acids* 16:429-438) for gene correction with single stranded oligonucleotides covering the 3 nucleotide-deletion site, via ssDNA-RecA-CPP nucleoprotein filaments correct the deletion mutation. This method can be used to edit genes while introducing fewer mutations than the CRISPR/Cas9 system. Since this system does not need to generate a dsDNA break near the mutation site, cas9 or any other nucleases are not needed. In addition, the single strand oligonucleotides are protected from nuclease digestion via formation of nucleoprotein filament with RecA polypeptide (see, e.g., Chen et al., 2008 *Nature* 453(7194): 761-764; and Lieber, 2010 *Annu Rev Biochem* 79:181-211) both in vitro (in the presence of ATP) and in vivo. In the meantime, binding of RecA to the single stranded oligonucleotide can promote HDR (see, e.g., Chen et al., 2008 *Nature* 453(7194):761-764; and Lieber, 2010 *Annu Rev Biochem* 79:181-211). To facilitate the entry of the nucleoprotein filament into the cells, RecA can be fused with cell-penetrating peptide (CPP) (see, e.g., Chang et al., 2018 *Int J Biochem Mol Biol* 9:1-10). Furthermore, a reporter protein (e.g., GFP) can also be included in the fusion protein so that the transfected cells can be sorted out. Thus, recombinant proteins described herein (e.g., CPP-RecA, CPP-GFP-RecA, RecA-CPP, and RecA-GFP-CPP) can be made (e.g., from N-terminus to C-terminus) and can be used in oligonucleotide-CPP-RecA nucleoprotein complex mediated transfection to treat a mammal in need thereof (e.g., to correct a disease-causing mutation in a mammal).

[0030] This document provides fusion proteins containing a RecA polypeptide and a CPP, nucleic acid constructs encoding a fusion protein comprising a RecA polypeptide and a CPP, and nucleoprotein filaments containing one or more (e.g., one, two, three, four, five, six, seven, eight, nine, or more) fusion proteins described herein (e.g., fusion proteins including a RecA polypeptide and a CPP) and a single stranded oligonucleotide.

[0031] A fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP) can include any appropriate RecA polypeptide. In some cases, a RecA polypeptide can be a bacterial RecA polypeptide (e.g., *Escherichia coli* RecA polypeptides, *Mycobacterium tuberculosis* RecA polypeptides, *Bacillus subtilis* RecA polypeptides, and *Yersinia* RecA polypeptides). In some cases, a RecA polypeptide can be a mammalian homolog of a RecA polypeptide (e.g., a RAD51 polypeptide such as a human RAD51 polypeptide). Examples of RecA polypeptides include, without limitation, polypeptide sequences set forth in the National Center for Biotechnology Information (NCBI) databases at GenBank Accession No. AML00775 (Version AML00775.1), GenBank Accession No. CAA41395 (Version CAA41395.1), GenBank Accession No. NP389576 (Version NP_389576.2), GenBank Accession No. WP_002209446 (Version WP_002209446.1), and GenBank Accession No. BAA03189 (Version BAA03189.1). In some cases, RecA polypeptides can be as shown in FIG. **8**. For example, a RecA polypeptide can include an amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4. In some cases, RecA polypeptides can be as described elsewhere (see, e.g., Chen et al., 2008 *Nature,* 453:489-4). A RecA polypeptide can be at either end of a fusion protein described herein. For example, a RecA polypeptide can be at the N-terminus of a fusion protein described herein. For example, a RecA polypeptide can be at the C-terminus of a fusion protein described herein.

[0032] In some cases, a RecA polypeptide in a fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP) can have a sequence that deviates from a wild type RecA polypeptide sequence, sometimes referred to as a variant sequence. For example, a RecA polypeptide sequence can have at least 80% sequence identity (e.g., at least 85% sequence identity, 90% sequence identity, 95% sequence identity, or at least 99% sequence identity) to SEQ ID NO:1 provided that it includes one or more amino acid additions, subtractions, or substitutions compared to SEQ ID NO:1. For example, a RecA polypeptide sequence can have at least 80% sequence identity (e.g., at least 85% sequence identity, 90% sequence identity, 95% sequence identity, or at least 99% sequence identity) to SEQ ID NO:2 provided that it includes one or more amino acid additions, subtractions, or substitutions compared to SEQ ID NO:2. For example, a RecA polypeptide sequence can have at least 80% sequence identity (e.g., at least 85% sequence identity, 90% sequence identity, 95% sequence identity, or at least 99% sequence identity) to SEQ ID NO:3 provided that it includes one or more amino acid additions, subtractions, or substitutions compared to SEQ ID NO:3. For example, a RecA polypeptide sequence can have at least 80% sequence identity (e.g., at least 85% sequence identity, 90% sequence

identity, 95% sequence identity, or at least 99% sequence identity) to SEQ ID NO:4 provided that it includes one or more amino acid additions, subtractions, or substitutions compared to SEQ ID NO:4. Percent sequence identity is calculated by determining the number of matched positions in aligned polypeptide sequences, dividing the number of matched positions by the total number of aligned amino acids, respectively, and multiplying by 100. A matched position refers to a position in which identical amino acids occur at the same position in aligned sequences. The total number of aligned amino acids refers to the minimum number of RecA amino acids that are necessary to align the second sequence, and does not include alignment (e.g., forced alignment) with non-RecA sequences, such as those fused to RecA. The total number of aligned amino acids may correspond to the entire RecA sequence or may correspond to fragments of the full-length RecA sequence as defined herein. Sequences can be aligned using the algorithm described by Altschul et al. (*Nucleic Acids Res.,* 25:3389-3402 (1997)) as incorporated into BLAST (basic local alignment search tool) programs, available at ncbi.nlm.nih.gov on the World Wide Web. BLAST searches or alignments can be performed to determine percent sequence identity between a RecA polypeptide and any other sequence or portion thereof using the Altschul et al. algorithm. For example, BLASTP can be used to align and compare the identity between amino acid sequences. When utilizing BLAST programs to calculate the percent identity between a RecA sequence and another sequence, the default parameters of the respective programs are used.

[0033] A fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP) can include any appropriate CPP. In some cases, a CPP can be a naturally occurring CPP. In some cases, a CPP can be an artificial CPP. In some cases, a CPP can be a synthetic CPP. Examples of CPPs include, without limitation, a TAT peptide sequence (e.g., YGRKKRRQRRR (SEQ ID NO:5)), a Pep-1 peptide sequence (e.g., KETWWETWWTEWSQPKKKRKV; SEQ ID NO:6), and a MPG peptide sequence (e.g., SVVDRVAEQDTQA; SEQ ID NO:7). In some cases, a CPP can be as described elsewhere (e.g., Okuyama et al., 2007 *Nat. Methods,* 4:153-9). A CPP can be at either end of a fusion protein described herein. For example, a CPP can be at the C-terminus of a fusion protein described herein. For example, a CPP can be at the N-terminus of a fusion protein described herein.

[0034] In some cases, a fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP) also can include one or more nuclear localization signal (NLS) polypeptides.

[0035] In some cases, a fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP) also can include one or more (e.g., one, two, three, or more) linkers. Examples of linkers include, without limitation, a peptide sequence including SGLRSRAAANT (SEQ ID NO:8), one or more alanine residues (e.g., 2 alanine residues), one or more glycine residues, and combinations thereof. In some cases, a linker can be as described elsewhere (e.g., Hou et al., 2009 *Biochemistry,* 48: 9122-9131). For example, a linker can be present between a RecA polypeptide and a CPP of a fusion protein described herein.

[0036] In some cases, a fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP) also can include one or more (e.g., one, two, three, or more) tags (e.g., detectable markers). Tags can be for detection, sorting, and/or purification of a protein. A tag can be any appropriate type of molecule (e.g., a protein tag). Examples of tags include, without limitation, fluorescent markers (e.g., GFP), epitopes (e.g., monoclonal antibody epitopes such as an MRP1 monoclonal antibody epitope), and histidine tags (e.g., a polyHis tag containing about 10 histidine residues). In cases where a fusion protein includes an MRP1 monoclonal antibody epitope, an MRP1 antibody (e.g., a monoclonal antibody such as MRP1 mAb 42.4) can be used to detect, sort, and/or purify the fusion protein (e.g., from bacterial cells and/or from mammalian cells). In some cases, a fusion protein provided herein can include a single tag. In some cases, a fusion protein provided herein can include two or more (e.g., two, three, or four) tags. A tag can be at any appropriate location within a fusion protein described herein. In some cases, a tag can be in the center (e.g., not at an end) of a fusion protein described herein. For example, a tag can be at any position between the N-terminus and C-terminus of the fusion protein. In some cases, a tag can be at an end of a fusion protein described herein. For example, a tag can be at the N-terminus of a fusion protein described herein. For example, a tag can be at the C-terminus of a fusion protein described herein.

[0037] In some cases, a fusion protein can include, from N-terminus to C-terminus, a CPP and a RecA polypeptide. In some cases, a fusion protein can include, from N-terminus to C-terminus, a CPP, a GFP, and a RecA polypeptide. In some cases, a fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide and a CPP. In some cases, a fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide, a GFP, and a CPP. In some cases, a fusion protein can include, from N-terminus to C-terminus, a RecA polypeptide, a first linker, a GFP, a second linker, an MRP1 monoclonal antibody epitope, 10 histidine residues, and a CPP. Exemplary fusion proteins can be as shown in FIG. **1**. For example, a fusion protein can include about 687 amino acids, and can contain (e.g., from N-terminus to C-terminus) a RecA, a linker (e.g., a first linker), a GFP, a linker (e.g., a second linker), an MRP1 monoclonal antibody epitope, about 10 histidine residues, and a CPP.

[0038] A nucleic acid construct provided herein (e.g., a nucleic acid construct encoding a fusion protein described herein (e.g., a fusion protein including a RecA polypeptide and a CPP)) can include any appropriate nucleic acid sequence encoding the fusion protein. In some cases, a nucleic acid construct can include a nucleic acid sequence (e.g., a RecA coding sequence) encoding a RecA polypeptide described herein. Examples of nucleic acid sequences encoding RecA polypeptides include, without limitation, nucleic acid sequences set forth in the NCBI databases at GenBank Accession No. NC_000913.3 (ID: 947170), GenBank Accession No. NC_000962.3 (ID: 888371), GenBank Accession No. NC_000964.3 (ID: 939497), and GenBank Accession No. DQ769876 (Version DQ769876.1). In some cases, RecA coding sequences can be as shown in FIG. **9**. For example, a RecA coding sequence can include a nucleic acid sequence set forth in SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12. In some cases, RecA coding sequences can be as described elsewhere (see, e.g., Chen et al., 2008 *Nature,* 453:489-4; Clone YpCD00014545 (Original Clone ID: FLH129217.01X) from the DNASU Plasmid Repository).

[0039] In some cases, a nucleic acid sequence encoding a RecA polypeptide in a nucleic acid construct described herein (e.g., a nucleic acid construct encoding a fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP)) can have a sequence that deviates from a wild type nucleic acid sequence encoding a RecA polypeptide, sometimes referred to as a variant sequence. For example, a nucleic acid sequence encoding a RecA polypeptide can have at least 80% sequence identity (e.g., at least 85% sequence identity, 90% sequence identity, 95% sequence identity, or at least 99% sequence identity) to SEQ ID NO:9 provided that it includes one or more nucleic acid additions, subtractions, or substitutions compared to SEQ ID NO:9. For example, a nucleic acid sequence encoding a RecA polypeptide can have at least 80% sequence identity (e.g., at least 85% sequence identity, 90% sequence identity, 95% sequence identity, or at least 99% sequence identity) to SEQ ID NO:10 provided that it includes one or more nucleic acid additions, subtractions, or substitutions compared to SEQ ID NO:10. For example, a nucleic acid sequence encoding a RecA polypeptide can have at least 80% sequence identity (e.g., at least 85% sequence identity, 90% sequence identity, 95% sequence identity, or at least 99% sequence identity) to SEQ ID NO:11 provided that it includes one or more nucleic acid additions, subtractions, or substitutions compared to SEQ ID NO:11. For example, a nucleic acid sequence encoding a RecA polypeptide can have at least 80% sequence identity (e.g., at least 85% sequence identity, 90% sequence identity, 95% sequence identity, or at least 99% sequence identity) to SEQ ID NO:12 provided that it includes one or more nucleic acid additions, subtractions, or substitutions compared to SEQ ID NO:12. Percent sequence identity is calculated by determining the number of matched positions in aligned nucleic acid or polypeptide sequences, dividing the number of matched positions by the total number of aligned nucleotides and multiplying by 100. A matched position refers to a position in which identical nucleotides occur at the same position in aligned sequences. The total number of aligned nucleotides refers to the minimum number of RecA nucleotides that are necessary to align the second sequence, and does not include alignment (e.g., forced alignment) with non-RecA sequences, such as those fused to RecA. The total number of aligned nucleotides may correspond to the entire RecA sequence or may correspond to fragments of the full-length RecA sequence as defined herein. Sequences can be aligned using the algorithm described by Altschul et al. (*Nucleic Acids Res.,* 25:3389-3402 (1997)) as incorporated into BLAST (basic local alignment search tool) programs, available at ncbi.nlm.nih.gov on the World Wide Web. BLAST searches or alignments can be performed to determine percent sequence identity between a RecA nucleic acid molecule and any other sequence or portion thereof using the Altschul et al. algorithm. For example, BLASTN can be used to align and compare the identity between nucleic acid sequences. When utilizing BLAST programs to calculate the percent identity between a RecA sequence and another sequence, the default parameters of the respective programs are used.

[0040] A nucleic acid construct provided herein (e.g., a nucleic acid construct encoding a fusion protein described herein (e.g., a fusion protein including a RecA polypeptide and a CPP)) can include any appropriate nucleic acid sequence encoding a CPP (e.g., any appropriate CPP coding sequence). In some cases, a nucleic acid construct can include a nucleic acid sequence (e.g., a coding sequence) encoding a CPP described herein. Examples of nucleic acid sequences encoding CPPs include, without limitation, a nucleic acid sequence encoding a TAT peptide sequence (e.g., a nucleic acid sequence including the sequence TACGGCAGGAAGAAGCGGAGACAGCGACGAAGA (SEQ ID NO:13)), a nucleic acid sequence encoding a Pep-1 peptide sequence, and a nucleic acid sequence encoding a MPG peptide sequence.

[0041] A nucleoprotein filament provided herein can include one or more (e.g., one, two, three, four, five, six, seven, eight, nine, or more) fusion proteins described herein (e.g., a fusion protein including a RecA polypeptide and a CPP) and a single stranded oligonucleotide. In some cases, a nucleoprotein filament can include one or more of the same fusion protein. A RecA polypeptide of a fusion protein described herein can interact with a single stranded oligonucleotide to form a nucleoprotein filament. In some cases, a RecA polypeptide of a fusion protein described herein can protect the single stranded oligonucleotide from degradation by, for example, DNAses. In some cases, a RecA polypeptide of a fusion protein described herein can promote homologous recombination. In some cases, a CPP of a fusion protein described herein can facilitate entry of the nucleoprotein filament into a cell (e.g., a cell having one or more mutations (e.g., one or more disease-causing mutations) in a nucleic acid sequence such as coding sequence).

[0042] A nucleoprotein filament provided herein (e.g., a nucleoprotein filament including one or more fusion proteins and a single stranded oligonucleotide) can include any appropriate single stranded oligonucleotide. A single stranded oligonucleotide can include DNA, RNA, or both. For example, a single stranded oligonucleotide can be a ssDNA. A single stranded oligonucleotide can be synthetic. A single stranded oligonucleotide can (e.g., can be designed to) hybridize to a target sequence (e.g., a nucleic acid sequence (e.g., an endogenous nucleic acid sequence) having one or more mutations such as disease-causing mutations). For example, a single stranded oligonucleotide can be (e.g., can include a nucleic acid sequence that is) sufficiently complementary to a target sequence such that the single stranded oligonucleotide can hybridize to and/or recognize the target sequence. In some cases, a target sequence can be a nucleic acid sequence (e.g., a coding sequence such as a gene) that contains one or more mutations (e.g., one or more disease-causing mutations). For example, when a target sequence is a nucleic acid sequence that contains one or more nucleotides, a single stranded oligonucleotide can include an alternative nucleic acid sequence (e.g., a sequence that, via HDR, can replace (e.g., correct) nucleotides in a target sequence). In some cases, a target sequence can be a portion of a gene (e.g., an endogenous gene) that contains one or more mutations (e.g., one or more disease-causing mutations). For example, when a target sequence is a portion of a gene that contains one or more mutations, a single stranded oligonucleotide can include a corrected gene sequence (e.g., a sequence that, via HDR, can replace (e.g., correct) one or more mutations in a target sequence) such as a sequence that does not include one or more disease-causing mutations (e.g., a wild type gene sequence).

[0043] This document also provides methods for editing a nucleic acid sequence (e.g., a coding sequence such as a gene). In some cases, a method for editing a nucleic acid

sequence can be used to edit a nucleic acid sequence containing one or more mutations (e.g., small deletions/insertions and/or point mutations) within a genome of a cell. For example, methods for editing a nucleic acid sequence within a cell can include contacting the cell with a fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP) and a single stranded oligonucleotide described herein (e.g., single stranded oligonucleotide capable of hybridizing to a target sequence and, optionally, including a corrected nucleic acid sequence). A cell can be any appropriate type of cell. In some cases, a cell can be a prokaryotic cell (e.g., a bacterial cell). In some cases, a cell can be a eukaryotic cell (e.g., a plant cell or a mammalian cell such as a human cell).

[0044] Any appropriate nucleic acid sequence can be edited (e.g., corrected) as described herein (e.g., by contacting a cell with a fusion protein described herein and a single stranded oligonucleotide described herein). In some cases, a nucleic acid sequence can be a coding sequence such as a gene. In some cases, a nucleic acid sequence can be an endogenous nucleic acid sequence. In some cases, a nucleic acid sequence can be within (e.g., a portion of) a gene associated with a genetic disease or genetic condition (e.g., a monogenetic disease or monogenetic condition). Examples of genes associated with a genetic disease or genetic condition include, without limitation, OPN1MW (associated with color blindness), CFTR/ABCC7 (associated with cystic fibrosis), HFE (associated with haemochromatosis), clotting factor 8 (associated with haemophilia A), clotting factor 9 (associated with haemophilia B), phenylalanine hydroxylase (associated with phenylketonuria), polycystic kidney disease 1 (PKD1; associated with polycystic kidney disease), PKD2 (associated with polycystic kidney disease), hemoglobin-Beta (associated with sickle-cell disease), Hex-A (associated with Tay-Sachs disease), huntingtin (associated with Huntington's disease), FBN1 (associated Marfan syndrome), dystrophin (associated with Duchene muscular dystrophy), and genes associated with cancers such as BRCA1, BRCA2, TP53, PTEN, MSH2, MLH1, MSH6, PMS2, EPCAM, APC, RB1, and PALB2.

[0045] A nucleic acid sequence (e.g., a coding sequence such as a gene) that can be edited as described herein can include one or more mutations. A mutation can be any appropriate type of mutation. Examples of mutations include, without limitation, deletions, insertions, and single nucleotide modifications (e.g., point mutations and single nucleotide polymorphisms (SNPs). In some cases, a deletion can include the deletion of from about 1 to about 100 nucleotides (e.g., from about 1 to about 90, from about 1 to about 80, from about 1 to about 70, from about 1 to about 60, from about 1 to about 50, from about 1 to about 40, from about 1 to about 30, from about 1 to about 20, from about 1 to about 10, from about 1 to about 5, from about 5 to about 100, from about 25 to about 100, from about 50 to about 100, from about 75 to about 100, from about 2 to about 75, from about 3 to about 50, from about 7 to about 40, from about 10 to about 30, from about 12 to about 25, from about 2 to about 10, from about 10 to about 20, from about 20 to about 30, from about 30 to about 40, or from about 40 to about 50 nucleotides). For example, a deletion can include the deletion of 3 nucleotides. In some cases, a insertion can include the insertion of from about 1 to about 100 nucleotides (e.g., from about 1 to about 90, from about 1 to about 80, from about 1 to about 70, from about 1 to about 60, from about 1 to about 50, from about 1 to about 40, from about 1 to about 30, from about 1 to about 20, from about 1 to about 10, from about 1 to about 5, from about 5 to about 100, from about 25 to about 100, from about 50 to about 100, from about 75 to about 100, from about 2 to about 75, from about 3 to about 50, from about 7 to about 40, from about 10 to about 30, from about 12 to about 25, from about 2 to about 10, from about 10 to about 20, from about 20 to about 30, from about 30 to about 40, or from about 40 to about 50 nucleotides). In cases where a nucleic acid sequence includes one or more mutations, the mutations can be disease-causing mutations. For example, a disease causing mutation can be deletion in CFTR (e.g., a three nucleotide deletion in the CFTR gene that causes a deletion of a phenylalanine residue at position of 508 of the CFTR polypeptide (ΔF508)) that causes cystic fibrosis.

[0046] This document also provides methods for treating a mammal having a genetic disease or genetic condition (e.g., a monogenetic disease or monogenetic condition) caused, at least in part, by one or more mutations in a nucleic acid sequence (e.g., a coding sequence such as a gene). For example, editing (e.g., correcting) one or more mutations in a nucleic acid sequence (e.g., one or more mutations in a gene associated with a genetic disease or genetic condition) can be effective to treat a mammal having a genetic disease or genetic condition. For example, methods for treating a mammal having a genetic disease or genetic condition can include contacting a cell of the mammal (e.g., a cell obtained from the mammal and/or a cell within the mammal) with a fusion protein described herein (e.g., a fusion protein containing a RecA polypeptide and a CPP) and a single stranded oligonucleotide described herein (e.g., single stranded oligonucleotide including a corrected nucleic acid sequence and capable of hybridizing to a target sequence).

[0047] A mammal having any appropriate genetic disease or genetic condition can be treated as described herein. In some cases, a genetic disease or genetic condition can be a monogenetic disease or monogenetic condition. Examples of monogenetic diseases and monogenetic conditions include, without limitation, color blindness, cystic fibrosis, haemochromatosis, haemophilia, phenylketonuria, polycystic kidney disease, sickle-cell disease, Tay-Sachs disease, Huntington's disease, Marfan syndrome, Duchene muscular dystrophy, and some cancers.

[0048] Any appropriate mammal (e.g., humans, non-human primates, monkeys, bovine species, pigs, horses, dogs, cats, sheep, goat, and rodents) having a monogenetic disease or monogenetic condition can be treated as described herein. In some cases, humans can be treated using the methods and materials provided herein. For example, a human having, or at risk of developing (e.g., based, at least in part, on the present of a disease-causing mutation in one or more cells within the human), cystic fibrosis can be treated by using the methods and materials provided herein to correct a CFTR coding sequence in one or more cells within the human. For example, a human having, or at risk of developing (e.g., based, at least in part, on the present of a disease-causing mutation in one or more cells within the human), Duchene muscular dystrophy can be treated by using the methods and materials provided herein to correct a dystrophin coding sequence in one or more cells within the human.

[0049] Any appropriate method can be used to deliver one or more nucleoprotein filaments described herein (e.g.,

nucleoprotein filaments including a fusion protein described herein and a single stranded oligonucleotide) to a cell (e.g., to a cell in a mammal).

[0050] In some cases, the methods and materials provided herein also can be used in other organisms. For example, the methods and materials provided herein can be used in plant cells, fungal cells, and/or bacterial cells.

[0051] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1. Materials

[0052] Most of the chemicals were purchased from Sigma; DMEM/F-12 medium and fetal bovine serum were derived from Thermo Scientific; Restriction endonucleases, from New England Biolabs; QuikChange site-directed mutagenesis kit, from Stratagene; Anti-mouse Ig conjugated with horseradish peroxidase, from Amersham Biosciences; Chemiluminescent substrates for western blotting, from Pierce; RecA DNA (pDONR221.RecA), from DNASU.

Example 2. RecA-CPP Fusion Nucleic Acid Construct

[0053] In order to express the RecA-CPP fusion protein in mammalian cells, the 5' part of the RecA DNA (pDONR221. RecA was used as template) was amplified by using the primers NutRecAfwasu and RecA324rvasu (Table 1); the fusion part between RecA and GFP was performed by two steps PCR, i.e., the 1st piece (pDONR221.RecA was used as template) was amplified by using RecA763fwasu and RecAlinkgfprvasu (Table 1), whereas the 2nd part (pCDH-CMV-MCS-EF1-copGFP was used as template) was amplified by using RecAlinkgfpfwasu and CDHGFP6658rv (Table 1); upon amplification of these two pieces DNA, they were used as templates to put them together by using RecA763fwasu and CDHGFP6658rv (Table 1) as primers; the 3' part of the fusion gene was amplified by three steps, i.e., the 1st piece (pCDH-CMV-MCS-EF1-copGFP was used as template) was amplified by using Gfp6302rv and 1st. CPPry (Table 1) as primers; the 2nd part (the 1st piece of the PCR product was used as template) was amplified by using Gfp6302rv and 2nd CPPrv (Table 1) as primers; whereas the 3rd part (the 2nd part of the PCR product was used as template) was amplified by using Gfp6302rv and 3rd.CPPrv (Table 1) as primers. All these pieces of PCR products were cloned into pBluescript and sequenced completely to make sure that there is no mutation occurred in the clones. Two bigger pieces, i.e., the N-terminal half (cloned by combining the XmaI-DraIII fragment from the 1st PCR clone, the DraIII-AseI fragment from pDONR221.RecA and the AseI-HindIII fragment from the RecA.GFP fusion clone) and C-terminal half (cloned by combining the HindIII-ApaL1 fragment from the RecA.GFP fusion clone, the ApaL1-BglI fragment from pCDH-CMV-MCS-EF1-copGFP and the Bgl1-HindIII fragment from the 3rd part of the clone), were cloned into pBluescript and sequenced completely. The N-terminal half and C-terminal half clones were used to make full length fusion gene in pNUT vector (see, e.g., Palmiter et al., 1987 Cell 50: 435-443). In order to make a shorter version of the fusion protein, the two primers, rmgfpbamhlfw and rmgfpbamhlry (Table 1), were used to delete the GFP gene from the full length fusion gene by employing the QuikChange Site-directed Mutagenesis kit (Stratagene). The longer version of the fusion gene (named as pNUT-RecA-GFP-CPP) and the shorter version of the fusion gene (named as pNUT-RecA-CPP) were sequenced completely to make sure that there is no mutation occurred in the final clones.

[0054] In order to express the RecA-CPP fusion proteins in bacteria, the two primers, ET32RecAfw1step and ET32RecArv1step (Table 1), were used to modify the 5' part of the N-terminal half clone by employing the QuikChange Site-directed Mutagenesis kit. The modified N-terminal half clone and the original C-terminal half clone were used to make full length fusion gene in pET32a expression vector. In order to make shorter version of the fusion protein, the two primers, rmgfpbamh1fw and rmgfpbamh1rv (Table 1), were used to delete the GFP gene from the full length fusion gene. The longer version of the fusion gene (named as pET32a. RecA-GFP-CPP) and the shorter version of the fusion gene (named as pET32a-RecA-CPP) were sequenced completely to make sure that there is no mutation occurred in the final clones.

TABLE 1

List of Oligonucleotides

SEQ ID NO	Name	Sequence
14	NutRecAfwasu	GCCCGGGACCATGGCTATTGATGAGAATAAAC
15	ET32RecAfwasu	CTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTATTGATGAGAATAAAC
16	RecA324rvasu	CAATTTCTTGGCATAGATTGG
17	RecA763fwasu	CCATTCAAACAAGCTGAATTC
18	RecAlinkGfpfwasu	GAAACCAACGAAGAATTTAGTGGCCTACGATCGCGAGCAGCTGCGAACACGATGAGTATTCAACATTTC
19	CDHGFP6658rv	CGGGATAATACCGCGCCAC
20	RecAlinkGfprvasu	GAAATGTTGAATACTCATCGTGTTCGCAGCTGCTCGCGATCGTAGGCCACTAAATTCTTCGTTGGTTTC
21	Gfp6302rv	GCTTCCCGGCAACAATTAATAG

TABLE 1-continued

List of Oligonucleotides

SEQ ID NO	Name	Sequence
22	Gfp6019fw	GAGTAAACTTGGTCTGACAG
23	1st.CPPrv	GTGAAGTTGACATCCAAAAAGGATGTTTTCTCGTGCTGCAGCCCAATGCTTAATCAGTGA
24	2nd.CPPrv	CTTCTTCCCTGCCGTAATGGTGATGGTGATGGTGATGGTGATGGTGAAGTTGACATCCAAA
25	3rd.CPPrv	GCGGCCGCCTATCTTCGTCGCTGTCTCCGCTTCTTCCTGCCGTAATG
26	ET32RecAfw1step	GGTGGCGGCCGCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTATTGATGAGAA TAAAC
27	ET32RecArv1step	GTTTATTCTCATCAATAGCCATATGTATATCTCCTTCTTAAAGTTAAACAAAATT ATTTCTAGACGGCCGCCACC
28	rmgfpbamh1fw	CGAGCAGCTGCGAACACGGGATCCGCTGCAGCACGAGAAAAC
29	rmgfpbamh1rv	GTTTTCTCGTGCTGCAGCGGATCCCGTGTTCGCAGCTGCTCG

[0055] Two versions of the RecA-CPP fusion proteins, i.e., shorter version (RecA-CPP) and longer version (RecA-GFP-CPP), were designed (FIG. **1**). RecA-CPP contains: 1) RecA; 2) an L1 linker (see, e.g., Orban et al., 2008 *Biochem Biophys Res Commun* 367:667-673; and Hou et al., 2009 *Biochemistry* 48:9122-9131); 3) Tag1, the epitope of the MRP1 mAb 42.4 (see, e.g., Hou et al., 2000 *J Biol Chem* 275:20280-20287); 4) Tag2, a ten histidine residue tag; 5) CPP, a cell-penetrating-peptide, i.e., transactivator of transcription (TAT) peptide (see, e.g., Frankel et al., 1988 *Cell* 55:1189-1193; Green et al., 1988 *Cell* 55:1179-1188; Debaisieux et al., 2012 *Traffic* 13:355-363; Schwarze et al., 2000 *Trends in cell biology* 10:290-295; and Dietz et al., 2004 *Molecular and cellular neurosciences* 27:85-131). The longer version, i.e., RecA-GFP-CPP, contains: 1) RecA; 2) L1; 3) GFP; 4) L2, a two-alanine residue short linker; 5) Tag1; 6) Tag2; and 7) CPP.

Example 3. Cell Culture and Transfection

[0056] Baby hamster kidney (BHK) cells were grown in DMEM/F-12 medium containing 5% fetal bovine serum at 37° C. in 5% CO2. Subconfluent cells were transfected with plasmid DNAs containing either longer version of the fusion gene (pNUT-RecA-GFP-CPP) or shorter version of the fusion gene (pNUT-RecA-CPP) in the presence of 20 mM HEPES (pH 7.05), 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM dextrose and 125 mM $CaCl_2$ (see, e.g., Chang et al., 1997 *J Biol Chem* 272:30962-30968). Whole mixture of the methotrexate-resistant cells was used to determine the expression of the fusion proteins with the MRP1 monoclonal antibody (mAb) 42.4 (see, e.g., Hou et al., 2000 *J Biol Chem* 275:20280-20287).

[0057] In order to express these fusion proteins in mammalian cells, the two fusion genes diagramed in FIG. **1** were inserted into a mammalian expression vector, i.e., pNUT (see, e.g., Palmiter et al., 1987 *Cell* 50:435-443). Upon transformation of BHK cells with these two constructs, i.e., pNUT-RecA-CPP and pNUT-RecA-GFP-CPP, the methotrexate resistant cells were used to determine the expression of these fusion proteins. The results in FIG. **5**A clearly indicated that RecA-CPP fusion protein is expressed in BHK cells. In addition, the amount of the fusion protein in cells lysed with SDS is similar to the cells lysed with NP40 or lysed in PBS, suggesting that majority of the fusion protein expressed in BHK cells is in soluble fraction. The expression of the longer version, i.e., RecA-GFP-CPP, in BHK cells is similar to the shorter version (FIG. **5**B).

[0058] Expression of fusion proteins in the cells was examined using western blotting as described in Example 5.

Example 4. Expression of the RecA-CPP Fusion Proteins in Prokaryotic DL21 Cells

[0059] The DL21 competent cells were transformed with either pET32a-RecA-GFP-CPP or pET32a-RecA-CPP. The freshly received ampicillin-resistant colonies were used to inoculate 1 mL of 50% Luria-Bertani Broth (LB) and 50% super LB (with 100 μg/mL ampicillin) and cells were grown at 37° C. for ~6 hours. 10-100 μL (depending on the cell density) of these bacteria were used to inoculate 100 mL of 50% LB and 50% super LB (with 100 μg/mL ampicillin) and the cells were grown overnight at 16° C. until the OD600 reaching 0.6-1.0. After adjusting temperature to 4° C., isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to 1 mM (final concentration) and cells were grown at this temperature for 16 hours. The cells were harvested by centrifugation at 5,000×g for 5 minutes at 4° C. and the pellets and supernatants were used to determine the expression of these fusion proteins.

[0060] Expression of fusion proteins in the cells was examined using western blotting as described in Example 5.

[0061] Expression of the fusion proteins completely blocked DL21 cell growth: The results in FIG. **3**A indicated that RecA-CPP fusion protein is clearly expressed in DL21 cells. The protein expressed in DL21 cells is not leaked out to the medium and it also clearly indicated that certain amount the fusion protein is in soluble fraction. The expression of the longer version, i.e., RecA-GFP-CPP, in DL21 cells is similar to the shorter version (FIG. **3**B).

[0062] In order to test whether the expression of the fusion proteins has effect on cell growth or not, the DL21 compe-

tent cells transformed with either pET32a-RecA-CPP or pET32a. RecA-GFP-CPP were plated out on the plates containing either 100 μg/mL ampicillin or 100 μg/mL ampicillin and 0.25 mM IPTG. Interestingly, regardless whether the shorter version or the longer version of the fusion constructs were used, the cells plated out on the plates containing only 100 μg/mL ampicillin grow very well, whereas the cells plated out on the plates containing 100 μg/mL ampicillin and 0.25 mM IPTG did not form visible colonies (FIGS. **4**A and **4**B), implying that IPTG induction of the fusion proteins significantly inhibited prokaryotic cell growth.

Example 5. Identification of RecA-CPP Fusion Proteins

[0063] Western blot was performed according to the routine protocol. For RecA-CPP fusion proteins expressed in BL21 cells, the following four samples were prepared: 1) the proteins in medium (the proteins in medium were precipitated with trichloroacetic acid and the pellets were dissolved in 1× sample buffer containing 1× protease inhibitor cocktail, i.e., Aprotonin, 2 μg/mL; Benzamide, 121 μg/mL; E64, 3.5 μg/mL; Leupeptin, 1 μg/mL; and Pefabloc, 50 μg/mL); 2) total proteins in bacteria [Cell pellets were re-suspended in 1× nickel bead binding buffer (20 mM Tris/HCl, pH7.9; 500 mM NaCl) containing 10% glycerol, 1× protease inhibitors and 20,000 units/mL of lysozyme, incubated at 37° C. for 15 minutes, added sodium dodecyl sulfate (SDS) to 2% (final concentration) and then sonicated for 20 bursts to break the DNA]; 3) proteins in soluble fraction (Cell pellets were re-suspended in 1× nickel bead binding buffer containing 10% glycerol, 1× protease inhibitors and 20,000 units/mL of lysozyme, incubated at 37° C. for 15 minutes, and then sonicated for 20 bursts to break the DNA. The soluble fraction was collected after centrifugation at 14,000 RPM for 10 minutes); 4) proteins in insoluble fraction (the pellets derived from previous step were dissolved in 1× nickel bead binding buffer containing 10% glycerol, 1× protease inhibitors and 2% SDS and then sonicated for 20 bursts to break the DNA).

[0064] For RecA-CPP fusion proteins expressed in BHK cells, the following three samples were prepared: 1) Cells lysed with SDS and sonication [Cells were lysed with phosphate buffered saline (PBS) containing 1× protease inhibitors and 2% SDS and then sonicated for 20 bursts to break the DNA]; 2) Cells lysed with sonication (Cells re-suspended in PBS containing 1× protease inhibitors were sonicated for 20 bursts to break the DNA); 3) Cells lysed with NP40 buffer [Cells were lysed with NP40 cell lysis buffer (0.1% NP40, 150 mM NaCl, 50 mM Tris, 10 mM Sodium Molybdate, pH 7.6) containing 1× protease inhibitors by shaking the plates in cold room for 30 minutes. The supernatants were collected after centrifugation at 14,000 RPM].

[0065] Samples were subjected to SDS-PAGE, followed by transferring the proteins to nitrocellulose membranes, probed with the MRP1 primary antibody 42.4 (see, e.g., Hou et al., 2000 *J Biol Chem* 275:20280-20287) overnight at 4° C., washed with PBS containing 0.1% Tween-20 and then incubated with anti-mouse Ig conjugated with horse radish peroxidase. Chemiluminescent film detection was performed according to the manufacturer's recommendations (Pierce).

[0066] Statistical Analysis: The results in FIG. **6** were presented as means±SD from the triplicate experiments. The two-tailed P values were calculated based on the unpaired t-test from GraphPad Software Quick Calcs. By conventional criteria, if P value is less than 0.05, the difference between two samples is considered to be statistically significant.

[0067] Expression of the fusion proteins significantly inhibited BHK cell growth: In order to test whether the expression of the fusion proteins has effect on mammalian cell growth or not, 10,000 BHK cells expressing either RecA-CPP or RecA-GFP-CPP were plated out on day 0 and counted on day 3. Interestingly, the number of BHK cells expressing RecA-CPP is similar to the cells expressing RecA-GFP-CPP, whereas the number of parental BHK cells is significantly higher than either cells expressing RecA-CPP or RecA-GFP-CPP (FIG. **6**), suggesting that expression of these fusion proteins significantly inhibited mammalian cell growth.

Example 6. Gene Editing Using the Nucleoprotein Filament

[0068] Ability of the present nucleoprotein filaments comprising fusion proteins and single stranded nucleotide to correct a mutation in a cell or a subject is studied in this example.

[0069] In one embodiment, CF ΔF508 mutation cell lines (i.e., cells containing a deletion of three nucleotides coding for phenylalanine at position of 508 (ΔF508) in CFTR) or cell lines having a mutation in the ATP-binding cassette transporter C7 (ABCC7) gene are treated with a fusion protein comprising a RecA, a CPP and a single stranded nucleotide comprising a sequence that is sufficiently complementary to the target sequence and a corrected sequence to correct the mutations.

[0070] In one embodiment, an animal model carrying a CF ΔF508 mutation is treated with the present nucleoprotein filament.

[0071] In one embodiment, a human having one or more cells having a CF ΔF508 mutation (i.e., cells containing a deletion of three nucleotides coding for phenylalanine at position of 508 (ΔF508) in CFTR are treated with nucleoprotein filaments containing one or more fusion proteins including a RecA polypeptide and a CPP, and a single stranded oligonucleotide comprising a sequence that is sufficiently complementary to the target sequence and a corrected sequence to correct the mutations.

Example 7. Model Systems to Test Frequencies of Homology-Directed Recombination (HDR)

[0072] A model system was established to test the efficiency of HDR in eukaryotic cells. A dual marker system in one construct was designed in which the expression of mouse DHFR in eukaryotic cells provides methotrexate resistance whereas the expression of GFP generates green cells. The construct was based on a dual promoter system in pNUT expression vector as described elsewhere (see, e.g., Palmiter et al., 1987 *Cell* 50:435-443). This system can be designed to express any appropriate polypeptide, such as MRP1 or CFTR.

GFP Model

[0073] When a cell expresses a wild-type DHFR and a methotrexate resistance phenotype, GFP is used to detect, and optionally evaluate, HDR.

[0074] A construct including a cDNA encoding a wild type DHFR and a cDNA encoding a loss-of-function mutated GFP is used. A deletion of nucleotides TGAT from 185 to 188 of a GFP cDNA generates a frame-shift mutation that leads to expression of a mutated GFP polypeptide and loss of function. An exemplary nucleotide sequence of wild-type GFP cDNA (SEQ ID NO:30) is shown in FIG. **10**A, and an exemplary nucleotide sequence of the frame-shift deletion mutated GFP (SEQ ID NO:31) is shown in FIG. **10**B.

[0075] Insertion of these 4 nucleotides via HDR using single stranded oligonucleotides corrects the deletion and restores GFP expression and function in the cell to provide a fluorescent phenotype. Exemplary single stranded oligonucleotides that can correct the deletion shown in FIG. **10**B are as set forth in Table 2.

[0076] Counting of the green cells (e.g., by fluorescence activated cell sorting (FACS)) is used to determine the efficiency of HDR mediated by ssDNA-RecA-CPP. This model can also be used to determine the efficiency of HDR by other gene editing systems such as ZFNs, TALENs, and CRISPR/Cas9.

TABLE 2

Single strand oligonucleotides used to correct the GFP cDNA frame-shift deletion shown in FIG. 10B (highlighted letters are the nucleotides inserted to correct the deletion mutation).

SEQ ID NO:	Name	Sequence
32	CR.TGAT.fw1	GGCGCCCTGACCTTCAGCCCCTACCTGCTGAGCCA CGTGATGGGCTACGGCTTCTACCAC
33	CR.TGAT.fw2	AACAAGATGAAGAGCACCAAAGGCGCCCTGACCTT CAGCCCCTACCTGCTGAGCCACGTGATGGGCTACG GCTTCTACCAC
34	CR.TGAT.fw3	CCCAAGCAGGGCCGCATGACCAACAAGATGAAGAG CACCAAAGGCGCCCTGACCTTCAGCCCCTACCTGC TGAGCCACGTGATGGGCTACGGCTTCTACCAC
35	CR.TGAT.rv1	ccgct ggggt aggtg ccgaa gtggt agaag ccgta gccc ATCA CGTG GCTC AGCA GGTA G
36	CR.TGAT.rv2	t gcagg aaggg gttct cgtag ccgct ggggt aggtg ccgaa gtggt agaag ccgta gccc ATCA CGTG GCTC AGCA GGTA G
37	CR.TGAT.rv3	tagcc gccgt tgttg atggc gt gcagg aaggg gttct cgtag ccgct ggggt aggtg ccgaa gtggt agaag ccgta gccc ATCA CGTG GCTC AGCA GGTA G

DHFR Model

[0077] When a cell expresses a wild-type GFP, DHFR is used to detect, and optionally evaluate, HDR.

[0078] A construct including a cDNA encoding a wild type GFP and a cDNA encoding a loss-of-function mutated DHFR is used. A deletion of nucleotides TG from 135 to 136 of a DHFR cDNA generates a frame-shift mutation that leads to expression of a mutated DHFR polypeptide and loss of function. An exemplary nucleotide sequence of wild-type DHFR cDNA (SEQ ID NO:38) is shown in FIG. **11**A, and an exemplary nucleotide sequence of the frame-shift deletion mutated DHFR (SEQ ID NO:39) is shown in FIG. **11**B.

[0079] Insertion of these 2 nucleotides via HDR using single stranded oligonucleotides corrects the deletion and restores DHFR expression and function in the cell to provide a methotrexate resistance phenotype. Exemplary single stranded oligonucleotides that can correct the deletion shown in FIG. **11**B are as set forth in Table 3.

[0080] Counting of methotrexate resistant colonies (e.g., compared to cells without methotrexate treatment) is used to determine the efficiency of HDR mediated by ssDNA-RecA-CPP nucleoprotein filaments. This model can also be used to determine the efficiency of HDR mediated by other gene editing systems such as ZFNs, TALENs, and CRISPR/Cas9.

TABLE 3

Single strand oligonucleotides used to correct the DHFR cDNA frame-shift deletion shown in FIG. 11B (highlighted letters are the nucleotides inserted to correct the deletion mutation).

SEQ ID NO:	Name	Sequence
40	CrdeTGinDHFRfw1	tggcct ccgctcagga acgagtggaa gtacttccaa agaatgacca caacctcttc agtg
41	CrdeTGinDHFRfw2	ggcaaga acggagacct accctggcct ccgctcagga acgagtggaa gtacttccaa agaatgacca caacctcttc agtg
42	CrdeTGinDHFRfw3	gtgtccca agatatgggg attggcaaga acggagacct accctggcct ccgctcagga acgagtggaa gtacttccaa agaatgacca caacctcttc agtg
43	CrdeTGinDHFRry1	TCACCA CATTCTGTTT ACCTTCCACT GAAGAGGTTG TGGTCATTCT TTGGAAGTAC TTCC
44	CrdeTGinDHFRry2	ACCAGGT TTTCCTACCC ATAATCACCA CATTCTGTTT ACCTTCCACT GAAGAGGTTG TGGTCATTCT TTGGAAGTAC TTCC
45	CrdeTGinDHFRry3	GATTCTTC TCAGGAATGG AGAACCAGGT TTTCCTACCC ATAATCACCA CATTCTGTTT ACCTTCCACT GAAGAGGTTG TGGTCATTCT TTGGAAGTAC TTCC

Other Embodiments

[0081] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 45

<210> SEQ ID NO 1
<211> LENGTH: 353
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 1

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Met Ala Ile Asp Glu Asn Lys Gln Lys Ala Leu Ala Ala Ala Leu Gly
1               5                   10                  15

Gln Ile Glu Lys Gln Phe Gly Lys Gly Ser Ile Met Arg Leu Gly Glu
            20                  25                  30

Asp Arg Ser Met Asp Val Glu Thr Ile Ser Thr Gly Ser Leu Ser Leu
        35                  40                  45

Asp Ile Ala Leu Gly Ala Gly Gly Leu Pro Met Gly Arg Ile Val Glu
    50                  55                  60

Ile Tyr Gly Pro Glu Ser Ser Gly Lys Thr Thr Leu Thr Leu Gln Val
65                  70                  75                  80

Ile Ala Ala Ala Gln Arg Glu Gly Lys Thr Cys Ala Phe Ile Asp Ala
                85                  90                  95

Glu His Ala Leu Asp Pro Ile Tyr Ala Arg Lys Leu Gly Val Asp Ile
            100                 105                 110

Asp Asn Leu Leu Cys Ser Gln Pro Asp Thr Gly Glu Gln Ala Leu Glu
        115                 120                 125

Ile Cys Asp Ala Leu Ala Arg Ser Gly Ala Val Asp Val Ile Val Val
    130                 135                 140

Asp Ser Val Ala Ala Leu Thr Pro Lys Ala Glu Ile Glu Gly Glu Ile
145                 150                 155                 160

Gly Asp Ser His Met Gly Leu Ala Ala Arg Met Met Ser Gln Ala Met
                165                 170                 175

Arg Lys Leu Ala Gly Asn Leu Lys Gln Ser Asn Thr Leu Leu Ile Phe
            180                 185                 190

Ile Asn Gln Ile Arg Met Lys Ile Gly Val Met Phe Gly Asn Pro Glu
        195                 200                 205

Thr Thr Thr Gly Gly Asn Ala Leu Lys Phe Tyr Ala Ser Val Arg Leu
    210                 215                 220

Asp Ile Arg Arg Ile Gly Ala Val Lys Glu Gly Glu Asn Val Val Gly
225                 230                 235                 240

Ser Glu Thr Arg Val Lys Val Val Lys Asn Lys Ile Ala Ala Pro Phe
                245                 250                 255

Lys Gln Ala Glu Phe Gln Ile Leu Tyr Gly Glu Gly Ile Asn Phe Tyr
            260                 265                 270

Gly Glu Leu Val Asp Leu Gly Val Lys Glu Lys Leu Ile Glu Lys Ala
        275                 280                 285

Gly Ala Trp Tyr Ser Tyr Lys Gly Glu Lys Ile Gly Gln Gly Lys Ala
    290                 295                 300

Asn Ala Thr Ala Trp Leu Lys Asp Asn Pro Glu Thr Ala Lys Glu Ile
305                 310                 315                 320

Glu Lys Lys Val Arg Glu Leu Leu Leu Ser Asn Pro Asn Ser Thr Pro
                325                 330                 335

Asp Phe Ser Val Asp Asp Ser Glu Gly Val Ala Glu Thr Asn Glu Asp
            340                 345                 350
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-continued

Phe

<210> SEQ ID NO 2
<211> LENGTH: 790
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 2

```
Met Thr Gln Thr Pro Asp Arg Glu Lys Ala Leu Glu Leu Ala Val Ala
1               5                   10                  15

Gln Ile Glu Lys Ser Tyr Gly Lys Gly Ser Val Met Arg Leu Gly Asp
            20                  25                  30

Glu Ala Arg Gln Pro Ile Ser Val Ile Pro Thr Gly Ser Ile Ala Leu
        35                  40                  45

Asp Val Ala Leu Gly Ile Gly Gly Leu Pro Arg Gly Arg Val Ile Glu
    50                  55                  60

Ile Tyr Gly Pro Glu Ser Ser Gly Lys Thr Thr Val Ala Leu His Ala
65                  70                  75                  80

Val Ala Asn Ala Gln Ala Ala Gly Gly Val Ala Ala Phe Ile Asp Ala
                85                  90                  95

Glu His Ala Leu Asp Pro Asp Tyr Ala Lys Lys Leu Gly Val Asp Thr
            100                 105                 110

Asp Ser Leu Leu Val Ser Gln Pro Asp Thr Gly Glu Gln Ala Leu Glu
        115                 120                 125

Ile Ala Asp Met Leu Ile Arg Ser Gly Ala Leu Asp Ile Val Val Ile
    130                 135                 140

Asp Ser Val Ala Ala Leu Val Pro Arg Ala Glu Leu Glu Gly Glu Met
145                 150                 155                 160

Gly Asp Ser His Val Gly Leu Gln Ala Arg Leu Met Ser Gln Ala Leu
                165                 170                 175

Arg Lys Met Thr Gly Ala Leu Asn Asn Ser Gly Thr Thr Ala Ile Phe
            180                 185                 190

Ile Asn Gln Leu Arg Asp Lys Ile Gly Val Met Phe Gly Ser Pro Glu
        195                 200                 205

Thr Thr Thr Gly Gly Lys Ala Leu Lys Phe Tyr Ala Ser Val Arg Met
    210                 215                 220

Asp Val Arg Arg Val Glu Thr Leu Lys Asp Gly Thr Asn Ala Val Gly
225                 230                 235                 240

Asn Arg Thr Arg Val Lys Val Val Lys Asn Lys Cys Leu Ala Glu Gly
                245                 250                 255

Thr Arg Ile Phe Asp Pro Val Thr Gly Thr Thr His Arg Ile Glu Asp
            260                 265                 270

Val Val Asp Gly Arg Lys Pro Ile His Val Val Ala Ala Ala Lys Asp
        275                 280                 285

Gly Thr Leu His Ala Arg Pro Val Val Ser Trp Phe Asp Gln Gly Thr
    290                 295                 300

Arg Asp Val Ile Gly Leu Arg Ile Ala Gly Gly Ala Ile Val Trp Ala
305                 310                 315                 320

Thr Pro Asp His Lys Val Leu Thr Glu Tyr Gly Trp Arg Ala Ala Gly
                325                 330                 335

Glu Leu Arg Lys Gly Asp Arg Val Ala Gln Pro Arg Arg Phe Asp Gly
            340                 345                 350
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Phe Gly Asp Ser Ala Pro Ile Pro Ala Asp His Ala Arg Leu Leu Gly
        355                 360                 365

Tyr Leu Ile Gly Asp Gly Arg Asp Gly Trp Val Gly Gly Lys Thr Pro
    370                 375                 380

Ile Asn Phe Ile Asn Val Gln Arg Ala Leu Ile Asp Asp Val Thr Arg
385                 390                 395                 400

Ile Ala Ala Thr Leu Gly Cys Ala Ala His Pro Gln Gly Arg Ile Ser
                405                 410                 415

Leu Ala Ile Ala His Arg Pro Gly Glu Arg Asn Gly Val Ala Asp Leu
            420                 425                 430

Cys Gln Gln Ala Gly Ile Tyr Gly Lys Leu Ala Trp Glu Lys Thr Ile
        435                 440                 445

Pro Asn Trp Phe Phe Glu Pro Asp Ile Ala Ala Asp Ile Val Gly Asn
    450                 455                 460

Leu Leu Phe Gly Leu Phe Glu Ser Asp Gly Trp Val Ser Arg Glu Gln
465                 470                 475                 480

Thr Gly Ala Leu Arg Val Gly Tyr Thr Thr Thr Ser Glu Gln Leu Ala
                485                 490                 495

His Gln Ile His Trp Leu Leu Leu Arg Phe Gly Val Gly Ser Thr Val
            500                 505                 510

Arg Asp Tyr Asp Pro Thr Gln Lys Arg Pro Ser Ile Val Asn Gly Arg
        515                 520                 525

Arg Ile Gln Ser Lys Arg Gln Val Phe Glu Val Arg Ile Ser Gly Met
    530                 535                 540

Asp Asn Val Thr Ala Phe Ala Glu Ser Val Pro Met Trp Gly Pro Arg
545                 550                 555                 560

Gly Ala Ala Leu Ile Gln Ala Ile Pro Glu Ala Thr Gln Gly Arg Arg
                565                 570                 575

Arg Gly Ser Gln Ala Thr Tyr Leu Ala Ala Glu Met Thr Asp Ala Val
            580                 585                 590

Leu Asn Tyr Leu Asp Glu Arg Gly Val Thr Ala Gln Glu Ala Ala Ala
        595                 600                 605

Met Ile Gly Val Ala Ser Gly Asp Pro Arg Gly Gly Met Lys Gln Val
    610                 615                 620

Leu Gly Ala Ser Arg Leu Arg Arg Asp Arg Val Gln Ala Leu Ala Asp
625                 630                 635                 640

Ala Leu Asp Asp Lys Phe Leu His Asp Met Leu Ala Glu Glu Leu Arg
                645                 650                 655

Tyr Ser Val Ile Arg Glu Val Leu Pro Thr Arg Arg Ala Arg Thr Phe
            660                 665                 670

Asp Leu Glu Val Glu Glu Leu His Thr Leu Val Ala Glu Gly Val Val
        675                 680                 685

Val His Asn Cys Ser Pro Pro Phe Lys Gln Ala Glu Phe Asp Ile Leu
    690                 695                 700

Tyr Gly Lys Gly Ile Ser Arg Glu Gly Ser Leu Ile Asp Met Gly Val
705                 710                 715                 720

Asp Gln Gly Leu Ile Arg Lys Ser Gly Ala Trp Phe Thr Tyr Glu Gly
                725                 730                 735

Glu Gln Leu Gly Gln Gly Lys Glu Asn Ala Arg Asn Phe Leu Val Glu
            740                 745                 750

Asn Ala Asp Val Ala Asp Glu Ile Glu Lys Lys Ile Lys Glu Lys Leu
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        755                 760                 765

Gly Ile Gly Ala Val Val Thr Asp Asp Pro Ser Asn Asp Gly Val Leu
    770                 775                 780

Pro Ala Pro Val Asp Phe
785                 790


<210> SEQ ID NO 3
<211> LENGTH: 348
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 3

Met Ser Asp Arg Gln Ala Ala Leu Asp Met Ala Leu Lys Gln Ile Glu
1               5                   10                  15

Lys Gln Phe Gly Lys Gly Ser Ile Met Lys Leu Gly Glu Lys Thr Asp
            20                  25                  30

Thr Arg Ile Ser Thr Val Pro Ser Gly Ser Leu Ala Leu Asp Thr Ala
        35                  40                  45

Leu Gly Ile Gly Gly Tyr Pro Arg Gly Arg Ile Ile Glu Val Tyr Gly
    50                  55                  60

Pro Glu Ser Ser Gly Lys Thr Thr Val Ala Leu His Ala Ile Ala Glu
65                  70                  75                  80

Val Gln Gln Gln Gly Gly Gln Ala Ala Phe Ile Asp Ala Glu His Ala
                85                  90                  95

Leu Asp Pro Val Tyr Ala Gln Lys Leu Gly Val Asn Ile Glu Glu Leu
            100                 105                 110

Leu Leu Ser Gln Pro Asp Thr Gly Glu Gln Ala Leu Glu Ile Ala Glu
        115                 120                 125

Ala Leu Val Arg Ser Gly Ala Val Asp Ile Val Val Val Asp Ser Val
    130                 135                 140

Ala Ala Leu Val Pro Lys Ala Glu Ile Glu Gly Asp Met Gly Asp Ser
145                 150                 155                 160

His Val Gly Leu Gln Ala Arg Leu Met Ser Gln Ala Leu Arg Lys Leu
                165                 170                 175

Ser Gly Ala Ile Asn Lys Ser Lys Thr Ile Ala Ile Phe Ile Asn Gln
            180                 185                 190

Ile Arg Glu Lys Val Gly Val Met Phe Gly Asn Pro Glu Thr Thr Pro
        195                 200                 205

Gly Gly Arg Ala Leu Lys Phe Tyr Ser Ser Val Arg Leu Glu Val Arg
    210                 215                 220

Arg Ala Glu Gln Leu Lys Gln Gly Asn Asp Val Met Gly Asn Lys Thr
225                 230                 235                 240

Lys Ile Lys Val Val Lys Asn Lys Val Ala Pro Pro Phe Arg Thr Ala
                245                 250                 255

Glu Val Asp Ile Met Tyr Gly Glu Gly Ile Ser Lys Glu Gly Glu Ile
            260                 265                 270

Ile Asp Leu Gly Thr Glu Leu Asp Ile Val Gln Lys Ser Gly Ser Trp
        275                 280                 285

Tyr Ser Tyr Glu Glu Glu Arg Leu Gly Gln Gly Arg Glu Asn Ala Lys
    290                 295                 300

Gln Phe Leu Lys Glu Asn Lys Asp Ile Met Leu Met Ile Gln Glu Gln
305                 310                 315                 320
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-continued

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Ile Arg Glu His Tyr Gly Leu Asp Asn Asn Gly Val Val Gln Gln Gln
                325                 330                 335

Ala Glu Glu Thr Gln Glu Glu Leu Glu Phe Glu Glu
            340                 345


<210> SEQ ID NO 4
<211> LENGTH: 356
<212> TYPE: PRT
<213> ORGANISM: Yersinia pseudotuberculosis

<400> SEQUENCE: 4

Met Ala Ile Asp Glu Asn Lys Gln Lys Ala Leu Ala Ala Ala Leu Gly
1               5                   10                  15

Gln Ile Glu Lys Gln Phe Gly Lys Gly Ser Ile Met Arg Leu Gly Glu
            20                  25                  30

Asp Arg Ser Met Asp Val Glu Thr Ile Ser Thr Gly Ser Leu Ser Leu
        35                  40                  45

Asp Ile Ala Leu Gly Ala Gly Gly Leu Pro Met Gly Arg Ile Val Glu
    50                  55                  60

Ile Tyr Gly Pro Glu Ser Ser Gly Lys Thr Thr Leu Thr Leu Gln Val
65                  70                  75                  80

Ile Ala Ala Ala Gln Arg Glu Gly Lys Thr Cys Ala Phe Ile Asp Ala
                85                  90                  95

Glu His Ala Leu Asp Pro Ile Tyr Ala Lys Lys Leu Gly Val Asp Ile
            100                 105                 110

Asp Asn Leu Leu Cys Ser Gln Pro Asp Thr Gly Glu Gln Ala Leu Glu
        115                 120                 125

Ile Cys Asp Ala Leu Thr Arg Ser Gly Ala Val Asp Val Ile Ile Val
    130                 135                 140

Asp Ser Val Ala Ala Leu Thr Pro Lys Ala Glu Ile Glu Gly Glu Ile
145                 150                 155                 160

Gly Asp Ser His Met Gly Leu Ala Ala Arg Met Met Ser Gln Ala Met
                165                 170                 175

Arg Lys Leu Ala Gly Asn Leu Lys Asn Ala Asn Thr Leu Leu Ile Phe
            180                 185                 190

Ile Asn Gln Ile Arg Met Lys Ile Gly Val Met Phe Gly Asn Pro Glu
        195                 200                 205

Thr Thr Thr Gly Gly Asn Ala Leu Lys Phe Tyr Ala Ser Val Arg Leu
    210                 215                 220

Asp Ile Arg Arg Ile Gly Ala Val Lys Asp Gly Asp Val Val Val Gly
225                 230                 235                 240

Ser Glu Thr Arg Val Lys Val Val Lys Asn Lys Ile Ala Ala Pro Phe
                245                 250                 255

Lys Gln Ala Glu Phe Gln Ile Leu Tyr Gly Glu Gly Ile Asn Ile Asn
            260                 265                 270

Gly Glu Leu Val Asp Leu Gly Val Lys His Lys Leu Ile Glu Lys Ala
        275                 280                 285

Gly Ala Trp Tyr Ser Tyr Asn Gly Asp Lys Ile Gly Gln Gly Lys Ala
    290                 295                 300

Asn Ala Ser Asn Tyr Leu Lys Glu Asn Pro Ala Ile Ala Ala Glu Leu
305                 310                 315                 320

Asp Lys Lys Leu Arg Glu Met Leu Leu Asn Gly Gly Asn Gly Glu Gln
                325                 330                 335
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Pro Val Ala Ala Ala Thr Ala Glu Phe Ala Asp Gly Ala Asp Glu Thr
340 345 350

Asn Glu Glu Phe
355

<210> SEQ ID NO 5
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TAT cell penetrating polypeptide

<400> SEQUENCE: 5

Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
1 5 10

<210> SEQ ID NO 6
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Pep-1 cell penetrating polypeptide

<400> SEQUENCE: 6

Lys Glu Thr Trp Trp Glu Thr Trp Trp Thr Glu Trp Ser Gln Pro Lys
1 5 10 15

Lys Lys Arg Lys Val
20

<210> SEQ ID NO 7
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MPG cell penetrating polypeptide

<400> SEQUENCE: 7

Ser Val Val Asp Arg Val Ala Glu Gln Asp Thr Gln Ala
1 5 10

<210> SEQ ID NO 8
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: peptide linker

<400> SEQUENCE: 8

Ser Gly Leu Arg Ser Arg Ala Ala Ala Asn Thr
1 5 10

<210> SEQ ID NO 9
<211> LENGTH: 1062
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 9

ttaaaaatct tcgttagttt ctgctacgcc ttcgctatca tctacagaga aatccggcgt 60

tgagttcggg ttgctcagca gcaactcacg tactttcttc tcgatctctt tcgcggtttc 120

cgggttatct ttcagccagg cagtcgcatt cgctttaccc tgaccgatct tctcaccttt 180

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gtagctgtac cacgcgcctg ctttctcgat cagcttctct tttacgccca ggtcaaccag 240

ttcgccgtag aagttgatac cttcgccgta gaggatctgg aattcagcct gtttaaacgg 300

cgcagcgatt ttgttcttca ccactttcac gcgggtttcg ctacccacca cgttttcgcc 360

ctctttcacc gcgccgatac gacggatgtc gagacgaaca gaggcgtaga atttcagcgc 420

gttaccaccg gtagtggttt ccgggttacc gaacatcaca ccaattttca tacggatctg 480

gttgatgaag atcagcagcg tgttggactg cttcaggtta cccgccagct tacgcatcgc 540

ctggctcatc atacgtgccg caaggcccat gtgagagtcg ccgatttcgc cttcgatttc 600

cgctttcggc gtcagtgccg ccacggagtc aacgacgata acgtctactg cgccagaacg 660

cgccagggcg tcacagattt ccagtgcctg ctcgccggtg tccggctggg agcacagcag 720

gttgtcgata tcgacgccca gtttacgtgc gtagattggg tccagcgcgt gttcagcatc 780

gataaacgca caggttttac cttcacgctg cgctgcggcg atcacctgca gcgtcagcgt 840

ggttttaccg gaagattccg gtccgtagat ttcgacgata cggcccatcg gcagaccacc 900

tgccccaagc gcgatatcca gtgaaagcga accggtagag atggtttcca catccatgga 960

acggtcttca cccaggcgca tgatggagcc tttaccaaat tgtttctcaa tctggcccag 1020

tgctgccgcc aacgctttct gtttgttttc gtcgatagcc at 1062

<210> SEQ ID NO 10
<211> LENGTH: 2373
<212> TYPE: DNA
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 10

tcagaagtcg acgggggcgg gcaggacacc gtcatttgag ggatcatcgg tcaccacggc 60

accaatgcca agcttttcct tgatcttctt ctcgatctcg tcagccacgt cggcgttctc 120

caccaagaag ttgcgggcat tctccttgcc ctggccgagc tgctcgccct cgtaggtgaa 180

ccaggcaccc gacttgcgga tgaggccctg atccacaccc atgtcgatca gcgagccctc 240

cctgctgatt cccttgccgt agaggatgtc gaactcggcc tgcttgaagg ggggcgaaca 300

gttgtgcacg acaacccctt cggcgacgag ggtgtgcagt tcctcgacct cgaggtcgaa 360

cgttcgtgcc cgccgcgttg gcagcacttc tcggatcacg gaatagcgga gttcttccgc 420

cagcatgtcg tgcaggaatt tgtcatccag ggcatccgcg agcgcctgca cgcgatcccg 480

acgaaggcgg ctggcaccta agacctgctt cattccaccg cgggggtccc cggaagctac 540

accgatcatg gccgcggcct cctgcgcggt cacgccgcgc tcgtccagat aattcagcac 600

ggcatcggtc atctctgcag ccagatatgt cgcttgcgat ccacgacgcc gcccctgcgt 660

ggcttctgga atcgcctgga taagcgcggc accgcgcggc ccccacatgg gaactgactc 720

cgcgaatgcc gtgacgttat ccatacccga gatccggacc tcgaacactt gacgtttgct 780

ctggatccgt cgaccgttga cgatgctcgg ccgcttctgg gtcggatcgt aatctcgaac 840

ggtgctcccg acaccgaacc gcagcagcag ccaatgaatc tgatgcgcga gttgttcaga 900

ggtcgtcgtg taaccgaccc gaagtgcccc ggtctgttcc cggctcaccc acccgtcgct 960

ttcgaacagg ccgaagagca gattgccgac aatgtcggcc gcgatgtccg gctcgaagaa 1020

ccaattcgga atcgtcttct cccacgcgag cttgccgtag ataccggcct gctgacaaag 1080

gtctgccaca ccgttgcgct caccgggtcg atgagcgatc gcgagtgaga tacgcccctg 1140

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cggatgggcc gcgcaaccga gcgtcgcagc gattcgcgtc acgtcgtcaa tgagcgcccg 1200

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gatcaggtag ccaagcagcc gggcatgatc cgccggaatc ggcgcactgt caccgaatcc 1320

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gatcgccgtg gtgcccgaat tattcagcgc gccggtcatt ttccgcagcg cctggctcat 1860

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caccagcgcc gccaccgagt cgatcaccac gatgtcaagc gcacccgagc ggatcagcat 1980

gtcggcgatc tcgagtgcct gttccccggt gtccggctgg ctgaccagca gcgaatcggt 2040

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cgcaacacca ccggcggcct gagcgttggc caccgcgtgc agcgccacgg tggtcttacc 2160

cgacgactcc gggccgtata tctctatcac ccggccacgc ggcaggccgc caatgcccag 2220

ggccacgtct agtgcgatgg atccggtcgg aatgaccgaa atcggctgac gcgcctcgtc 2280

gccgaggcgc atcaccgaac ctttgccgta actcttctcg atctgggcca ctgccagctc 2340

gagcgccttt tcccgatcgg gggtctgcgt cat 2373

<210> SEQ ID NO 11
<211> LENGTH: 1047
<212> TYPE: DNA
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 11

atgagtgatc gtcaggcagc cttagatatg gctcttaaac aaatagaaaa acagttcggc 60

aaaggttcca ttatgaaact gggagaaaag acagatacaa gaatttctac tgtaccaagc 120

ggctccctcg ctcttgatac agcactggga attggcggat atcctcgcgg acggattatt 180

gaagtatacg gtcctgaaag ctcaggtaaa acaactgtgg cgcttcatgc gattgctgaa 240

gttcagcagc agggcggaca agccgcgttt atcgatgcgg agcatgcgtt agatccggta 300

tacgcgcaaa agctcggtgt taacatcgaa gagcttttac tgtctcagcc tgacacaggc 360

gagcaggcgc ttgaaattgc ggaagcattg gttcgaagcg gggcagttga cattgtcgtt 420

gtcgactctg tagccgctct cgttccgaaa gcggaaattg aaggcgacat gggagattcg 480

catgtcggtt tacaagcacg cttaatgtct caagcgcttc gtaagctttc aggggccatt 540

aacaaatcga agacaatcgc gattttcatt aaccaaattc gtgaaaaagt cggtgttatg 600

ttcgggaacc cggaaacaac acctggcggc cgtgcgttga aattctattc ttccgtgcgt 660

cttgaagtgc gccgtgctga acagctgaaa caaggcaacg acgtaatggg gaacaaaacg 720

aaaatcaaag tcgtgaaaaa caaggtggct ccgccgttcc gtacagccga ggttgacatt 780

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atgtacggag aaggcatttc aaaagaaggc gaaatcattg atctaggaac tgaacttgat 840

atcgtgcaaa aaagcggttc atggtactct tatgaagaag agcgtcttgg ccaaggccgt 900

gaaaatgcaa aacaattcct gaaagaaaat aaagatatca tgctgatgat ccaggagcaa 960

attcgcgaac attacggctt ggataataac ggagtagtgc agcagcaagc tgaagagaca 1020

caagaagaac tcgaatttga agaataa 1047

<210> SEQ ID NO 12
<211> LENGTH: 1071
<212> TYPE: DNA
<213> ORGANISM: Yersinia pseudotuberculosis

<400> SEQUENCE: 12

atggctattg atgagaataa acaaaaggcg ttagcagcag cactgggcca aattgaaaaa 60

caattcggta aaggctctat tatgcgcctt ggcgaagacc gctcaatgga tgttgaaacc 120

atctctaccg gctccctttc ccttgatatt gcactggggg ctggtggctt accaatgggg 180

cgtatcgttg agatttatgg cccagaatca tcaggtaaga cgacactgac attacaggtt 240

atcgccgccg cacagcgtga aggcaaaacg tgtgcattta tcgatgccga acatgccctt 300

gacccaatct atgccaagaa attgggtgta gatattgata acctattgtg ttctcagcca 360

gatactggcg agcaggcact ggaaatttgt gatgcgctga ctcgctctgg tgcggttgac 420

gttatcatcg ttgactccgt agcggcattg acaccaaaag ctgaaattga aggtgaaatt 480

ggcgattctc atatgggcct tgccgcgcgt atgatgagcc aggctatgcg taagctggcg 540

ggtaacctga agaatgcgaa taccttactg atttttatca accaaatccg catgaaaatt 600

ggcgtgatgt ttggtaaccc agaaaccact accggtggca acgctcttaa attttacgct 660

tctgtacgtt tggatatccg ccgtattggt gcagtaaaag atggtgatgt ggtcgtgggg 720

agtgaaaccc gcgttaaagt cgttaaaaac aagattgctg cgccattcaa acaagctgaa 780

ttccagatcc tctacggtga aggcattaat atcaacggtg aactggttga cttaggtgtt 840

aaacacaaac tgattgagaa agctggcgca tggtatagct ataacggtga taaaattggt 900

cagggtaaag ccaatgccag caactattta aaagaaaacc cagccattgc tgctgagtta 960

gataaaaaac tgcgtgaaat gctacttaat ggcggcaatg gtgaacaacc tgttgctgcg 1020

gcaacagcag aattcgccga tggtgcagat gaaaccaacg aagaatttta g 1071

<210> SEQ ID NO 13
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid encoding a TAT cell penetrating polypeptide

<400> SEQUENCE: 13

tacggcagga agaagcggag acagcgacga aga 33

<210> SEQ ID NO 14
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 14

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gcccgggacc atggctattg atgagaataa ac 32

<210> SEQ ID NO 15
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 15

ctctagaaat aattttgttt aactttaaga aggagatata catatggcta ttgatgagaa 60

taaac 65

<210> SEQ ID NO 16
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 16

caatttcttg gcatagattg g 21

<210> SEQ ID NO 17
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 17

ccattcaaac aagctgaatt c 21

<210> SEQ ID NO 18
<211> LENGTH: 69
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 18

gaaaccaacg aagaatttag tggcctacga tcgcgagcag ctgcgaacac gatgagtatt 60

caacatttc 69

<210> SEQ ID NO 19
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 19

cgggataata ccgcgccac 19

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

<400> SEQUENCE: 20

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gaaatgttga atactcatcg tgttcgcagc tgctcgcgat cgtaggccac taaattcttc 60

gttggtttc 69

<210> SEQ ID NO 21
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 21

gcttcccggc aacaattaat ag 22

<210> SEQ ID NO 22
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 22

gagtaaactt ggtctgacag 20

<210> SEQ ID NO 23
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 23

gtgaagttga catccaaaaa ggatgttttc tcgtgctgca gcccaatgct taatcagtga 60

<210> SEQ ID NO 24
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 24

cttcttcctg ccgtaatggt gatggtgatg gtgatggtga tggtgaagtt gacatccaaa 60

<210> SEQ ID NO 25
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 25

gcggccgcct atcttcgtcg ctgtctccgc ttcttcctgc cgtaatg 47

<210> SEQ ID NO 26
<211> LENGTH: 76
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 26

ggtggcggcc gctctagaaa taattttgtt taactttaag aaggagatat acatatggct 60

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attgatgaga ataaac 76

<210> SEQ ID NO 27
<211> LENGTH: 76
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 27

gtttattctc atcaatagcc atatgtatat ctccttctta aagttaaaca aaattatttc 60

tagagcggcc gccacc 76

<210> SEQ ID NO 28
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 28

cgagcagctg cgaacacggg atccgctgca gcacgagaaa ac 42

<210> SEQ ID NO 29
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide primer

<400> SEQUENCE: 29

gttttctcgt gctgcagcgg atcccgtgtt cgcagctgct cg 42

<210> SEQ ID NO 30
<211> LENGTH: 1055
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence encoding a wild type GFP

<400> SEQUENCE: 30

atggagagcg acgagagcgg cctgcccgcc atggagatcg agtgccgcat caccggcacc 60

ctgaacggcg tggagttcga gctggtgggc ggcggagagg gcacccccaa gcagggccgc 120

atgaccaaca agatgaagag caccaaaggc gccctgacct tcagccccta cctgctgagc 180

cacgtgatgg gctacggctt ctaccacttc ggcacctacc ccagcggcta cgagaacccc 240

ttcctgcacg ccatcaacaa cggcggctac accaacaccc gcatcgagaa gtacgaggac 300

ggcggcgtgc tgcacgtgag cttcagctac cgctacgagg ccggccgcgt gatcggcgac 360

ttcaaggtgg tgggcaccgg cttccccgag gacagcgtga tcttcaccga caagatcatc 420

cgcagcaacg ccaccgtgga gcacctgcac cccatgggcg ataacgtgct ggtgggcagc 480

ttcgcccgca ccttcagcct gcgcgacggc ggctactaca gcttcgtggt ggacagccac 540

atgcacttca agagcgccat ccaccccagc atcctgcaga acgggggccc catgttcgcc 600

ttccgccgcg tggaggagct gcacagcaac accgagctgg gcatcgtgga gtaccagcac 660

gccttcaaga cccccattgc cttcgccaga tcccgcgctc agtcgtccaa ttctgccgtg 720

gacggcaccg ccggacccgg ctccaccgga tctcgctaag aattcgtcga caatcaacct 780

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ctggattaca aaatttgtga aagattgact ggtattctta actatgttgc tccttttacg 840

ctatgtggat acgctgcttt aatgcctttg tatcatgcta ttgcttcccg tatggctttc 900

attttctcct ccttgtataa atcctggttg ctgtctcttt atgaggagtt gtggcccgtt 960

gtcaggcaac gtggcgtggt gtgcactgtg tttgctgacg caacccccac tggttggggc 1020

attgccacca cctgtcagct cctttccggg acttt 1055

<210> SEQ ID NO 31
<211> LENGTH: 1051
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence encoding a loss-of-function GFP

<400> SEQUENCE: 31

atggagagcg acgagagcgg cctgcccgcc atggagatcg agtgccgcat caccggcacc 60

ctgaacggcg tggagttcga gctggtgggc ggcggagagg gcacccccaa gcagggccgc 120

atgaccaaca agatgaagag caccaaaggc gccctgacct tcagccccta cctgctgagc 180

cacggggcta cggcttctac cacttcggca cctaccccag cggctacgag aaccccttcc 240

tgcacgccat caacaacggc ggctacacca acacccgcat cgagaagtac gaggacggcg 300

gcgtgctgca cgtgagcttc agctaccgct acgaggccgg ccgcgtgatc ggcgacttca 360

aggtggtggg caccggcttc cccgaggaca gcgtgatctt caccgacaag atcatccgca 420

gcaacgccac cgtggagcac ctgcacccca tgggcgataa cgtgctggtg ggcagcttcg 480

cccgcacctt cagcctgcgc gacggcggct actacagctt cgtggtggac agccacatgc 540

acttcaagag cgccatccac cccagcatcc tgcagaacgg gggccccatg ttcgccttcc 600

gccgcgtgga ggagctgcac agcaacaccg agctgggcat cgtggagtac cagcacgcct 660

tcaagacccc cattgccttc gccagatccc gcgctcagtc gtccaattct gccgtggacg 720

gcaccgccgg acccggctcc accggatctc gctaagaatt cgtcgacaat caacctctgg 780

attacaaaat ttgtgaaaga ttgactggta ttcttaacta tgttgctcct tttacgctat 840

gtggatacgc tgctttaatg cctttgtatc atgctattgc ttcccgtatg gctttcattt 900

tctcctcctt gtataaatcc tggttgctgt ctctttatga ggagttgtgg cccgttgtca 960

ggcaacgtgg cgtggtgtgc actgtgtttg ctgacgcaac ccccactggt tggggcattg 1020

ccaccacctg tcagctcctt tccgggactt t 1051

<210> SEQ ID NO 32
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a GFP cDNA frame shift deletion

<400> SEQUENCE: 32

ggcgccctga ccttcagccc ctacctgctg agccacgtga tgggctacgg cttctaccac 60

<210> SEQ ID NO 33
<211> LENGTH: 81
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a GFP cDNA frame shift deletion

<400> SEQUENCE: 33

aacaagatga agagcaccaa aggcgccctg accttcagcc cctacctgct gagccacgtg 60

atgggctacg gcttctacca c 81

<210> SEQ ID NO 34
<211> LENGTH: 102
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a GFP cDNA frame shift deletion

<400> SEQUENCE: 34

cccaagcagg gccgcatgac caacaagatg aagagcacca aaggcgccct gaccttcagc 60

ccctacctgc tgagccacgt gatgggctac ggcttctacc ac 102

<210> SEQ ID NO 35
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a GFP cDNA frame shift deletion

<400> SEQUENCE: 35

ccgctggggt aggtgccgaa gtggtagaag ccgtagccca tcacgtggct cagcaggtag 60

<210> SEQ ID NO 36
<211> LENGTH: 81
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a GFP cDNA frame shift deletion

<400> SEQUENCE: 36

tgcaggaagg ggttctcgta gccgctgggg taggtgccga agtggtagaa gccgtagccc 60

atcacgtggc tcagcaggta g 81

<210> SEQ ID NO 37
<211> LENGTH: 102
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a GFP cDNA frame shift deletion

<400> SEQUENCE: 37

tagccgccgt tgttgatggc gtgcaggaag gggttctcgt agccgctggg gtaggtgccg 60

aagtggtaga agccgtagcc catcacgtgg ctcagcaggt ag 102

<210> SEQ ID NO 38
<211> LENGTH: 671
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 38

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aagctttatc cccgctgcca tcatggttcg accattgaac tgcatcgtcg ccgtgtccca 60

agatatgggg attggcaaga acggagacct accctggcct ccgctcagga acgagtggaa 120

gtacttccaa agaatgacca caacctcttc agtggaaggt aaacagaatc tggtgattat 180

gggtaggaaa acctggttct ccattcctga gaagaatcga cctttaaagg acagaattaa 240

tatagttctc agtagagaac tcaaagaacc accacgagga gctcattttc ttgccaaaag 300

tttggatgat gccttaagac ttattgaaca accggaattg gcaagtaaag tagacatggt 360

ttggatagtc ggaggcagtt ctgtttacca ggaagccatg aatcaaccag gccacctcag 420

actctttgtg acaaggatca tgcaggaatt tgaaagtgac acgtttttcc cagaaattga 480

tttggggaaa tataaacttc tcccagaata cccaggcgtc ctctctgagg tccaggagga 540

aaaaggcatc aagtataagt ttgaagtcta cgagaagaaa gactaacagg aagatgcttt 600

caagttctct gctcccctcc taaagctatg catttttata agaccatggg acttttgctg 660

gctttagatc t 671

<210> SEQ ID NO 39
<211> LENGTH: 669
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence encoding a loss-of-function mouse dihydrofolate reductase

<400> SEQUENCE: 39

aagctttatc cccgctgcca tcatggttcg accattgaac tgcatcgtcg ccgtgtccca 60

agatatgggg attggcaaga acggagacct accctggcct ccgctcagga acgagtggaa 120

gtacttccaa agaaaccaca acctcttcag tggaaggtaa acagaatctg gtgattatgg 180

gtaggaaaac ctggttctcc attcctgaga agaatcgacc tttaaaggac agaattaata 240

tagttctcag tagagaactc aaagaaccac cacgaggagc tcattttctt gccaaaagtt 300

tggatgatgc cttaagactt attgaacaac cggaattggc aagtaaagta gacatggttt 360

ggatagtcgg aggcagttct gtttaccagg aagccatgaa tcaaccaggc cacctcagac 420

tctttgtgac aaggatcatg caggaatttg aaagtgacac gtttttccca gaaattgatt 480

tggggaaata taaacttctc ccagaatacc caggcgtcct ctctgaggtc caggaggaaa 540

aaggcatcaa gtataagttt gaagtctacg agaagaaaga ctaacaggaa gatgctttca 600

agttctctgc tcccctccta aagctatgca tttttataag accatgggac ttttgctggc 660

tttagatct 669

<210> SEQ ID NO 40
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a DHFR cDNA frame shift deletion

<400> SEQUENCE: 40

tggcctccgc tcaggaacga gtggaagtac ttccaaagaa tgaccacaac ctcttcagtg 60

<210> SEQ ID NO 41
<211> LENGTH: 81
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a DHFR cDNA frame shift deletion

<400> SEQUENCE: 41

ggcaagaacg gagacctacc ctggcctccg ctcaggaacg agtggaagta cttccaaaga 60

atgaccacaa cctcttcagt g 81

<210> SEQ ID NO 42
<211> LENGTH: 102
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligonucleotide to correct a DHFR cDNA frame shift deletion

<400> SEQUENCE: 42

gtgtcccaag atatggggat tggcaagaac ggagacctac cctggcctcc gctcaggaac 60

gagtggaagt acttccaaag aatgaccaca acctcttcag tg 102

<210> SEQ ID NO 43
<211> LENGTH: 60
<212> TYPE: DNA
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1. A fusion protein comprising a RecA polypeptide and cell penetrating peptide (CPP).

2. The fusion protein of claim **1**, wherein said RecA polypeptide comprises an amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

3. (canceled)

4. The fusion protein of claim **1**, wherein said CPP is selected from the group consisting of a trans-activating transcriptional activator (TAT) peptide sequence, a Pep-1 peptide sequence, and a MPG peptide sequence.

5. The fusion protein of claim **4**, wherein said CPP is a TAT peptide, and wherein said TAT peptide sequence comprises the amino acid sequence YGRKKRRQRRR (SEQ ID NO:5).

6. The fusion protein of claim **4**, wherein said CPP is a Pep-1 peptide, and wherein said Pep-1 peptide sequence comprises the amino acid sequence KETWWETWW-TEWSQPKKKRKV (SEQ ID NO:6).

7. The fusion protein of claim **4**, wherein said CPP is a MPG peptide, and wherein said MPG peptide sequence comprises the amino acid sequence SVVDRVAEQDTQA (SEQ ID NO:7).

8. (canceled)

9. The fusion protein of claim **1**, said fusion protein further comprising a peptide linker present between said RecA polypeptide and said CPP.

10. The fusion protein of claim **9**, wherein said peptide linker is selected from the group consisting of a peptide sequence including SGLRSRAAANT (SEQ ID NO:8), one or more alanine residues, one or more glycine residues, and combinations thereof.

11. The fusion protein of claim **1**, said fusion protein further comprising a peptide tag, wherein said peptide tag is an antibody epitope or a fluorescent protein.

12. (canceled)

13. The fusion protein of claim **11**, wherein said antibody epitope is a multidrug resistance protein 1 (MRP1) antibody epitope.

14. (canceled)

15. The fusion protein of claim **11**, wherein said fluorescent protein is a green fluorescent protein.

16. The fusion protein of claim **11**, said fusion protein comprising an antibody epitope or a fluorescent protein, wherein said antibody epitope is a MRP1 antibody epitope, and wherein said fluorescent protein is a green fluorescent protein.

17-20. (canceled)

21. A nucleic acid construct encoding the fusion protein of claim **1**.

22-25. (canceled)

26. A nucleoprotein filament comprising:

one or more fusion proteins of claim **1**; and

a single stranded oligonucleotide, wherein said single stranded oligonucleotide can hybridize to a target sequence having one or more mutations, and wherein said single stranded oligonucleotide comprises a corrected nucleic acid sequence.

27. A method for editing the genome of a cell, said method comprising:

contacting the cell with a) a fusion protein comprising a RecA polypeptide and cell penetrating peptide (CPP); and b) a single stranded oligonucleotide, wherein said single stranded oligonucleotide can hybridize to a target sequence having one or more mutations, and wherein said single stranded oligonucleotide comprises a corrected nucleic acid sequence.

28. The method of claim **27**, wherein said cell is a prokaryotic cell.

29. The method of claim **27**, wherein said cell is a eukaryotic cell.

30. (canceled)

31. A method for treating a mammal having a monogenetic disease, the method comprising:

contacting a cell in the mammal with a) a fusion protein comprising a RecA polypeptide and cell penetrating peptide; and b) a single stranded oligonucleotide, wherein said single stranded oligonucleotide can hybridize to a target sequence in a genome within said cell, wherein said target sequence comprises a nucleic acid sequence comprising one or more disease-causing mutations, and wherein said single stranded oligonucleotide comprises a corrected nucleic sequence.

32. The method of claim **31**, wherein said mammal is a human.

33. The method of claim **31**, wherein said monogenetic disease is selected from the group consisting of color blindness, cystic fibrosis, haemochromatosis, haemophilia, phenylketonuria, polycystic kidney disease, Tay-Sachs disease, Huntington's disease, Marfan syndrome, sickle-cell disease, duchenne muscular dystrophy, and cancer.

34-43. (canceled)

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