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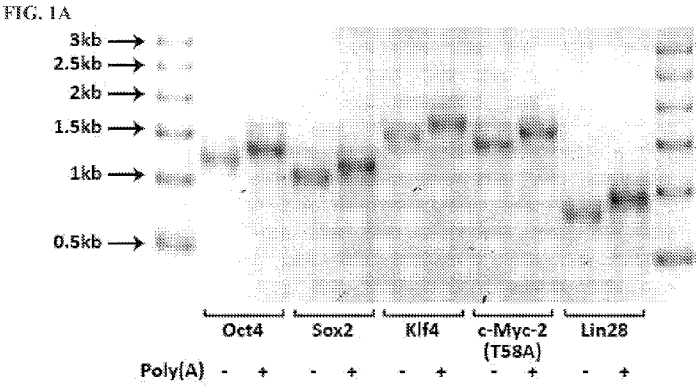
(54) **Title**: METHODS AND PRODUCTS FOR EXPRESSING PROTEINS IN CELLS

FIG. 1A



(57) **Abstract**: The present invention relates in part to nucleic acids encoding proteins, therapeutics comprising nucleic acids encoding proteins, methods for inducing cells to express proteins using nucleic acids, methods, kits and devices for transfecting, gene editing, and reprogramming cells, and cells, organisms, and therapeutics produced using these methods, kits, and devices. Methods and products for altering the DNA sequence of a cell are described, as are methods and products for inducing cells to express proteins using synthetic RNA molecules. Therapeutics comprising nucleic acids encoding gene-editing proteins are also described.

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METHODS AND PRODUCTS FOR EXPRESSING PROTEINS IN CELLS

PRIORITY

The present application claims priority to U.S. Provisional Application No. 61/721,302, filed on November 1, 2012, U.S. Provisional Application No. 61/785,404, filed on March 14, 2013, and U.S. Provisional Application No. 61/842,874, filed on July 3, 2013, the contents of which are herein incorporated by reference in their entireties. The present application is related to U.S. Application No. 13/465,490, filed on May 7, 2012, International Application No. PCT/US2012/067966, filed on December 5, 2012, and U.S. Application No. 13/931,251, filed on June 28, 2013, the contents of which are herein incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates in part to nucleic acids encoding proteins, therapeutics comprising nucleic acids encoding proteins, methods for inducing cells to express proteins using nucleic acids, methods, kits and devices for transfecting, gene editing, and reprogramming cells, and cells, organisms, and therapeutics produced using these methods, kits, and devices.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: FABI_005_02WO_SeqList_ST25.txt; date recorded: October 30, 2013; file size: 255 KB).

BACKGROUND

Synthetic RNA and RNA Therapeutics

Ribonucleic acid (RNA) is ubiquitous in both prokaryotic and eukaryotic cells, where it encodes genetic information in the form of messenger RNA, binds and transports amino acids in the form of transfer RNA, assembles amino acids into proteins in the form of ribosomal RNA, and performs numerous other functions including gene expression regulation in the forms of microRNA and long non-coding RNA. RNA can be produced synthetically by methods including direct chemical synthesis and *in vitro* transcription, and can be administered to patients for therapeutic use.

Cell Reprogramming and Cell-Based Therapies

Cells can be reprogrammed by exposing them to specific extracellular cues and/or by ectopic expression of specific proteins, microRNAs, etc. While several reprogramming methods have been previously described, most that rely on ectopic expression require the introduction of exogenous DNA, which can carry mutation risks. DNA-free reprogramming methods based on direct delivery of

reprogramming proteins have been reported. However, these methods are too inefficient and unreliable for commercial use. In addition, RNA-based reprogramming methods have been described (*See, e.g.*, Angel. MIT Thesis. 2008. 1-56; Angel *et al.* PLoS ONE. 2010. 5,107; Warren *et al.* Cell Stem Cell. 2010. 7,618-630; Angel. MIT Thesis. 2011. 1-89; and Lee *et al.* Cell. 2012. 151,547-558; the contents of all of which are hereby incorporated by reference). However, existing RNA-based reprogramming methods are slow, unreliable, and inefficient when performed on adult cells, require many transfections (resulting in significant expense and opportunity for error), can reprogram only a limited number of cell types, can reprogram cells to only a limited number of cell types, require the use of immunosuppressants, and require the use of multiple human-derived components, including blood-derived HSA and human fibroblast feeders. The many drawbacks of previously disclosed RNA-based reprogramming methods make them undesirable for both research and therapeutic use.

Gene Editing

Several naturally occurring proteins contain DNA-binding domains that can recognize specific DNA sequences, for example, zinc fingers (ZFs) and transcription activator-like effectors (TALEs). Fusion proteins containing one or more of these DNA-binding domains and the cleavage domain of FokI endonuclease can be used to create a double-strand break in a desired region of DNA in a cell (*See, e.g.*, US Patent Appl. Pub. No. US 2012/0064620, US Patent Appl. Pub. No. US 2011/0239315, US Patent No. 8,470,973, US Patent Appl. Pub. No. US 2013/0217119, US Patent No. 8,420,782, US Patent Appl. Pub. No. US 2011/0301073, US Patent Appl. Pub. No. US 2011/0145940, US Patent No. 8,450,471, US Patent No. 8,440,431, US Patent No. 8,440,432, and US Patent Appl. Pub. No. 2013/0122581, the contents of all of which are hereby incorporated by reference). However, current methods for gene editing cells are inefficient and carry a risk of uncontrolled mutagenesis, making them undesirable for both research and therapeutic use. Methods for DNA-free gene editing of somatic cells have not been previously explored, nor have methods for simultaneous or sequential gene editing and reprogramming of somatic cells. In addition, methods for directly gene editing cells in patients (*i.e., in vivo*) have not been previously explored, and the development of such methods has been limited by a lack of acceptable targets, inefficient delivery, inefficient expression of the gene-editing protein/proteins, inefficient gene editing by the expressed gene-editing protein/proteins, due in part to poor binding of DNA-binding domains, excessive off-target effects, due in part to non-directed dimerization of the FokI cleavage domain and poor specificity of DNA-binding domains, and other factors. Finally, the use of gene editing in anti-bacterial, anti-viral, and anti-cancer treatments has not been previously explored.

Accordingly, there remains a need for improved compositions and methods for the expression of proteins in cells.

SUMMARY OF THE INVENTION

The present invention provides, in part, compositions, methods, articles, and devices for inducing cells to express proteins, methods, articles, and devices for producing these compositions, methods, articles, and devices, and compositions and articles, including cells, organisms, and therapeutics, produced using these compositions, methods, articles, and devices. Unlike previously reported methods, certain embodiments of the present invention do not involve exposing cells to exogenous DNA or to allogeneic or animal-derived materials, making products produced according to the methods of the present invention useful for therapeutic applications.

In some aspects, synthetic RNA molecules with low toxicity and high translation efficiency are provided. In one aspect, a cell-culture medium for high-efficiency transfection, reprogramming, and gene editing of cells is provided. Other aspects pertain to methods for producing synthetic RNA molecules encoding reprogramming proteins. Still further aspects pertain to methods for producing synthetic RNA molecules encoding gene-editing proteins.

In one aspect, the invention provides high-efficiency gene-editing proteins comprising engineered nuclease cleavage domains. In another aspect, the invention provides high-fidelity gene-editing proteins comprising engineered nuclease cleavage domains. Other aspects relate to high-efficiency gene-editing proteins comprising engineered DNA-binding domains. Still further aspects pertain to high-fidelity gene-editing proteins comprising engineered DNA-binding domains. Still further aspects relate to gene-editing proteins comprising engineered repeat sequences. Some aspects relate to methods for altering the DNA sequence of a cell by transfecting the cell with or inducing the cell to express a gene-editing protein. Other aspects relate to methods for altering the DNA sequence of a cell that is present in an *in vitro* culture. Still further aspects relate to methods for altering the DNA sequence of a cell that is present *in vivo*.

In some aspects, the invention provides methods for treating cancer comprising administering to a patient a therapeutically effective amount of a gene-editing protein or a nucleic-acid encoding a gene-editing protein. In one aspect, the gene-editing protein is capable of altering the DNA sequence of a cancer associated gene. In another aspect, the cancer-associated gene is the BIRC5 gene. Still other aspects relate to therapeutics comprising nucleic acids and/or cells and methods of using therapeutics comprising nucleic acids and/or cells for the treatment of, for example, type 1 diabetes, heart disease, including ischemic and dilated cardiomyopathy, macular degeneration, Parkinson's disease, cystic fibrosis, sickle-cell anemia, thalassemia, Fanconi anemia, severe combined immunodeficiency, hereditary sensory neuropathy, xeroderma pigmentosum, Huntington's disease, muscular dystrophy, amyotrophic lateral sclerosis, Alzheimer's disease, cancer, and infectious diseases including hepatitis and HIV/AIDS. In some aspects, the nucleic acids comprise synthetic RNA. In other aspects, the nucleic acids are delivered to cells using a virus. In some aspects, the virus is a replication-competent virus. In other aspects, the virus is a replication-incompetent virus.

The details of the invention are set forth in the accompanying description below. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, illustrative methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, 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 belongs.

DETAILED DESCRIPTION OF THE FIGURES

FIG. 1A depicts RNA encoding the indicated proteins and containing adenosine, 50% guanosine, 50% 7-deazaguanosine, 70% uridine, 30% 5-methyluridine, and 5-methylcytidine, resolved on a denaturing formaldehyde-agarose gel.

FIG. 1B depicts RNA encoding the indicated proteins and containing adenosine, 50% guanosine, 50% 7-deazaguanosine, 50% uridine, 50% 5-methyluridine, and 5-methylcytidine, resolved on a denaturing formaldehyde-agarose gel.

FIG. 2 depicts primary human neonatal fibroblasts reprogrammed by five transfections with RNA encoding reprogramming proteins. Cells were fixed and stained for Oct4 protein. Nuclei were counterstained with Hoechst 33342.

FIG. 3A depicts primary human adult fibroblasts.

FIG. 3B depicts the primary human adult fibroblasts shown in **FIG. 3A**, reprogrammed by seven transfections with RNA encoding reprogramming proteins. Arrows indicate colonies of reprogrammed cells.

FIG. 3C depicts a large colony of reprogrammed primary human adult fibroblasts.

FIG. 4A depicts the location of a TALEN pair targeting the human CCR5 gene. Single-lines indicate the TALEN binding sites. Double-lines indicate the location of the Δ32 mutation.

FIG. 4B depicts synthetic RNA encoding the TALEN pair of **FIG. 4A**, resolved on a denaturing formaldehyde-agarose gel.

FIG. 4C depicts the results of a SURVEYOR assay testing the functionality of the RNA of **FIG. 4B** on human dermal fibroblasts (GM00609). The appearance of the 760bp and 200bp bands in the sample generated from cells transfected with RNA indicates successful gene editing. The percentage below each lane indicates the efficiency of gene editing (percentage of edited alleles).

FIG. 4D depicts a line-profile graph of the "Neg" and "TALENs" lanes of **FIG. 4C**. Numbers indicate the integrated intensity of the three bands, relative to the total integrated intensity.

FIG. 4E depicts the results of a SURVEYOR assay performed as in **FIG. 4C**, and also including a sample generated from cells that were transfected twice with RNA (the lane labeled "2x").

FIG. 4F depicts simultaneous gene editing and reprogramming of primary human cells (GM00609) using synthetic RNA. Images show representative colonies of reprogrammed cells.

FIG. 4G depicts the results of direct sequencing of the CCR5 gene in gene-edited, reprogrammed cells generated as in **FIG. 4F**. Four of the nine lines tested contained a deletion between the TALEN binding sites, indicating efficient gene editing.

FIG. 5 depicts the results of a SURVEYOR assay performed as in **FIG. 4C**, except using RNA targeting the human MYC gene, and containing either canonical nucleotides ("A,G,U,C") or non-canonical nucleotides ("A,7dG,5mU,5mC"). The dark bands at 470bp and 500bp indicate high-efficiency gene editing.

FIG. 6 depicts the results of a SURVEYOR assay performed as in **FIG. 4C**, except using RNA targeting the human BIRC5 gene, and containing either canonical nucleotides ("A,G,U,C") or non-canonical nucleotides ("A,7dG,5mU,5mC"). The dark band at 710bp indicates high-efficiency gene editing.

FIG. 7A depicts HeLa cells (cervical carcinoma) transfected with RNA targeting the human BIRC5 gene (RiboSlice). Cells were transfected with either a single RNA ("2x Survivin L") or equal amounts of each member of an RNA pair ("Survivin L + R"), with the same total amount of RNA delivered in each case. As shown in the right panel, cells transfected with the RNA pair became enlarged, and exhibited fragmented nuclei and markedly reduced proliferation, demonstrating the potent anti-cancer activity of RiboSlice.

FIG. 7B depicts HeLa cells transfected with RNA targeting the human BIRC5 gene as in **FIG. 7A**. Cells were subsequently fixed and stained for survivin protein. Nuclei were counterstained with Hoechst 33342. The large, fragmented nuclei of cells transfected with RiboSlice are indicated with arrows.

FIG. 8 depicts primary human adult fibroblasts reprogrammed using synthetic RNA. Arrows indicate compact colonies of cells that exhibit a morphology indicative of reprogramming.

FIG. 9 depicts synthetic RNA encoding the indicated gene-editing proteins, resolved on a denaturing formaldehyde-agarose gel.

FIG. 10A depicts the results of a SURVEYOR assay testing the effectiveness of the RNA of **FIG. 9** on human dermal fibroblasts. Cells were lysed approximately 48h after transfection. Bands corresponding to digestion products resulting from successful gene editing are indicated with asterisks. Lane labels are of the form "X.Y", where X refers to the exon from which DNA was amplified, and Y

refers to the gene-editing protein pair. For example, "1.1" refers to the gene-editing protein pair targeting the region of exon 1 closest to the start codon. "X.N" refers to untransfected cells.

FIG. 10B depicts the results of a SURVEYOR assay testing the toxicity of the RNA of **FIG. 9** on human dermal fibroblasts. Cells were lysed 11 days after transfection. Lanes and bands are labeled as in **FIG. 10A**. The appearance of the bands indicated with asterisks demonstrates that the transfected cells retained high viability.

FIG. 11 depicts the results of a study designed to test the safety of RNA encoding gene-editing proteins *in vivo*. The graph shows the mean body weight of four groups of mice (10 animals in each group), including one untreated group, one vehicle-only group, one group treated with RiboSlice via intratumoral injection, and one group treated with RiboSlice via intravenous injection. For all treated groups, animals were given 5 doses, every other day, from day 1 to day 9. Animals were followed until day 17. The lack of a statistically significant difference between the mean body weights of the four groups demonstrates the *in vivo* safety of RiboSlice.

FIG. 12A depicts the results of a SURVEYOR assay testing the effectiveness of gene-editing proteins comprising various 36 amino-acid-long repeat sequences. Human dermal fibroblasts were lysed approximately 48h after transfection with RNA encoding gene-editing proteins containing the indicated repeat sequence. The band corresponding to the digestion product resulting from successful gene editing is indicated with an asterisk. Lane labels refer to the amino acids at the C-terminus of the repeat sequence. "Neg." refers to untransfected cells.

FIG. 12B depicts the results of a SURVEYOR assay testing the effectiveness of gene-editing proteins in which every other repeat sequence is 36 amino acids long. Human dermal fibroblasts were lysed approximately 48h after transfection with RNA encoding gene-editing proteins containing the indicated repeat sequence. The band corresponding to the digestion product resulting from successful gene editing is indicated with an asterisk. Lane labels refer to the amino acids at the C-terminus of the repeat sequences. "Neg." refers to untransfected cells.

FIG. 13A depicts the results of a study designed to test the safety and efficacy of RiboSlice AAV replication-incompetent virus carrying nucleic acids encoding gene-editing proteins *in vivo*. The graph shows the mean body weight of three groups of mice carrying subcutaneous tumors comprising human glioma cells, including one untreated group (no treatment control, "NTC", n = 6), one group treated with AAV encoding GFP ("GFP", n=2) via intratumoral injection, and one group treated with RiboSlice AAV encoding gene-editing proteins targeting the BIRC5 gene ("RiboSlice", n=2) via intratumoral injection. Animals were dosed on day 1 for the GFP group, and days 1 and 15 for the RiboSlice group. Animals were followed until day 25. The lack of a statistically significant difference between the mean body weights of the three groups demonstrates the *in vivo* safety of RiboSlice AAV.

FIG. 13B depicts the normalized tumor volumes of the animals in the study shown in **FIG. 13A**. The slower increase in normalized tumor volume in the group treated with RiboSlice AAV compared to both the NTC and GFP groups demonstrates the *in vivo* efficacy of RiboSlice AAV.

FIG. 14 depicts the results of a SURVEYOR assay testing the effectiveness of gene-editing proteins, as in **FIG. 12B**. "RiboSlice" refers to gene-editing proteins in which every other repeat sequence is 36 amino acids long. "w.t." refers to untransfected cells.

FIG. 15 depicts RNA encoding the indicated proteins and containing adenosine, 50% guanosine, 50% 7-deazaguanosine, 60% uridine, 40% 5-methyluridine, and 5-methylcytidine, resolved on a denaturing formaldehyde-agarose gel.

FIG. 16 depicts the results of an assay testing the integration of a repair template into the APP gene. The appearance of the 562bp and 385bp bands in the sample generated from cells transfected with RNA and a repair template indicates successful integration of a PstI restriction site. "-" refers to an undigested sample, "+" refers to a sample treated with PstI restriction nuclease.

Definitions

By "molecule" is meant a molecular entity (molecule, ion, complex, etc.).

By "RNA molecule" is meant a molecule that comprises RNA.

By "synthetic RNA molecule" is meant an RNA molecule that is produced outside of a cell or that is produced inside of a cell using bioengineering, by way of non-limiting example, an RNA molecule that is produced in an *in vitro*-transcription reaction, an RNA molecule that is produced by direct chemical synthesis or an RNA molecule that is produced in a genetically-engineered *E.coli* cell.

By "transfection" is meant contacting a cell with a molecule, wherein the molecule is internalized by the cell.

By "upon transfection" is meant during or after transfection.

By "transfection reagent" is meant a substance or mixture of substances that associates with a molecule and facilitates the delivery of the molecule to and/or internalization of the molecule by a cell, by way of non-limiting example, a cationic lipid, a charged polymer or a cell-penetrating peptide.

By "reagent-based transfection" is meant transfection using a transfection reagent.

By "cell-culture medium" is meant a medium that can be used for cell culture, by way of non-limiting example, Dulbecco's Modified Eagle's Medium (DMEM) or DMEM + 10% fetal bovine serum (FBS).

By "complexation medium" is meant a medium to which a transfection reagent and a molecule to be transfected are added and in which the transfection reagent associates with the molecule to be transfected.

By "transfection medium" is meant a medium that can be used for transfection, by way of non-limiting example, Dulbecco's Modified Eagle's Medium (DMEM) or DMEM/F12.

By "recombinant protein" is meant a protein or peptide that is not produced in animals or humans. Non-limiting examples include human transferrin that is produced in bacteria, human fibronectin that is produced in an *in vitro* culture of mouse cells, and human serum albumin that is produced in a rice plant.

By "lipid carrier" is meant a substance that can increase the solubility of a lipid or lipid-soluble molecule in an aqueous solution, by way of non-limiting example, human serum albumin or methyl-beta-cyclodextrin.

By "Oct4 protein" is meant a protein that is encoded by the POU5F1 gene, or a natural or engineered variant, family-member, orthologue, fragment or fusion construct thereof, by way of non-limiting example, human Oct4 protein (SEQ ID NO: 8), mouse Oct4 protein, Oct1 protein, a protein encoded by POU5F1 pseudogene 2, a DNA-binding domain of Oct4 protein or an Oct4-GFP fusion protein. In some embodiments the Oct4 protein comprises an amino acid sequence that has at least 70% identity with SEQ ID NO: 8, or in other embodiments, at least 75%, 80%, 85%, 90%, or 95% identity with SEQ ID NO: 8. In some embodiments, the Oct4 protein comprises an amino acid sequence having from 1 to 20 amino acid insertions, deletions, or substitutions (collectively) with respect to SEQ ID NO: 8. Or in other embodiments, the Oct4 protein comprises an amino acid sequence having from 1 to 15 or from 1 to 10 amino acid insertions, deletions, or substitutions (collectively) with respect to SEQ ID NO: 8.

By "Sox2 protein" is meant a protein that is encoded by the SOX2 gene, or a natural or engineered variant, family-member, orthologue, fragment or fusion construct thereof, by way of non-limiting example, human Sox2 protein (SEQ ID NO: 9), mouse Sox2 protein, a DNA-binding domain of Sox2 protein or a Sox2-GFP fusion protein. In some embodiments the Sox2 protein comprises an amino acid sequence that has at least 70% identity with SEQ ID NO: 9, or in other embodiments, at least 75%, 80%, 85%, 90%, or 95% identity with SEQ ID NO: 9. In some embodiments, the Sox2 protein comprises an amino acid sequence having from 1 to 20 amino acid insertions, deletions, or substitutions (collectively) with respect to SEQ ID NO: 9. Or in other embodiments, the Sox2 protein comprises an amino acid sequence having from 1 to 15 or from 1 to 10 amino acid insertions, deletions, or substitutions (collectively) with respect to SEQ ID NO: 9.

By "Klf4 protein" is meant a protein that is encoded by the KLF4 gene, or a natural or engineered variant, family-member, orthologue, fragment or fusion construct thereof, by way of non-limiting example, human Klf4 protein (SEQ ID NO: 10), mouse Klf4 protein, a DNA-binding domain of Klf4 protein or a Klf4-GFP fusion protein. In some embodiments the Klf4 protein comprises an amino acid sequence that has at least 70% identity with SEQ ID NO: 10, or in other embodiments, at least 75%, 80%, 85%, 90%, or 95% identity with SEQ ID NO: 10. In some embodiments, the Klf4 protein comprises an amino acid sequence having from 1 to 20 amino acid insertions, deletions, or substitutions (collectively) with respect to SEQ ID NO: 10. Or in other embodiments, the Klf4 protein comprises an amino acid sequence having from 1 to 15 or from 1 to 10 amino acid insertions, deletions, or substitutions (collectively) with respect to SEQ ID NO: 10.

By "c-Myc protein" is meant a protein that is encoded by the MYC gene, or a natural or engineered variant, family-member, orthologue, fragment or fusion construct thereof, by way of non-limiting example, human c-Myc protein (SEQ ID NO: 11), mouse c-Myc protein, l-Myc protein, c-Myc (T58A) protein, a DNA-binding domain of c-Myc protein or a c-Myc-GFP fusion protein. In some embodiments the c-Myc protein comprises an amino acid sequence that has at least 70% identity with SEQ ID NO: 11, or in other embodiments, at least 75%, 80%, 85%, 90%, or 95% identity with SEQ ID NO: 11. In some embodiments, the c-Myc protein comprises an amino acid having from 1 to 20 amino acid insertions, deletions, or substitutions (collectively) with respect to SEQ ID NO: 11. Or in other embodiments, the c-Myc protein comprises an amino acid sequence having from 1 to 15 or from 1 to 10 amino acid insertions, deletions, or substitutions (collectively) with respect to SEQ ID NO: 11.

By "reprogramming" is meant causing a change in the phenotype of a cell, by way of non-limiting example, causing a β-cell progenitor to differentiate into a mature β-cell, causing a fibroblast to dedifferentiate into a pluripotent stem cell, causing a keratinocyte to transdifferentiate into a cardiac stem cell or causing the axon of a neuron to grow.

By "reprogramming factor" is meant a molecule that, when a cell is contacted with the molecule and/or the cell expresses the molecule, can, either alone or in combination with other molecules, cause reprogramming, by way of non-limiting example, Oct4 protein.

By "feeder" is meant a cell that can be used to condition medium or to otherwise support the growth of other cells in culture.

By "conditioning" is meant contacting one or more feeders with a medium.

By "fatty acid" is meant a molecule that comprises an aliphatic chain of at least two carbon atoms, by way of non-limiting example, linoleic acid, α-linolenic acid, octanoic acid, a leukotriene, a prostaglandin, cholesterol, a glucocorticoid, a resolvin, a protectin, a thromboxane, a lipoxin, a maresin, a sphingolipid, tryptophan, N-acetyl tryptophan or a salt, methyl ester or derivative thereof.

By "short-chain fatty acid" is meant a fatty acid that comprises an aliphatic chain of between two and 30 carbon atoms.

By "albumin" is meant a protein that is highly soluble in water, by way of non-limiting example, human serum albumin.

By "associated molecule" is meant a molecule that is non-covalently bound to another molecule.

By "associated-molecule-component of albumin" is meant one or more molecules that are bound to an albumin polypeptide, by way of non-limiting example, lipids, hormones, cholesterol, calcium ions, etc. that are bound to an albumin polypeptide.

By "treated albumin" is meant albumin that is treated to reduce, remove, replace or otherwise inactivate the associated-molecule-component of the albumin, by way of non-limiting example, human serum albumin that is incubated at an elevated temperature, human serum albumin that is contacted with sodium octanoate or human serum albumin that is contacted with a porous material.

By "ion-exchange resin" is meant a material that, when contacted with a solution containing ions, can replace one or more of the ions with one or more different ions, by way of non-limiting example, a material that can replace one or more calcium ions with one or more sodium ions.

By "germ cell" is meant a sperm cell or an egg cell.

By "pluripotent stem cell" is meant a cell that can differentiate into cells of all three germ layers (endoderm, mesoderm, and ectoderm) *in vivo*.

By "somatic cell" is meant a cell that is not a pluripotent stem cell or a germ cell, by way of non-limiting example, a skin cell.

By "glucose-responsive insulin-producing cell" is meant a cell that, when exposed to a certain concentration of glucose, can produce and/or secrete an amount of insulin that is different from (either less than or more than) the amount of insulin that the cell produces and/or secretes when the cell is exposed to a different concentration of glucose, by way of non-limiting example, a β-cell.

By "hematopoietic cell" is meant a blood cell or a cell that can differentiate into a blood cell, by way of non-limiting example, a hematopoietic stem cell or a white blood cell.

By "cardiac cell" is meant a heart cell or a cell that can differentiate into a heart cell, by way of non-limiting example, a cardiac stem cell or a cardiomyocyte.

By "retinal cell" is meant a cell of the retina or a cell that can differentiate into a cell of the retina, by way of non-limiting example, a retinal pigmented epithelial cell.

By "skin cell" is meant a cell that is normally found in the skin, by way of non-limiting example, a fibroblast, a keratinocyte, a melanocyte, an adipocyte, a mesenchymal stem cell, an adipose stem cell or a blood cell.

By "Wnt signaling agonist" is meant a molecule that can perform one or more of the biological functions of one or more members of the Wnt family of proteins, by way of non-limiting example, Wnt1, Wnt2, Wnt3, Wnt3a or 2-amino-4-[3,4-(methylenedioxy)benzylamino]-6-(3-methoxyphenyl)pyrimidine.

By "IL-6 signaling agonist" is meant a molecule that can perform one or more of the biological functions of IL-6 protein, by way of non-limiting example, IL-6 protein or IL-6 receptor (also known as soluble IL-6 receptor, IL-6R, IL-6R alpha, etc.).

By "TGF-β signaling agonist" is meant a molecule that can perform one or more of the biological functions of one or more members of the TGF-β superfamily of proteins, by way of non-limiting example, TGF-β1, TGF-β3, Activin A, BMP-4 or Nodal.

By "immunosuppressant" is meant a substance that can suppress one or more aspects of an immune system, and that is not normally present in a mammal, by way of non-limiting example, B18R or dexamethasone.

By "single-strand break" is meant a region of single-stranded or double-stranded DNA in which one or more of the covalent bonds linking the nucleotides has been broken in one of the one or two strands.

By "double-strand break" is meant a region of double-stranded DNA in which one or more of the covalent bonds linking the nucleotides has been broken in each of the two strands.

By "nucleotide" is meant a nucleotide or a fragment or derivative thereof, by way of non-limiting example, a nucleobase, a nucleoside, a nucleotide-triphosphate, etc.

By "nucleoside" is meant a nucleotide or a fragment or derivative thereof, by way of non-limiting example, a nucleobase, a nucleoside, a nucleotide-triphosphate, etc.

By "gene editing" is meant altering the DNA sequence of a cell, by way of non-limiting example, by transfecting the cell with a protein that causes a mutation in the DNA of the cell.

By "gene-editing protein" is meant a protein that can, either alone or in combination with one or more other molecules, alter the DNA sequence of a cell, by way of non-limiting example, a nuclease, a transcription activator-like effector nuclease (TALEN), a zinc-finger nuclease, a meganuclease, a nickase, a clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein or a natural or engineered variant, family-member, orthologue, fragment or fusion construct thereof.

By "repair template" is meant a nucleic acid containing a region of at least about 70% homology with a sequence that is within 10kb of a target site of a gene-editing protein.

By "repeat sequence" is meant an amino-acid sequence that is present in more than one copy in a protein, to within at least about 10% homology, by way of non-limiting example, a monomer repeat of a transcription activator-like effector.

By "DNA-binding domain" is meant a region of a molecule that is capable of binding to a DNA molecule, by way of non-limiting example, a protein domain comprising one or more zinc fingers, a protein domain comprising one or more transcription activator-like (TAL) effector repeat sequences or a binding pocket of a small molecule that is capable of binding to a DNA molecule.

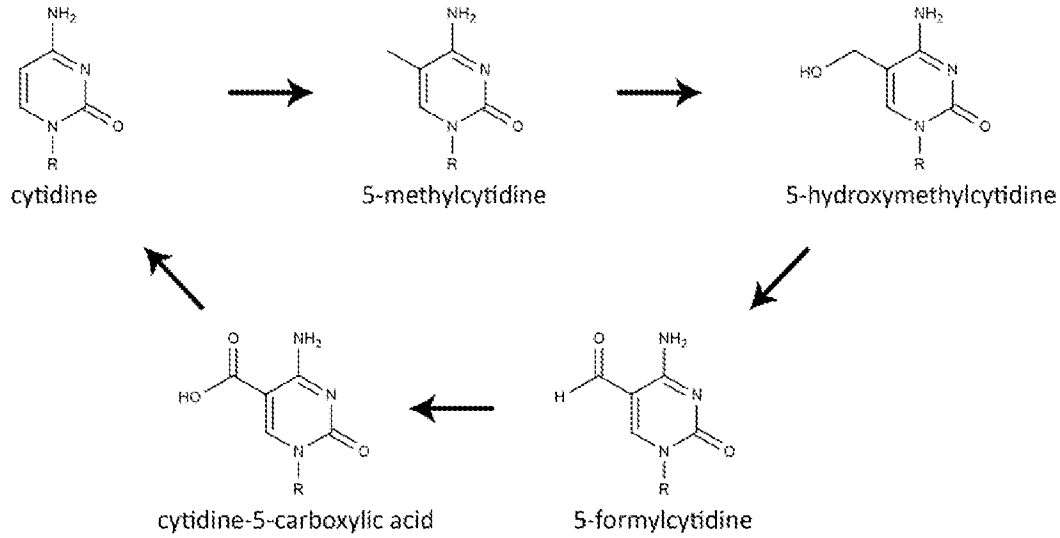
By "binding site" is meant a nucleic-acid sequence that is capable of being recognized by a gene-editing protein, DNA-binding protein, DNA-binding domain or a biologically active fragment or variant thereof or a nucleic-acid sequence for which a gene-editing protein, DNA-binding protein, DNA-binding domain or a biologically active fragment or variant thereof has high affinity, by way of non-limiting example, an about 20-base-pair sequence of DNA in exon 1 of the human BIRC5 gene.

By "target" is meant a nucleic acid that contains a binding site.

Other definitions are set forth in U.S. Application No. 13/465,490, U.S. Provisional Application No. 61/664,494, U.S. Provisional Application No. 61/721,302, International Application No. PCT/US12/67966, U.S. Provisional Application No. 61/785,404, and U.S. Provisional Application No. 61/842,874, the contents of which are hereby incorporated by reference in their entireties.

It has now been discovered that the non-canonical nucleotide members of the 5-methylcytidine de-methylation pathway, when incorporated into synthetic RNA, can increase the efficiency with which the synthetic RNA can be translated into protein, and can decrease the toxicity of the synthetic RNA. These non-canonical nucleotides include, for example: 5-methylcytidine, 5-hydroxymethylcytidine, 5-formylcytidine, and 5-carboxycytidine (a.k.a. "cytidine-5-carboxylic acid"). Certain embodiments are therefore directed to a nucleic acid. In one embodiment, the nucleic acid is a synthetic RNA molecule. In another embodiment, the nucleic acid comprises one or more non-canonical nucleotides. In one embodiment, the nucleic acid comprises one or more non-canonical nucleotide members of the 5-methylcytidine de-methylation pathway. In another embodiment, the nucleic acid comprises at least one of: 5-methylcytidine, 5-hydroxymethylcytidine, 5-formylcytidine, and 5-carboxycytidine or a derivative thereof. In a further embodiment, the nucleic acid comprises at least one of: pseudouridine, 5-methylpseudouridine, 5-methyluridine, 5-methylcytidine, 5-hydroxymethylcytidine, N4-methylcytidine, N4-acetylcytidine, and 7-deazaguanosine or a derivative thereof.

5-methylcytidine De-Methylation Pathway



Certain embodiments are directed to a protein. Other embodiments are directed to a nucleic acid that encodes a protein. In one embodiment, the protein is a protein of interest. In another embodiment, the protein is selected from: a reprogramming protein and a gene-editing protein. In one embodiment, the nucleic acid is a plasmid. In another embodiment, the nucleic acid is present in a virus or viral vector. In a further embodiment, the virus or viral vector is replication incompetent. In a still further embodiment, the virus or viral vector is replication competent. In one embodiment, the virus or viral vector includes at least one of: an adenovirus, a retrovirus, a lentivirus, a herpes virus, an adeno-associated virus or a natural or engineered variant thereof, and an engineered virus.

It has also been discovered that certain combinations of non-canonical nucleotides can be particularly effective at increasing the efficiency with which synthetic RNA can be translated into protein, and decreasing the toxicity of synthetic RNA, for example, the combinations: 5-methyluridine and 5-methylcytidine, 5-methyluridine and 7-deazaguanosine, 5-methylcytidine and 7-deazaguanosine, 5-methyluridine, 5-methylcytidine, and 7-deazaguanosine, and 5-methyluridine, 5-hydroxymethylcytidine, and 7-deazaguanosine. Certain embodiments are therefore directed to a nucleic acid comprising at least two of: 5-methyluridine, 5-methylcytidine, 5-hydroxymethylcytidine, and 7-deazaguanosine or one or more derivatives thereof. Other embodiments are directed to a nucleic acid comprising at least three of: 5-methyluridine, 5-methylcytidine, 5-hydroxymethylcytidine, and 7-deazaguanosine or one or more derivatives thereof. Other embodiments are directed to a nucleic acid comprising all of: 5-methyluridine, 5-methylcytidine, 5-hydroxymethylcytidine, and 7-deazaguanosine or one or more derivatives thereof. In one embodiment, the nucleic acid comprises one or more 5-methyluridine residues, one or more 5-methylcytidine residues, and one or more 7-deazaguanosine residues or one or more 5-methyluridine residues, one or more 5-hydroxymethylcytidine residues, and one or more 7-deazaguanosine residues.

It has been further discovered that synthetic RNA molecules containing certain fractions of certain non-canonical nucleotides and combinations thereof can exhibit particularly high translation efficiency and low toxicity. Certain embodiments are therefore directed to a nucleic acid comprising at least one of: one or more uridine residues, one or more cytidine residues, and one or more guanosine residues, and comprising one or more non-canonical nucleotides. In one embodiment, between about 20% and about 80% of the uridine residues are 5-methyluridine residues. In another embodiment, between about 30% and about 50% of the uridine residues are 5-methyluridine residues. In a further embodiment, about 40% of the uridine residues are 5-methyluridine residues. In one embodiment, between about 60% and about 80% of the cytidine residues are 5-methylcytidine residues. In another embodiment, between about 80% and about 100% of the cytidine residues are 5-methylcytidine residues. In a further embodiment, about 100% of the cytidine residues are 5-methylcytidine residues. In a still further embodiment, between about 20% and about 100% of the cytidine residues are 5-hydroxymethylcytidine residues. In one embodiment, between about 20% and about 80% of the guanosine residues are 7-deazaguanosine residues. In another embodiment, between about 40% and about 60% of the guanosine residues are 7-deazaguanosine residues. In a further embodiment, about 50% of the guanosine residues are 7-deazaguanosine residues. In one embodiment, between about 20% and about 80% or between about 30% and about 60% or about 40% of the cytidine residues are N4-methylcytidine and/or N4-acetylcytidine residues. In another embodiment, each cytidine residue is a 5-methylcytidine residue. In a further embodiment, about 100% of the cytidine residues are 5-methylcytidine residues and/or 5-hydroxymethylcytidine residues and/or N4-methylcytidine residues and/or N4-acetylcytidine residues and/or one or more derivatives thereof. In a still further embodiment, about 40% of the uridine residues are 5-methyluridine residues, between about 20% and about 100% of the cytidine residues are N4-methylcytidine and/or N4-acetylcytidine residues, and about 50% of the guanosine residues are 7-deazaguanosine residues. In one embodiment, about 40% of the uridine residues are 5-methyluridine residues and about 100% of the cytidine residues are 5-methylcytidine residues. In another embodiment, about 40% of the uridine residues are 5-methyluridine residues and about 50% of the guanosine residues are 7-deazaguanosine residues. In a further embodiment, about 100% of the cytidine residues are 5-methylcytidine residues and about 50% of the guanosine residues are 7-deazaguanosine residues. In one embodiment, about 40% of the uridine residues are 5-methyluridine residues, about 100% of the cytidine residues are 5-methylcytidine residues, and about 50% of the guanosine residues are 7-deazaguanosine residues. In another embodiment, about 40% of the uridine residues are 5-methyluridine residues, between about 20% and about 100% of the cytidine residues are 5-hydroxymethylcytidine residues, and about 50% of the guanosine residues are 7-deazaguanosine residues. In some embodiments, less than 100% of the cytidine residues are 5-methylcytidine residues. In other embodiments, less than 100% of the cytidine residues are 5-hydroxymethylcytidine residues. In one embodiment, each uridine residue in the

synthetic RNA molecule is a pseudouridine residue or a 5-methylpseudouridine residue. In another embodiment, about 100% of the uridine residues are pseudouridine residues and/or 5-methylpseudouridine residues. In a further embodiment, about 100% of the uridine residues are pseudouridine residues and/or 5-methylpseudouridine residues, about 100% of the cytidine residues are 5-methylcytidine residues, and about 50% of the guanosine residues are 7-deazaguanosine residues.

Other non-canonical nucleotides that can be used in place of or in combination with 5-methyluridine include, but are not limited to: pseudouridine and 5-methylpseudouridine (a.k.a. "1-methylpseudouridine", a.k.a. "N1-methylpseudouridine") or one or more derivatives thereof. Other non-canonical nucleotides that can be used in place of or in combination with 5-methylcytidine and/or 5-hydroxymethylcytidine include, but are not limited to: pseudoisocytidine, 5-methylpseudoisocytidine, 5-hydroxymethylcytidine, 5-formylcytidine, 5-carboxycytidine, N4-methylcytidine, N4-acetylcytidine or one or more derivatives thereof. In certain embodiments, for example, when performing only a single transfection or when the cells being transfected are not particularly sensitive to transfection-associated toxicity or innate-immune signaling, the fractions of non-canonical nucleotides can be reduced. Reducing the fraction of non-canonical nucleotides can be beneficial, in part, because reducing the fraction of non-canonical nucleotides can reduce the cost of the nucleic acid. In certain situations, for example, when minimal immunogenicity of the nucleic acid is desired, the fractions of non-canonical nucleotides can be increased.

Enzymes such as T7 RNA polymerase may preferentially incorporate canonical nucleotides in an *in vitro*-transcription reaction containing both canonical and non-canonical nucleotides. As a result, an *in vitro*-transcription reaction containing a certain fraction of a non-canonical nucleotide may yield RNA containing a different, often lower, fraction of the non-canonical nucleotide than the fraction at which the non-canonical nucleotide was present in the reaction. In certain embodiments, references to nucleotide incorporation fractions (for example, "50% 5-methyluridine") therefore can refer both to nucleic acids containing the stated fraction of the nucleotide, and to nucleic acids synthesized in a reaction containing the stated fraction of the nucleotide (or nucleotide derivative, for example, nucleotide-triphosphate), even though such a reaction may yield a nucleic acid containing a different fraction of the nucleotide than the fraction at which the non-canonical nucleotide was present in the reaction. In addition, different nucleotide sequences can encode the same protein by utilizing alternative codons. In certain embodiments, references to nucleotide incorporation fractions therefore can refer both to nucleic acids containing the stated fraction of the nucleotide, and to nucleic acids encoding the same protein as a different nucleic acid, wherein the different nucleic acid contains the stated fraction of the nucleotide.

The DNA sequence of a cell can be altered by contacting the cell with a gene-editing protein or by inducing the cell to express a gene-editing protein. However, previously disclosed gene-editing proteins suffer from low binding efficiency and excessive off-target activity, which can introduce undesired mutations in the DNA of the cell, severely limiting their use in therapeutic applications, in which the introduction of undesired mutations in a patient's cells could lead to the development of cancer. It has now been discovered that gene-editing proteins that comprise the StsI endonuclease cleavage domain (SEQ ID NO: 1) can exhibit substantially lower off-target activity than previously disclosed gene-editing proteins, while maintaining a high level of on-target activity. Other novel engineered proteins have also been discovered that can exhibit high on-target activity, low off-target activity, small size, solubility, and other desirable characteristics when they are used as the nuclease domain of a gene-editing protein: StsI-HA (SEQ ID NO: 2), StsI-HA2 (SEQ ID NO: 3), StsI-UHA (SEQ ID NO: 4), StsI-UHA2 (SEQ ID NO: 5), StsI-HF (SEQ ID NO: 6), and StsI-UHF (SEQ ID NO: 7). StsI-HA, StsI-HA2 (high activity), StsI-UHA, and StsI-UHA2 (ultra-high activity) can exhibit higher on-target activity than both wild-type StsI and wild-type FokI, due in part to specific amino-acid substitutions within the N-terminal region at the 34 and 61 positions, while StsI-HF (high fidelity) and StsI-UHF (ultra-high fidelity) can exhibit lower off-target activity than both wild-type StsI and wild-type FokI, due in part to specific amino-acid substitutions within the C-terminal region at the 141 and 152 positions. Certain embodiments are therefore directed to a protein that comprises a nuclease domain. In one embodiment, the nuclease domain comprises one or more of: the cleavage domain of FokI endonuclease (SEQ ID NO: 53), the cleavage domain of StsI endonuclease (SEQ ID NO: 1), StsI-HA (SEQ ID NO: 2), StsI-HA2 (SEQ ID NO: 3), StsI-UHA (SEQ ID NO: 4), StsI-UHA2 (SEQ ID NO: 5), StsI-HF (SEQ ID NO: 6), and StsI-UHF (SEQ ID NO: 7) or a biologically active fragment or variant thereof.

It has also been discovered that engineered gene-editing proteins that comprise DNA-binding domains comprising certain novel repeat sequences can exhibit lower off-target activity than previously disclosed gene-editing proteins, while maintaining a high level of on-target activity. Certain of these engineered gene-editing proteins can provide several advantages over previously disclosed gene-editing proteins, including, for example, increased flexibility of the linker region connecting repeat sequences, which can result in increased binding efficiency. Certain embodiments are therefore directed to a protein comprising a plurality of repeat sequences. In one embodiment, at least one of the repeat sequences contains the amino-acid sequence: GabG, where "a" and "b" each represent any amino acid. In one embodiment, the protein is a gene-editing protein. In another embodiment, one or more of the repeat sequences are present in a DNA-binding domain. In a further embodiment, "a" and "b" are each independently selected from the group: H and G. In a still further embodiment, "a" and "b" are H and G, respectively. In one embodiment, the amino-acid sequence is present within about 5 amino acids of the C-terminus of the repeat sequence. In another embodiment, the amino-acid

sequence is present at the C-terminus of the repeat sequence. In some embodiments, one or more G in the amino-acid sequence GabG is replaced with one or more amino acids other than G, for example A, H or GG. In one embodiment, the repeat sequence has a length of between about 32 and about 40 amino acids or between about 33 and about 39 amino acids or between about 34 and 38 amino acids or between about 35 and about 37 amino acids or about 36 amino acids or greater than about 32 amino acids or greater than about 33 amino acids or greater than about 34 amino acids or greater than about 35 amino acids. Other embodiments are directed to a protein comprising one or more transcription activator-like effector domains. In one embodiment, at least one of the transcription activator-like effector domains comprises a repeat sequence. Other embodiments are directed to a protein comprising a plurality of repeat sequences generated by inserting one or more amino acids between at least two of the repeat sequences of a transcription activator-like effector domain. In one embodiment, one or more amino acids is inserted about 1 or about 2 or about 3 or about 4 or about 5 amino acids from the C-terminus of at least one repeat sequence. Still other embodiments are directed to a protein comprising a plurality of repeat sequences, wherein about every other repeat sequence has a different length than the repeat sequence immediately preceding or following the repeat sequence. In one embodiment, every other repeat sequence is about 36 amino acids long. In another embodiment, every other repeat sequence is 36 amino acids long. Still other embodiments are directed to a protein comprising a plurality of repeat sequences, wherein the plurality of repeat sequences comprises at least two repeat sequences that are each at least 36 amino acids long, and wherein at least two of the repeat sequences that are at least 36 amino acids long are separated by at least one repeat sequence that is less than 36 amino acids long. Some embodiments are directed to a protein that comprises one or more sequences selected from, for example, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, and SEQ ID NO: 60.

Other embodiments are directed to a protein that comprises a DNA-binding domain. In some embodiments, the DNA-binding domain comprises a plurality of repeat sequences. In one embodiment, the plurality of repeat sequences enables high-specificity recognition of a binding site in a target DNA molecule. In another embodiment, at least two of the repeat sequences have at least about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 98%, or about 99% homology to each other. In a further embodiment, at least one of the repeat sequences comprises one or more regions capable of binding to a binding site in a target DNA molecule. In a still further embodiment, the binding site comprises a defined sequence of between about 1 to about 5 bases in length. In one embodiment, the DNA-binding domain comprises a zinc finger. In another embodiment, the DNA-binding domain comprises a transcription activator-like effector (TALE). In a further embodiment, the plurality of repeat sequences includes at least one repeat sequence having at least about 50% or about 60% or about 70% or about 80% or about 90% or about 95% or about 98%, or about 99% homology to a TALE. In a still further embodiment, the gene-editing protein comprises

a clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein. In one embodiment, the gene-editing protein comprises a nuclear-localization sequence. In another embodiment, the nuclear-localization sequence comprises the amino-acid sequence: PKKKRKV. In one embodiment, the gene-editing protein comprises a mitochondrial-localization sequence. In another embodiment, the mitochondrial-localization sequence comprises the amino-acid sequence: LGRVIPRKIASRASLM. In one embodiment, the gene-editing protein comprises a linker. In another embodiment, the linker connects a DNA-binding domain to a nuclease domain. In a further embodiment, the linker is between about 1 and about 10 amino acids long. In some embodiments, the linker is about 1, about 2, or about 3, or about 4, or about 5, or about 6, or about 7, or about 8, or about 9, or about 10 amino acids long. In one embodiment, the gene-editing protein is capable of generating a nick or a double-strand break in a target DNA molecule.

Certain embodiments are directed to a method for modifying the genome of a cell, the method comprising introducing into the cell a nucleic acid molecule encoding a non-naturally occurring fusion protein comprising an artificial transcription activator-like (TAL) effector repeat domain comprising one or more repeat units 36 amino acids in length and an endonuclease domain, wherein the repeat domain is engineered for recognition of a predetermined nucleotide sequence, and wherein the fusion protein recognizes the predetermined nucleotide sequence. In one embodiment, the cell is a eukaryotic cell. In another embodiment, the cell is an animal cell. In a further embodiment, the cell is a mammalian cell. In a still further embodiment, the cell is a human cell. In one embodiment, the cell is a plant cell. In another embodiment, the cell is a prokaryotic cell. In some embodiments, the fusion protein introduces an endonucleolytic cleavage in a nucleic acid of the cell, whereby the genome of the cell is modified.

Other embodiments are directed to a nucleic acid molecule encoding a non-naturally occurring fusion protein comprising an artificial transcription activator-like (TAL) effector repeat domain comprising one or more repeat units 36 amino acids in length and restriction endonuclease activity, wherein the repeat domain is engineered for recognition of a predetermined nucleotide sequence and wherein the fusion protein recognizes the predetermined nucleotide sequence. In one embodiment, the repeat units differ by no more than about seven amino acids. In another embodiment, each of the repeat units contains the amino acid sequence: LTPXQVVAIAS where X can be either E or Q, and the amino acid sequence: LTPXQVVAIAS is followed on the carboxyl terminus by either one or two amino acids that determine recognition for one of adenine, cytosine, guanine or thymine. In one embodiment, the nucleic acid encodes about 1.5 to about 28.5 repeat units. In another embodiment, the nucleic acid encodes about 11.5, about 14.5, about 17.5 or about 18.5 repeat units. In a further embodiment, the predetermined nucleotide sequence is a promoter region. Some embodiments are directed to a vector comprising a nucleic acid molecule or sequence. In one embodiment, the vector is a viral vector. In another embodiment, the viral vector comprises one or more of: an adenovirus, a retrovirus, a

lentivirus, a herpes virus, an adeno-associated virus or a natural or engineered variant thereof, and an engineered virus.

Certain embodiments are directed to a nucleic acid molecule encoding a non-naturally occurring fusion protein comprising a first region that recognizes a predetermined nucleotide sequence and a second region with endonuclease activity, wherein the first region contains an artificial TAL effector repeat domain comprising one or more repeat units about 36 amino acids in length which differ from each other by no more than seven amino acids, and wherein the repeat domain is engineered for recognition of the predetermined nucleotide sequence. In one embodiment, the first region contains the amino acid sequence: LTPXQVVAIAS where X can be either E or Q. In another embodiment, the amino acid sequence LTPXQVVAIAS of the encoded non-naturally occurring fusion protein is immediately followed by an amino acid sequence selected from: HD, NG, NS, NI, NN, and N. In a further embodiment, the fusion protein comprises restriction endonuclease activity. Some embodiments are directed to a nucleic acid molecule encoding a protein that comprises one or more sequences selected from: SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60.

In one embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzHG, wherein "v" is D or E, "w" is S or N, "x" is N, H or I, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQDHG, GGKQALETVQRLLPVLCQAHG, GKQALETVQRLLPVLCQDHG or GKQALETVQRLLPVLCQAHG. In another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzHG, wherein "v" is D or E, "w" is S or N, "x" is N, H or I, "y" is selected from: D, A, I, N, H, K, S, and G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQDHG, GGKQALETVQRLLPVLCQAHG, GKQALETVQRLLPVLCQDHG or GKQALETVQRLLPVLCQAHG. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzHG, wherein "v" is D or E, "w" is S or N, "x" is any amino acid other than N, H and I, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQDHG, GGKQALETVQRLLPVLCQAHG, GKQALETVQRLLPVLCQDHG or GKQALETVQRLLPVLCQAHG. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwIyzHG, wherein "v" is D or E, "w" is S or N, "y" is any amino acid other than G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQDHG, GGKQALETVQRLLPVLCQAHG, GKQALETVQRLLPVLCQDHG or GKQALETVQRLLPVLCQAHG. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwIAzHG, wherein "v" is D or E, "w" is S or N, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQDHG, GGKQALETVQRLLPVLCQAHG, GKQALETVQRLLPVLCQDHG or GKQALETVQRLLPVLCQAHG. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzHG, wherein "v" is D or E, "w" is S or N, "x" is

S, T or Q, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQDHG, GGKQALETVQRLLPVLCQAHG, GKQALETVQRLLPVLCQDHG or GKQALETVQRLLPVLCQAHG. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzHG, wherein "v" is D or E, "w" is S or N, "x" is S, T or Q, "y" is selected from: D, A, I, N, H, K, S, and G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQDHG, GGKQALETVQRLLPVLCQAHG, GKQALETVQRLLPVLCQDHG or GKQALETVQRLLPVLCQAHG. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwx, wherein "v" is D or E, "w" is S or N, and "x" is S, T or Q. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxy, wherein "v" is D or E, "w" is S or N, "x" is S, T or Q, and "y" is selected from: D, A, I, N, H, K, S, and G. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is N, H or I, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is N, H or I, "y" is selected from: D, A, I, N, H, K, S, and G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is any amino acid other than N, H and I, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwIyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "y" is any amino acid other than G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwIAzGHGG, wherein "v" is Q, D or E, "w" is S or N, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is S, T or Q, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is S, T or Q, "y" is selected from: D, A, I, N, H, K, S, and G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA. In yet another embodiment, the repeat sequence comprises:

LTPvQVVAIAwx, wherein "v" is Q, D or E, "w" is S or N, and "x" is S, T or Q. In yet another embodiment, the repeat sequence comprises: LTPvQVVAIAwxy, wherein "v" is Q, D or E, "w" is S or N, "x" is S, T or Q, and "y" is selected from: D, A, I, N, H, K, S, and G.

Certain fragments of an endonuclease cleavage domain, including fragments that are truncated at the N-terminus, fragments that are truncated at the C-terminus, fragments that have internal deletions, and fragments that combine N-terminus, C-terminus, and/or internal deletions, can maintain part or all of the catalytic activity of the full endonuclease cleavage domain. Determining whether a fragment can maintain part or all of the catalytic activity of the full domain can be accomplished by, for example, synthesizing a gene-editing protein that contains the fragment according to the methods of the present invention, inducing cells to express the gene-editing protein according to the methods of the present invention, and measuring the efficiency of gene editing. In this way, a measurement of gene-editing efficiency can be used to ascertain whether any specific fragment can maintain part or all of the catalytic activity of the full endonuclease cleavage domain. Certain embodiments are therefore directed to a biologically active fragment of an endonuclease cleavage domain. In one embodiment, the endonuclease cleavage domain is selected from: FokI, StsI, StsI-HA, StsI-HA2, StsI-UHA, StsI-UHA2, StsI-HF, and StsI-UHF or a natural or engineered variant or biologically active fragment thereof.

Certain fragments of a DNA-binding domain or repeat sequence, including fragments that are truncated at the N-terminus, fragments that are truncated at the C-terminus, fragments that have internal deletions, and fragments that combine N-terminus, C-terminus, and/or internal deletions, can maintain part or all of the binding activity of the full DNA-binding domain or repeat sequence. Examples of fragments of DNA-binding domains or repeat sequences that can maintain part or all of the binding activity of the full repeat sequence include *Ralstonia solanacearum* TALE-like proteins (RTLs). Determining whether a fragment can maintain part or all of the binding activity of the full DNA-binding domain or repeat sequence can be accomplished by, for example, synthesizing a gene-editing protein that contains the fragment according to the methods of the present invention, inducing cells to express the gene-editing protein according to the methods of the present invention, and measuring the efficiency of gene editing. In this way, a measurement of gene-editing efficiency can be used to ascertain whether any specific fragment can maintain part or all of the binding activity of the full DNA-binding domain or repeat sequence. Certain embodiments are therefore directed to a biologically active fragment of a DNA-binding domain or repeat sequence. In one embodiment, the fragment enables high-specificity recognition of a binding site in a target DNA molecule. In another embodiment, the fragment comprises a sequence that encodes a *Ralstonia solanacearum* TALE-like protein or a biologically active fragment thereof.

Certain embodiments are directed to a composition for altering the DNA sequence of a cell comprising a nucleic acid, wherein the nucleic acid encodes a gene-editing protein. Other embodiments are directed to a composition for altering the DNA sequence of a cell comprising a nucleic-acid mixture, wherein the nucleic-acid mixture comprises: a first nucleic acid that encodes a first gene-editing protein, and a second nucleic acid that encodes a second gene-editing protein. In one embodiment, the binding site of the first gene-editing protein and the binding site of the second gene-editing protein are present in the same target DNA molecule. In another embodiment, the binding site of the first gene-editing protein and the binding site of the second gene-editing protein are separated by less than about 50 bases, or less than about 40 bases, or less than about 30 bases or less than about 20 bases, or less than about 10 bases, or between about 10 bases and about 25 bases or about 15 bases. In one embodiment, the nuclease domain of the first gene-editing protein and the nuclease domain of the second gene-editing protein are capable of forming a dimer. In another embodiment, the dimer is capable of generating a nick or double-strand break in a target DNA molecule. In one embodiment, the composition is a therapeutic composition. In another embodiment, the composition comprises a repair template. In a further embodiment, the repair template is a single-stranded DNA molecule or a double-stranded DNA molecule.

Other embodiments are directed to an article of manufacture for synthesizing a protein or a nucleic acid encoding a protein. In one embodiment, the article is a nucleic acid. In another embodiment, the protein comprises a DNA-binding domain. In a further embodiment, the nucleic acid comprises a nucleotide sequence encoding a DNA-binding domain. In one embodiment, the protein comprises a nuclease domain. In another embodiment, the nucleic acid comprises a nucleotide sequence encoding a nuclease domain. In one embodiment, the protein comprises a plurality of repeat sequences. In another embodiment, the nucleic acid encodes a plurality of repeat sequences. In a further embodiment, the nuclease domain is selected from: FokI, StsI, StsI-HA, StsI-HA2, StsI-UHA, StsI-UHA2, StsI-HF, and StsI-UHF or a natural or engineered variant or biologically active fragment thereof. In one embodiment, the nucleic acid comprises an RNA-polymerase promoter. In another embodiment, the RNA-polymerase promoter is a T7 promoter or a SP6 promoter. In a further embodiment, the nucleic acid comprises a viral promoter. In one embodiment, the nucleic acid comprises an untranslated region. In another embodiment, the nucleic acid is an *in vitro*-transcription template.

Certain embodiments are directed to a method for inducing a cell to express a protein. Other embodiments are directed to a method for altering the DNA sequence of a cell comprising transfecting the cell with a gene-editing protein or inducing the cell to express a gene-editing protein. Still other embodiments are directed to a method for reducing the expression of a protein of interest in a cell. In one embodiment, the cell is induced to express a gene-editing protein, wherein the gene-editing protein is capable of creating a nick or a double-strand break in a target DNA molecule. In another

embodiment, the nick or double-strand break results in inactivation of a gene. Still other embodiments are directed to a method for generating an inactive, reduced-activity or dominant-negative form of a protein. In one embodiment, the protein is survivin. Still other embodiments are directed to a method for repairing one or more mutations in a cell. In one embodiment, the cell is contacted with a repair template. In another embodiment, the repair template is a DNA molecule. In a further embodiment, the repair template does not contain a binding site of the gene-editing protein. In a still further embodiment, the repair template encodes an amino-acid sequence that is encoded by a DNA sequence that comprises a binding site of the gene-editing protein.

Other embodiments are directed to a method for treating a patient comprising administering to the patient a therapeutically effective amount of a protein or a nucleic acid encoding a protein. In one embodiment, the treatment results in one or more of the patient's symptoms being ameliorated. Certain embodiments are directed to a method for treating a patient comprising: a. removing a cell from the patient, b. inducing the cell to express a gene-editing protein by transfecting the cell with a nucleic acid encoding a gene-editing protein, c. reprogramming the cell, and e. introducing the cell into the patient. In one embodiment, the cell is reprogrammed to a less differentiated state. In another embodiment, the cell is reprogrammed by transfecting the cell with one or more synthetic RNA molecules encoding one or more reprogramming proteins. In a further embodiment, the cell is differentiated. In a still further embodiment, the cell is differentiated into one of: a skin cell, a glucose-responsive insulin-producing cell, a hematopoietic cell, a cardiac cell, a retinal cell, a renal cell, a neural cell, a stromal cell, a fat cell, a bone cell, a muscle cell, an oocyte, and a sperm cell. Other embodiments are directed to a method for treating a patient comprising: a. removing a hematopoietic cell or a stem cell from the patient, b. inducing the cell to express a gene-editing protein by transfecting the cell with a nucleic acid encoding a gene-editing protein, and c. introducing the cell into the patient.

It has now been discovered that a cell-culture medium consisting essentially of or comprising: DMEM/F12, ascorbic acid, insulin, transferrin, sodium selenite, ethanolamine, basic fibroblast growth factor, and transforming growth factor-beta is sufficient to sustain pluripotent stem cells, including human pluripotent stem cells, *in vitro*. Certain embodiments are therefore directed to a cell-culture medium consisting essentially of or comprising: DMEM/F12, ascorbic acid, insulin, transferrin, sodium selenite, ethanolamine, basic fibroblast growth factor, and transforming growth factor-beta. In one embodiment, the ascorbic acid is present at about 50μg/mL. In another embodiment, the insulin is present at about 10μg/mL. In a further embodiment, the transferrin is present at about 5.5μg/mL. In a still further embodiment, the sodium selenite is present at about 6.7ng/mL. In a still further embodiment, the ethanolamine is present at about 2μg/mL. In a still further embodiment, the basic fibroblast growth factor is present at about 20ng/mL. In a still further embodiment, the transforming growth factor-beta is present at about 2ng/mL. In one embodiment, the ascorbic acid is ascorbic acid-

2-phosphate. In another embodiment, the transforming growth factor-beta is transforming growth factor-beta 1 or transforming growth factor-beta 3. In one embodiment, the cell-culture medium is used for the culture of pluripotent stem cells. In another embodiment, the pluripotent stem cells are human pluripotent stem cells. In a further embodiment, the cell-culture medium is used for the culture of cells during or after reprogramming. In one embodiment, the cell-culture medium contains no animal-derived components. In another embodiment, the cell-culture medium is manufactured according to a manufacturing standard. In a further embodiment, the manufacturing standard is GMP. In one embodiment, the cells are contacted with a cell-adhesion molecule. In another embodiment, the cell-adhesion molecule is selected from: fibronectin and vitronectin or a biologically active fragment thereof. In a further embodiment, the cells are contacted with fibronectin and vitronectin. In a still further embodiment, the cell-adhesion molecule is recombinant.

In certain situations, for example, when producing a therapeutic, it can be beneficial to replace animal-derived components with non-animal-derived components, in part to reduce the risk of contamination with viruses and/or other animal-borne pathogens. It has now been discovered that synthetic cholesterol, including semi-synthetic plant-derived cholesterol, can be substituted for animal-derived cholesterol in transfection medium without decreasing transfection efficiency or increasing transfection-associated toxicity. Certain embodiments are therefore directed to a transfection medium containing synthetic or semi-synthetic cholesterol. In one embodiment, the semi-synthetic cholesterol is plant-derived. In another embodiment, the transfection medium does not contain animal-derived cholesterol. In a further embodiment, the transfection medium is a reprogramming medium. Other embodiments are directed to a complexation medium. In one embodiment, the complexation medium has a pH greater than about 7, or greater than about 7.2, or greater than about 7.4, or greater than about 7.6, or greater than about 7.8, or greater than about 8.0, or greater than about 8.2, or greater than about 8.4, or greater than about 8.6, or greater than about 8.8, or greater than about 9.0. In another embodiment, the complexation medium comprises transferrin. In a further embodiment, the complexation medium comprises DMEM. In a still further embodiment, the complexation medium comprises DMEM/F12. Still other embodiments are directed to a method for forming nucleic-acid-transfection-reagent complexes. In one embodiment, the transfection reagent is incubated with a complexation medium. In another embodiment, the incubation occurs before a mixing step. In a further embodiment, the incubation step is between about 5 seconds and about 5 minutes or between about 10 seconds and about 2 minutes or between about 15 seconds and about 1 minute or between about 30 seconds and about 45 seconds. In one embodiment, the transfection reagent is selected from Table 1. In another embodiment, the transfection reagent is a lipid or lipidoid. In a further embodiment, the transfection reagent comprises a cation. In a still further embodiment, the cation is a multivalent cation. In a still further embodiment, the transfection reagent is N1-[2-

((1S)-1-[(3-aminopropyl)amino]-4-[di(3-amino-propyl)amino]butylcarboxamido)ethyl]-3,4-di[oleyloxy]-benzamide (a.k.a. MVL5) or a derivative thereof.

Certain embodiments are directed to a method for inducing a cell to express a protein by contacting the cell with a nucleic acid. In one embodiment, the cell is a mammalian cell. In another embodiment, the cell is a human cell or a rodent cell. Other embodiments are directed to a cell produced using one or more of the methods of the present invention. In one embodiment, the cell is present in a patient. In another embodiment, the cell is isolated from a patient. Other embodiments are directed to a screening library comprising a cell produced using one or more of the methods of the present invention. In one embodiment, the screening library is used for at least one of: toxicity screening, including: cardiotoxicity screening, neurotoxicity screening, and hepatotoxicity screening, efficacy screening, high-throughput screening, high-content screening, and other screening.

Other embodiments are directed to a kit containing a nucleic acid. In one embodiment, the kit contains a delivery reagent (a.k.a. "transfection reagent"). In another embodiment, the kit is a reprogramming kit. In a further embodiment, the kit is a gene-editing kit. Other embodiments are directed to a kit for producing nucleic acids. In one embodiment, the kit contains at least two of: pseudouridine-triphosphate, 5-methyluridine triphosphate, 5-methylcytidine triphosphate, 5-hydroxymethylcytidine triphosphate, N4-methylcytidine triphosphate, N4-acetylcytidine triphosphate, and 7-deazaguanosine triphosphate or one or more derivatives thereof. Other embodiments are directed to a therapeutic comprising a nucleic acid. In one embodiment, the therapeutic is a pharmaceutical composition. In another embodiment, the pharmaceutical composition is formulated. In a further embodiment, the formulation comprises an aqueous suspension of liposomes. Example liposome components are set forth in Table 1, and are given by way of example, and not by way of limitation. In one embodiment, the liposomes include one or more polyethylene glycol (PEG) chains. In another embodiment, the PEG is PEG2000. In a further embodiment, the liposomes include 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) or a derivative thereof. In one embodiment, the therapeutic comprises one or more ligands. In another embodiment, the therapeutic comprises at least one of: androgen, CD30 (TNFRSF8), a cell-penetrating peptide, CXCR, estrogen, epidermal growth factor, EGFR, HER2, folate, insulin, insulin-like growth factor-I, interleukin-13, integrin, progesterone, stromal-derived-factor-1, thrombin, vitamin D, and transferrin or a biologically active fragment or variant thereof. Still other embodiments are directed to a therapeutic comprising a cell generated using one or more of the methods of the present invention. In one embodiment, the therapeutic is administered to a patient for the treatment of at least one of: type 1 diabetes, heart disease, including ischemic and dilated cardiomyopathy, macular degeneration, Parkinson's disease, cystic fibrosis, sickle-cell anemia, thalassemia, Fanconi anemia, severe combined immunodeficiency, hereditary sensory neuropathy, xeroderma pigmentosum, Huntington's disease, muscular dystrophy, amyotrophic lateral sclerosis, Alzheimer's disease, cancer, and infectious diseases including: hepatitis and HIV/AIDS.

Table 1. Exemplary Biocompatible Lipids

1	3β -[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Cholesterol)
2	1,2-dioleoyl-3-trimethylammonium-propane (DOTAP / 18:1 TAP)
3	N-(4-carboxybenzyl)-N,N-dimethyl-2,3-bis(oleoyloxy)propan-1-aminium (DOBAQ)
4	1,2-dimyristoyl-3-trimethylammonium-propane (14:0 TAP)
5	1,2-dipalmitoyl-3-trimethylammonium-propane (16:0 TAP)
6	1,2-stearoyl-3-trimethylammonium-propane (18:0 TAP)
7	1,2-dioleoyl-3-dimethylammonium-propane (DODAP / 18:1 DAP)
8	1,2-dimyristoyl-3-dimethylammonium-propane (14:0 DAP)
9	1,2-dipalmitoyl-3-dimethylammonium-propane (16:0 DAP)
10	1,2-distearoyl-3-dimethylammonium-propane (18:0 DAP)
11	dimethyldioctadecylammonium (18:0 DDAB)
12	1,2-dilauroyl-sn-glycero-3-ethylphosphocholine (12:0 EthylPC)
13	1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine (14:0 EthylPC)
14	1,2-dimyristoleoyl-sn-glycero-3-ethylphosphocholine (14:1 EthylPC)
15	1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (16:0 EthylPC)
16	1,2-distearoyl-sn-glycero-3-ethylphosphocholine (18:0 EthylPC)
17	1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (18:1 EthylPC)
18	1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:1-18:1 EthylPC)
19	1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA)
20	N1-[2-((1S)-1-[(3-aminopropyl)amino]-4-[di(3-amino-propyl)amino]butylcarboxamido)ethyl]-3,4-di[oleyloxy]-benzamide (MVL5)
21	2,3-dioleyloxy-N-[2-spermine carboxamide]ethyl-N,N-dimethyl-1-propanammonium trifluoroacetate (DOSPA)
22	1,3-di-oleoyloxy-2-(6-carboxy-spermyl)-propylamid (DOSPER)
23	N-[1-(2,3-dimyristyloxy)propyl]-N,N-dimethyl-N-(2-hydroxyethyl)ammonium bromide (DMRIE)
24	dioctadecyl amidoglyceryl spermine (DOGS)
25	dioleoyl phosphatidyl ethanolamine (DOPE)

Certain embodiments are directed to a nucleic acid comprising a 5′-cap structure selected from Cap 0, Cap 1, Cap 2, and Cap 3 or a derivative thereof. In one embodiment, the nucleic acid comprises one or more UTRs. In another embodiment, the one or more UTRs increase the stability of the nucleic acid. In a further embodiment, the one or more UTRs comprise an alpha-globin or beta-globin 5′-UTR. In a still further embodiment, the one or more UTRs comprise an alpha-globin or beta-globin 3′-UTR. In a still further embodiment, the synthetic RNA molecule comprises an alpha-globin or beta-globin 5′-UTR and an alpha-globin or beta-globin 3′-UTR. In one embodiment, the 5′-UTR comprises a Kozak sequence that is substantially similar to the Kozak consensus sequence. In another embodiment, the nucleic acid comprises a 3′-poly(A) tail. In a further embodiment, the 3′-poly(A) tail is between about 20nt and about 250nt or between about 120nt and about 150nt long. In a further embodiment, the 3′-

poly(A) tail is about 20nt, or about 30nt, or about 40nt, or about 50nt, or about 60nt, or about 70nt, or about 80nt, or about 90nt, or about 100nt, or about 110nt, or about 120nt, or about 130nt, or about 140nt, or about 150nt, or about 160nt, or about 170nt, or about 180nt, or about 190nt, or about 200nt, or about 210nt, or about 220nt, or about 230nt, or about 240nt, or about 250nt long.

Other embodiments are directed to a method for reprogramming a cell. In one embodiment, the cell is reprogrammed by contacting the cell with one or more nucleic acids. In one embodiment, the cell is contacted with a plurality of nucleic acids encoding at least one of: Oct4 protein, Sox2 protein, Klf4 protein, c-Myc protein, Lin28 protein or a biologically active fragment, variant or derivative thereof. In another embodiment, the cell is contacted with a plurality of nucleic acids encoding a plurality of proteins including: Oct4 protein, Sox2 protein, Klf4 protein, and c-Myc protein or one or more biologically active fragments, variants or derivatives thereof. Still other embodiments are directed to a method for gene editing a cell. In one embodiment, the cell is gene-edited by contacting the cell with one or more nucleic acids.

Animal models are routinely used to study the effects of biological processes. In certain situations, for example, when studying a human disease, an animal model containing a modified genome can be beneficial, in part because such an animal model may more closely mimic the human disease phenotype. Certain embodiments are therefore directed to a method for creating an organism containing one or more genetic modifications (a.k.a. "mutations", a.k.a. "gene edits"). In one embodiment, the one or more genetic modifications is generated by transfecting a cell with one or more nucleic acids encoding one or more gene-editing proteins. In another embodiment, the one or more nucleic acids include a synthetic RNA molecule. In one embodiment, the one or more gene-editing proteins include at least one of: a zinc finger nuclease, a TALEN, a clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein, a nuclease, a meganuclease, and a nickase or a biologically active fragment or variant thereof. In one embodiment, the cell is a pluripotent cell. In another embodiment, the cell is an embryonic stem cell. In a further embodiment, the cell is an embryo. In a still further embodiment, the cell is a member of: an animal cell, a plant cell, a yeast cell, and a bacterial cell. In one embodiment, the cell is a rodent cell. In another embodiment, the cell is a human cell. In certain embodiments, the cell is transfected with one or more nucleic acids encoding one or more gene-editing proteins and one or more nucleic acids encoding one or more repair templates. In one embodiment, the cell is introduced into a blastocyst. In another embodiment, the cell is introduced into a pseudopregnant female. In a further embodiment, the presence or absence of the genetic modification in the offspring is determined. In a still further embodiment, the determining is by direct sequencing. In one embodiment, the organism is livestock, for example, a pig, a cow, etc. In another embodiment, the organism is a pet, for example, a dog, a cat, a fish, etc.

In certain situations, for example, when modifying the genome of a target cell by the addition of a nucleic-acid sequence, it can be advantageous to insert the nucleic-acid sequence into a safe-harbor location, in part to reduce the risks associated with random insertion. Certain embodiments are therefore directed to a method for inserting a nucleic-acid sequence into a safe-harbor location. In one embodiment, the cell is a human cell and the safe-harbor location is the AAVS1 locus. In another embodiment, the cell is a rodent cell and the safe-harbor location is the Rosa26 locus. In one embodiment, the cell is further contacted with one or more nucleic acids encoding one or more repair templates. Other embodiments are directed to a kit for altering the DNA sequence of a cell. In one embodiment, the cell is a human cell, and the target DNA molecule comprises a nucleotide sequence that encodes the AAVS1 locus. In another embodiment, the cell is a rodent cell, and the target DNA molecule comprises a nucleotide sequence that encodes the Rosa26 locus. Other embodiments are directed to a method for generating a reporter cell by contacting the cell with one or more nucleic acids encoding one or more gene-editing proteins and one or more nucleic acids encoding one or more repair templates. In one embodiment, the one or more repair templates comprise DNA. In another embodiment, the one or more repair templates encode one or more fluorescent proteins. In a further embodiment, the one or more repair templates encode at least part of the promoter region of a gene.

In certain situations, for example, when generating a library of gene-edited cells, it can be beneficial to increase the efficiency of gene editing, in part to reduce the cost of cell characterization. It has now been discovered that gene-editing efficiency can be increased by repeatedly contacting a cell with synthetic RNA encoding one or more gene-editing proteins. Certain embodiments are therefore directed to a method for gene editing a cell by repeatedly contacting the cell with one or more nucleic acids encoding one or more gene-editing proteins. In one embodiment, the cell is contacted at least twice during five consecutive days. In another embodiment, the cell is contacted twice at an interval of between about 24 hours and about 48 hours.

In cancer, the survival and proliferation of malignant cells can be due in part to the presence of specific genetic abnormalities that are not generally present in the patient. It has now been discovered that gene-editing proteins can be used to target survival and proliferation-associated pathways, and that when used in this manner, gene-editing proteins and nucleic acids encoding gene-editing proteins can constitute potent anti-cancer therapeutics. Certain embodiments are therefore directed to an anti-cancer therapeutic. In one embodiment, the therapeutic is a therapeutic composition that inhibits the survival and/or prevents, slows or otherwise limits the proliferation of a cell. In another embodiment, the cell is a cancer cell. In a further embodiment, the therapeutic comprises one or more gene-editing proteins or a nucleic acid that encodes one or more gene-editing proteins. In a still further embodiment, the one or more gene-editing proteins target one or more sequences that promote survival and/or proliferation of the cell. Such sequences include, but are not limited to: apoptosis-related genes, including genes of the inhibitor of apoptosis (IAP) family (*See, e.g.*, Table 2 and Table

2 of U.S. Provisional Application No. 61/721,302, the contents of which are hereby incorporated by reference), such as BIRC5, sequences associated with telomere maintenance, such as the gene telomerase reverse transcriptase (TERT) and the telomerase RNA component (TERC), sequences affecting angiogenesis, such as the gene VEGF, and other cancer-associated genes, including: BRAF, BRCA1, BRCA2, CDKN2A, CTNNB1, EGFR, the MYC family, the RAS family, PIK3CA, PIK3R1, PKN3, TP53, PTEN, RET, SMAD4, KIT, MET, APC, RB1, the VEGF family, TNF, and genes of the ribonucleotide reductase family. Example gene-editing protein target sequences for BIRC5 are set forth in Table 3 and in Table 3 of U.S. Provisional Application No. 61/721,302, the contents of which are hereby incorporated by reference, and are given by way of example, and not by way of limitation. In one embodiment, at least one of the one or more sequences is present in both malignant and non-malignant cells. In another embodiment, at least one of the one or more sequences is enriched in malignant cells. In a further embodiment, at least one of the one or more sequences is enriched in non-malignant cells. In one embodiment, the therapeutic composition further comprises a nucleic acid encoding one or more repair templates. In another embodiment, the one or more gene-editing proteins induce the cells to express an inactive or dominant-negative form of a protein. In a further embodiment, the protein is a member of the IAP family. In a still further embodiment, the protein is survivin.

Table 2. Exemplary Inhibitor of Apoptosis (IAP) Genes

Name	Length/aa	BIR Domains	CARD Domain	RING Domain
BIRC1 (neuronal apoptosis-inhibitory protein)	1,403	3	N	N
BIRC2 (c-IAP1 protein)	604	3	Y	Y
BIRC3 (c-IAP2 protein)	618	3	Y	Y
BIRC4 (X-linked IAP)	497	3	N	Y
BIRC5 (survivin protein)	142	1	N	N
BIRC6 (BRUCE/apollon protein)	4845	1	N	N
BIRC7 (livin protein)	298	1	N	Y
ILP2 (tissue-specific homolog of BIRC4)	236	1	N	Y

Table 3. Exemplary Gene Editing-Protein Target Sequences for BIRC5

Target	Left	Right
UTR	TAAGAGGGCGTGCGCTCCCG	TCAAATCTGGCGGTTAATGG
Start Codon	TTGGCAGAGGTGGCGGCGGC	TGCCAGGCAGGGGGCAACGT
Exon 1	TTGCCCCCTGCCTGGCAGCC	TTCTTGAATGTAGAGATGCG
Exon 2	TCCACTGCCCCACTGAGAAC	TCCTTGAAGCAGAAGAAACA
Exon 4	TAAAAAGCATTCGTCCGGTT	TTCTTCAAACTGCTTCTTGA
Exon 5	TTGAGGAAACTGCGGAGAAA	TCCATGGCAGCCAGCTGCTC

Other embodiments are directed to a method for treating cancer comprising administering to a patient a therapeutically effective amount of a gene-editing protein or a nucleic acid encoding one or more gene-editing proteins. In one embodiment, the treatment results in the growth of cancer cells in the patient being reduced or halted. In another embodiment, the treatment results in delayed progression or remission of the cancer. In one embodiment, the target DNA molecule comprises the BIRC5 gene. In another embodiment, the target DNA molecule comprises a sequence selected from: SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15. In a further embodiment, a plurality of adjacent binding sites are at least about 50% or at least about 60% or at least about 70% or at least about 80% or at least about 90% or at least about 95% or at least about 98%, or at least about 99% homologous to one or more sequences listed in Table 3, Table 4, Table 3 of U.S. Provisional Application No. 61/721,302, the contents of which are hereby incorporated by reference, Table 1 of U.S. Provisional Application No. 61/785,404, the contents of which are hereby incorporated by reference or Table 1 of U.S. Provisional Application No. 61/842,874, the contents of which are hereby incorporated by reference. In certain situations, a gene-editing protein with a truncated N-terminal domain can be used to eliminate the first-base-T restriction on the binding-site sequence. In some embodiments, the cancer is glioma. In one embodiment, the patient has previously undergone surgery and/or radiation therapy and/or concurrently undergoes surgery and/or radiation therapy. In another embodiment, the administering is by one or more of: intrathecal injection, intracranial injection, intravenous injection, perfusion, subcutaneous injection, intraperitoneal injection, intraportal injection, and topical delivery.

Table 4. Exemplary BIRC5 Binding Sites

Gene	#	Left	Right	Spacing
BIRC5	1	TGGGTGCCCCGACGTTGCCC	TGCGGTGGTCCTTGAGAAAG	14
BIRC5	2	TGGGTGCCCCGACGTTGCCC	TAGAGATGCGGTGGTCCTTG	20
BIRC5	3	TGCCCCGACGTTGCCCCCTG	TAGAGATGCGGTGGTCCTTG	16
BIRC5	4	TGCCCCGACGTTGCCCCCTG	TGTAGAGATGCGGTGGTCCT	18
BIRC5	5	TCAAGGACCACCGCATCTCT	TGCAGGCGCAGCCCTCCAAG	20
BIRC5	6	TCTCTACATTCAAGAACTGG	TCACCCGCTCCGGGGTGCAG	20
BIRC5	7	TCTACATTCAAGAACTGGCC	TCACCCGCTCCGGGGTGCAG	18
BIRC5	8	TCTACATTCAAGAACTGGCC	TCTCACCCGCTCCGGGGTGC	20
BIRC5	9	TACATTCAAGAACTGGCCCT	TCACCCGCTCCGGGGTGCAG	16
BIRC5	10	TACATTCAAGAACTGGCCCT	TCTCACCCGCTCCGGGGTGC	18
BIRC5	11	TTCAAGAACTGGCCCTTCTT	TCTCACCCGCTCCGGGGTGC	14
BIRC5	1	TCCCTTGCAGATGGCCGAGG	TGGCTCGTTCTCAGTGGGGC	15
BIRC5	2	TCCCTTGCAGATGGCCGAGG	TCTGGCTCGTTCTCAGTGGG	17
BIRC5	3	TGGCCGAGGCTGGCTTCATC	TGGGCCAAGTCTGGCTCGTT	15
BIRC5	4	TCCACTGCCCCACTGAGAAC	TCCTTGAAGCAGAAGAAACA	18
BIRC5	5	TGCCCCACTGAGAACGAGCC	TCCAGCTCCTTGAAGCAGAA	19

BIRC5	6	TGCCCCACTGAGAACGAGCC	TTCCAGCTCCTTGAAGCAGA	20
BIRC5	7	TTGGCCCAGTGTTTCTTCTG	TCGTCATCTGGCTCCCAGCC	16
BIRC5	8	TGGCCCAGTGTTTCTTCTGC	TCGTCATCTGGCTCCCAGCC	15
BIRC5	9	TGGCCCAGTGTTTCTTCTGC	TGGGGTCGTCATCTGGCTCC	20
BIRC5	10	TGTTTCTTCTGCTTCAAGGA	TACATGGGGTCGTCATCTGG	16
BIRC5	11	TGTTTCTTCTGCTTCAAGGA	TTACATGGGGTCGTCATCTG	17
BIRC5	12	TTTCTTCTGCTTCAAGGAGC	TACATGGGGTCGTCATCTGG	14
BIRC5	13	TTTCTTCTGCTTCAAGGAGC	TTACATGGGGTCGTCATCTG	15
BIRC5	14	TTCTTCTGCTTCAAGGAGCT	TTACATGGGGTCGTCATCTG	14
BIRC5	1	TTTTCTAGAGAGGAACATAA	TGACAGAAAGGAAAGCGCAA	15
BIRC5	2	TTTTCTAGAGAGGAACATAA	TTGACAGAAAGGAAAGCGCA	16
BIRC5	3	TTTTCTAGAGAGGAACATAA	TCTTGACAGAAAGGAAAGCG	18
BIRC5	4	TAGAGAGGAACATAAAAAGC	TGCTTCTTGACAGAAAGGAA	17
BIRC5	5	TAAAAAGCATTCGTCCGGTT	TCTTCAAACTGCTTCTTGAC	14
BIRC5	6	TAAAAAGCATTCGTCCGGTT	TTCTTCAAACTGCTTCTTGA	15
BIRC5	7	TAAAAAGCATTCGTCCGGTT	TAATTCTTCAAACTGCTTCT	18
BIRC5	8	TAAAAAGCATTCGTCCGGTT	TTAATTCTTCAAACTGCTTC	19
BIRC5	9	TTCGTCCGGTTGCGCTTTCC	TCACCAAGGGTTAATTCTTC	20
BIRC5	10	TCGTCCGGTTGCGCTTTCCT	TCACCAAGGGTTAATTCTTC	19
BIRC5	11	TCGTCCGGTTGCGCTTTCCT	TTCACCAAGGGTTAATTCTT	20
BIRC5	12	TCCGGTTGCGCTTTCCTTTC	TCACCAAGGGTTAATTCTTC	16
BIRC5	13	TCCGGTTGCGCTTTCCTTTC	TTCACCAAGGGTTAATTCTT	17
BIRC5	14	TTGCGCTTTCCTTTCTGTCA	TCAAAAATTCACCAAGGGTT	19
BIRC5	15	TTGCGCTTTCCTTTCTGTCA	TTCAAAAATTCACCAAGGGT	20
BIRC5	16	TGCGCTTTCCTTTCTGTCAA	TCAAAAATTCACCAAGGGTT	18
BIRC5	17	TGCGCTTTCCTTTCTGTCAA	TTCAAAAATTCACCAAGGGT	19
BIRC5	18	TGCGCTTTCCTTTCTGTCAA	TTTCAAAAATTCACCAAGGG	20
BIRC5	19	TTTCCTTTCTGTCAAGAAGC	TTCAAAAATTCACCAAGGGT	14
BIRC5	20	TTTCCTTTCTGTCAAGAAGC	TTTCAAAAATTCACCAAGGG	15
BIRC5	21	TTTCCTTTCTGTCAAGAAGC	TCCAGTTTCAAAAATTCACC	20
BIRC5	22	TTCCTTTCTGTCAAGAAGCA	TTTCAAAAATTCACCAAGGG	14
BIRC5	23	TTCCTTTCTGTCAAGAAGCA	TCCAGTTTCAAAAATTCACC	19
BIRC5	24	TCCTTTCTGTCAAGAAGCAG	TCCAGTTTCAAAAATTCACC	18
BIRC5	25	TCCTTTCTGTCAAGAAGCAG	TGTCCAGTTTCAAAAATTCA	20
BIRC5	26	TTTCTGTCAAGAAGCAGTTT	TCCAGTTTCAAAAATTCACC	15
BIRC5	27	TTTCTGTCAAGAAGCAGTTT	TGTCCAGTTTCAAAAATTCA	17
BIRC5	28	TTTCTGTCAAGAAGCAGTTT	TCTGTCCAGTTTCAAAAATT	19
BIRC5	29	TTCTGTCAAGAAGCAGTTTG	TCCAGTTTCAAAAATTCACC	14
BIRC5	30	TTCTGTCAAGAAGCAGTTTG	TGTCCAGTTTCAAAAATTCA	16
BIRC5	31	TTCTGTCAAGAAGCAGTTTG	TCTGTCCAGTTTCAAAAATT	18
BIRC5	32	TTCTGTCAAGAAGCAGTTTG	TCTCTGTCCAGTTTCAAAAA	20
BIRC5	33	TCTGTCAAGAAGCAGTTTGA	TGTCCAGTTTCAAAAATTCA	15

BIRC5	34	TCTGTCAAGAAGCAGTTTGA	TCTGTCCAGTTTCAAAAATT	17
BIRC5	35	TCTGTCAAGAAGCAGTTTGA	TCTCTGTCCAGTTTCAAAAA	19
BIRC5	36	TCTGTCAAGAAGCAGTTTGA	TTCTCTGTCCAGTTTCAAAA	20
BIRC5	37	TGTCAAGAAGCAGTTTGAAG	TCTGTCCAGTTTCAAAAATT	15
BIRC5	38	TGTCAAGAAGCAGTTTGAAG	TCTCTGTCCAGTTTCAAAAA	17
BIRC5	39	TGTCAAGAAGCAGTTTGAAG	TTCTCTGTCCAGTTTCAAAA	18
BIRC5	40	TGTCAAGAAGCAGTTTGAAG	TTTCTCTGTCCAGTTTCAAA	19
BIRC5	41	TCAAGAAGCAGTTTGAAGAA	TCTCTGTCCAGTTTCAAAAA	15
BIRC5	42	TCAAGAAGCAGTTTGAAGAA	TTCTCTGTCCAGTTTCAAAA	16
BIRC5	43	TCAAGAAGCAGTTTGAAGAA	TTTCTCTGTCCAGTTTCAAA	17
BIRC5	44	TTTGAAGAATTAACCCTTGG	TCTTGGCTCTTTCTCTGTCC	15
BIRC5	45	TTGAAGAATTAACCCTTGGT	TCTTGGCTCTTTCTCTGTCC	14
BIRC5	46	TTGAAGAATTAACCCTTGGT	TTCTTGGCTCTTTCTCTGTC	15
BIRC5	47	TGAAGAATTAACCCTTGGTG	TTCTTGGCTCTTTCTCTGTC	14
BIRC5	48	TGAAGAATTAACCCTTGGTG	TGTTCTTGGCTCTTTCTCTG	16
BIRC5	49	TTAACCCTTGGTGAATTTTT	TACAATTTTGTTCTTGGCTC	17
BIRC5	50	TAACCCTTGGTGAATTTTTG	TACAATTTTGTTCTTGGCTC	16
BIRC5	51	TAACCCTTGGTGAATTTTTG	TACATACAATTTTGTTCTTG	20
BIRC5	52	TTGGTGAATTTTTGAAACTG	TACATACAATTTTGTTCTTG	14
BIRC5	1	TTATTTCCAGGCAAAGGAAA	TCCGCAGTTTCCTCAAATTC	17
BIRC5	2	TTATTTCCAGGCAAAGGAAA	TCTCCGCAGTTTCCTCAAAT	19
BIRC5	3	TTATTTCCAGGCAAAGGAAA	TTCTCCGCAGTTTCCTCAAA	20
BIRC5	4	TATTTCCAGGCAAAGGAAAC	TCCGCAGTTTCCTCAAATTC	16
BIRC5	5	TATTTCCAGGCAAAGGAAAC	TCTCCGCAGTTTCCTCAAAT	18
BIRC5	6	TATTTCCAGGCAAAGGAAAC	TTCTCCGCAGTTTCCTCAAA	19
BIRC5	7	TATTTCCAGGCAAAGGAAAC	TTTCTCCGCAGTTTCCTCAA	20
BIRC5	8	TCCAGGCAAAGGAAACCAAC	TCTCCGCAGTTTCCTCAAAT	14
BIRC5	9	TCCAGGCAAAGGAAACCAAC	TTCTCCGCAGTTTCCTCAAA	15
BIRC5	10	TCCAGGCAAAGGAAACCAAC	TTTCTCCGCAGTTTCCTCAA	16
BIRC5	11	TTTGAGGAAACTGCGGAGAA	TCCATGGCAGCCAGCTGCTC	16
BIRC5	12	TTTGAGGAAACTGCGGAGAA	TCAATCCATGGCAGCCAGCT	20
BIRC5	13	TTGAGGAAACTGCGGAGAAA	TCCATGGCAGCCAGCTGCTC	15
BIRC5	14	TTGAGGAAACTGCGGAGAAA	TCAATCCATGGCAGCCAGCT	19
BIRC5	15	TGAGGAAACTGCGGAGAAAG	TCCATGGCAGCCAGCTGCTC	14
BIRC5	16	TGAGGAAACTGCGGAGAAAG	TCAATCCATGGCAGCCAGCT	18

Certain embodiments are directed to a method for treating cancer comprising: a. removing a biopsy containing one or more cancerous cells from a patient, b. determining the sequence of a cancer-associated genetic marker in the one or more cancerous cells, and c. administering to the patient a therapeutically effective amount of a gene-editing protein or a nucleic acid encoding a gene-editing protein, wherein the sequence of the target DNA molecule is at least about 50% or about 60% or

about 70% or about 80% or about 90% or about 95% or about 98%, or about 99% homologous to the sequence of the cancer-associated genetic marker. In one embodiment, the method further comprises comparing the sequence of one or more cancer-associated genetic markers in the one or more cancerous cells to the sequence of the same cancer-associated genetic markers in one or more non-cancerous cells, selecting a cancer-associated genetic marker having a sequence that is different in the one or more cancerous cells and the one or more non-cancerous cells, and wherein the sequence of the target DNA molecule or binding site is at least about 50% or about 60% or about 70% or about 80% or about 90% or about 95% or about 98% or about 99% homologous to the sequence of the selected cancer-associated genetic marker.

Many cancer cells express survivin, a member of the inhibitor of apoptosis (IAP) protein family that, in humans, is encoded by the BIRC5 gene. Using RNA interference to reduce expression of certain mRNA molecules, including survivin mRNA, can transiently inhibit the growth of certain cancer cells. However, previous methods of using RNA interference to reduce expression of survivin mRNA yield temporary effects, and result in only a short increase in mean time-to-death (TTD) in animal models. It has now been discovered that inducing a cell to express one or more gene-editing proteins that target the BIRC5 gene can result in disruption of the BIRC5 gene, can induce the cell to express and/or secrete a non-functional variant of survivin protein, can induce the cell to express and/or secrete a dominant-negative variant of survivin protein, can trigger activation of one or more apoptosis pathways in the cell and nearby cells, can slow or halt the growth of the cell and nearby cells, can result in the death of the cell and nearby cells, can inhibit the progression of cancer, and can result in remission in a cancer patient. Certain embodiments are therefore directed to a gene-editing protein that targets the BIRC5 gene. In one embodiment, the gene-editing protein binds to one or more regions in the BIRC5 gene. In another embodiment, the gene-editing protein binds to one or more regions of a sequence selected from: SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15. In a further embodiment, the gene-editing protein binds to one or more sequences selected from: SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27. In a still further embodiment, the gene-editing protein binds to one or more nucleic-acid sequences that encode SEQ ID NO: 34 or a biologically active fragment, variant or analogue thereof. In a still further embodiment, the gene-editing protein binds to one or more sequences selected from Table 3, Table 4, Table 3 of U.S. Provisional Application No. 61/721,302, the contents of which are hereby incorporated by reference, Table 1 of U.S. Provisional Application No. 61/785,404, the contents of which are hereby incorporated by reference or Table 1 of U.S. Provisional Application No. 61/842,874, the contents of which are hereby incorporated by reference or to one or more sequences that is at least about 50% or at least about 60% or at least about 70% or at least about 80% or at least about 90% or at least about 95% or at least about 98%, or about 99%

homologous to one or more sequences selected from Table 3, Table 4, Table 3 of U.S. Provisional Application No. 61/721,302, the contents of which are hereby incorporated by reference, Table 1 of U.S. Provisional Application No. 61/785,404, the contents of which are hereby incorporated by reference or Table 1 of U.S. Provisional Application No. 61/842,874, the contents of which are hereby incorporated by reference. In one embodiment, the gene-editing protein creates one or more nicks or double-strand breaks in the DNA of the cell. In another embodiment, the one or more nicks or double-strand breaks is created in the BIRC5 gene. In a further embodiment, the one or more nicks or double-strand breaks is created in one or more exons of the BIRC5 gene. In a still further embodiment, the one or more nicks or double-strand breaks is created in a sequence selected from: SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15. In a still further embodiment, the one or more nicks or double-strand breaks is created within a sequence that encodes an inhibitor of apoptosis domain (aka. "IAP", "IAP domain", "IAP repeat", "baculovirus inhibitor of apoptosis protein repeat", "BIR", etc.). In a still further embodiment, the gene-editing protein binds to one or more sequences selected from Table 5, Table 2 of U.S. Provisional Application No. 61/785,404, the contents of which are hereby incorporated by reference or Table 2 of U.S. Provisional Application No. 61/842,874, the contents of which are hereby incorporated by reference or to one or more sequences that is at least about 50% or at least about 60% or at least about 70% or at least about 80% or at least about 90% or at least about 95% or at least about 98% homologous to one or more sequences selected from Table 5, Table 2 of U.S. Provisional Application No. 61/785,404, the contents of which are hereby incorporated by reference or Table 2 of U.S. Provisional Application No. 61/842,874, the contents of which are hereby incorporated by reference. In yet another embodiment, the gene editing protein binds to a sequence that encodes one or more genes selected from Table 2, Table 5, Table 6, Table 7, Table 4 of U.S. Provisional Application No. 61/721,302, the contents of which are hereby incorporated by reference, Table 2 of U.S. Provisional Application No. 61/785,404, the contents of which are hereby incorporated by reference or Table 2 of U.S. Provisional Application No. 61/842,874, the contents of which are hereby incorporated by reference.

Table 5. Exemplary Cancer-Associated Gene Binding Sites

Gene	#	Left	Right	Spacing
CDK1	1	TTTAGGATCTACCATACCCA	TCTCTATTTTGGTATAATCT	15
CDK1	2	TTTAGGATCTACCATACCCA	TTCTCTATTTTGGTATAATC	16
CDK1	3	TTTAGGATCTACCATACCCA	TTTCTCTATTTTGGTATAAT	17
CDK1	4	TTAGGATCTACCATACCCAT	TCTCTATTTTGGTATAATCT	14
CDK1	5	TTAGGATCTACCATACCCAT	TTCTCTATTTTGGTATAATC	15
CDK1	1	TCACACAGCATATTATTTAC	TACCCTTATACACAACTCCA	17
CDK1	2	TCACACAGCATATTATTTAC	TCTACCCTTATACACAACTC	19
CDK1	3	TACTTTGTTTCAGGTACCTA	TGTAGTTTTGTGTCTACCCT	14
CDK1	4	TACTTTGTTTCAGGTACCTA	TGACCTGTAGTTTTGTGTCT	19

CDK1	5	TTTGTTTCAGGTACCTATGG	TGACCTGTAGTTTTGTGTCT	16
CDK2	1	TGACCCGACTCGCTGGCGCT	TCCGATCTTTTCCACCTTTT	15
CDK2	2	TGACCCGACTCGCTGGCGCT	TCTCCGATCTTTTCCACCTT	17
CDK2	3	TCGCTGGCGCTTCATGGAGA	TACGTGCCCTCTCCGATCTT	17
CDK2	4	TTCATGGAGAACTTCCAAAA	TACACAACTCCGTACGTGCC	19
CDK2	5	TCATGGAGAACTTCCAAAAG	TACACAACTCCGTACGTGCC	18
CDK2	1	TTTCCCAACCTCTCCAAGTG	TCTCGGATGGCAGTACTGGG	14
CDK2	2	TTCCCAACCTCTCCAAGTGA	TCTCTCGGATGGCAGTACTG	15
CDK2	3	TCCCAACCTCTCCAAGTGAG	TCTCTCGGATGGCAGTACTG	14
CDK2	4	TCTCCAAGTGAGACTGAGGG	TAAGCAGAGAGATCTCTCGG	18
CDK2	5	TCTCCAAGTGAGACTGAGGG	TTAAGCAGAGAGATCTCTCG	19
CDK3	1	TGTTTCCCAGGCAGCTCTGT	TCTCCGATCTTCTCTACCTT	19
CDK3	2	TTTCCCAGGCAGCTCTGTGG	TCTCCGATCTTCTCTACCTT	17
CDK3	3	TTCCCAGGCAGCTCTGTGGC	TCTCCGATCTTCTCTACCTT	16
CDK3	4	TCCCAGGCAGCTCTGTGGCC	TCTCCGATCTTCTCTACCTT	15
CDK3	5	TGGATATGTTCCAGAAGGTA	TACACCACCCCATAGGTGCC	15
CDK3	1	TGCCCACGGCTGTGCCCTTG	TGGCAGTGCTTGGGACCCCC	19
CDK3	2	TGTGCCCTTGTTTCTTGCAG	TCCCTGATGGCAGTGCTTGG	16
CDK3	3	TTTCTTGCAGGGAGATGGAG	TGAGCAGCGAGATCTCCCTG	20
CDK3	4	TTCTTGCAGGGAGATGGAGG	TGAGCAGCGAGATCTCCCTG	19
CDK3	5	TTCTTGCAGGGAGATGGAGG	TTGAGCAGCGAGATCTCCCT	20
CDK4	1	TGTGATTGTAGGGTCTCCCT	TGGCTCATATCGAGAGGTAG	14
CDK4	2	TGATTGTAGGGTCTCCCTTG	TCAGCCACTGGCTCATATCG	20
CDK4	3	TTGTAGGGTCTCCCTTGATC	TCAGCCACTGGCTCATATCG	17
CDK4	4	TGTAGGGTCTCCCTTGATCT	TCAGCCACTGGCTCATATCG	16
CDK4	5	TAGGGTCTCCCTTGATCTGA	TCAGCCACTGGCTCATATCG	14
CDK4	1	TTGAAAAGTGAGCATTTACT	TCGGGATGTGGCACAGACGT	16
CDK4	2	TTGAAAAGTGAGCATTTACT	TTCGGGATGTGGCACAGACG	17
CDK4	3	TGAAAAGTGAGCATTTACTC	TCGGGATGTGGCACAGACGT	15
CDK4	4	TGAAAAGTGAGCATTTACTC	TTCGGGATGTGGCACAGACG	16
CDK4	5	TGAAAAGTGAGCATTTACTC	TCAGTTCGGGATGTGGCACA	20
CDK5	1	TACGAGAAACTGGAAAAGAT	TGCAGGAACATCTCGAGATT	15
CDK5	2	TACGAGAAACTGGAAAAGAT	TTGCAGGAACATCTCGAGAT	16
CDK5	3	TACGAGAAACTGGAAAAGAT	TCTTGCAGGAACATCTCGAG	18
CDK5	1	TCCTTCCCCTAGGCACCTAC	TGAGTCTCCCGGTTTTTGGC	15
CDK5	2	TCCTTCCCCTAGGCACCTAC	TCATGAGTCTCCCGGTTTTT	18
CDK5	3	TCCTTCCCCTAGGCACCTAC	TCTCATGAGTCTCCCGGTTT	20
CDK5	4	TTCCCCTAGGCACCTACGGA	TCATGAGTCTCCCGGTTTTT	15
CDK5	5	TTCCCCTAGGCACCTACGGA	TCTCATGAGTCTCCCGGTTT	17
CDK6	1	TGTGCCGCGCTGACCAGCAG	TAGGCGCCCTCCCCGATCTC	15
CDK6	2	TGTGCCGCGCTGACCAGCAG	TCCCATAGGCGCCCTCCCCG	20
CDK6	3	TGCCGCGCTGACCAGCAGTA	TCCCATAGGCGCCCTCCCCG	18

CDK6	4	TGCCGCGCTGACCAGCAGTA	TTCCCATAGGCGCCCTCCCC	19
CDK6	5	TGACCAGCAGTACGAATGCG	TGAACACCTTCCCATAGGCG	19
CDK6	1	TCTAGGTTGTTTGATGTGTG	TAGTTTGGTTTCTCTGTCTG	14
CDK6	2	TCTAGGTTGTTTGATGTGTG	TAAAGTTAGTTTGGTTTCTC	20
CDK6	3	TAGGTTGTTTGATGTGTGCA	TAAAGTTAGTTTGGTTTCTC	18
CDK6	4	TTGTTTGATGTGTGCACAGT	TAAAGTTAGTTTGGTTTCTC	14
CDK6	5	TTGATGTGTGCACAGTGTCA	TCAAACACTAAAGTTAGTTT	18
EGFR	1	TCCGGGACGGCCGGGGCAGC	TCGCCGGGCAGAGCGCAGCC	15
EGFR	1	TCTTCCAGTTTGCCAAGGCA	TCAAAAGTGCCCAACTGCGT	14
EGFR	2	TCTTCCAGTTTGCCAAGGCA	TGATCTTCAAAAGTGCCCAA	20
EGFR	3	TTCCAGTTTGCCAAGGCACG	TGATCTTCAAAAGTGCCCAA	18
EGFR	4	TCCAGTTTGCCAAGGCACGA	TGATCTTCAAAAGTGCCCAA	17
EGFR	5	TCACGCAGTTGGGCACTTTT	TGAACATCCTCTGGAGGCTG	14
HIF1A	1	TGAAGACATCGCGGGGACCG	TGTCGTTCGCGCCGCCGGCG	15
HIF1A	2	TGAAGACATCGCGGGGACCG	TTGTCGTTCGCGCCGCCGGC	16
HIF1A	3	TGAAGACATCGCGGGGACCG	TCTTGTCGTTCGCGCCGCCG	18
HIF1A	4	TGAAGACATCGCGGGGACCG	TTCTTGTCGTTCGCGCCGCC	19
HIF1A	5	TGAAGACATCGCGGGGACCG	TTTCTTGTCGTTCGCGCCGC	20
HIF1A	1	TCTCGTGTTTTTCTTGTTGT	TCTTTTCGACGTTCAGAACT	14
HIF1A	2	TCTCGTGTTTTTCTTGTTGT	TTCTTTTCGACGTTCAGAAC	15
HIF1A	3	TCTCGTGTTTTTCTTGTTGT	TTTCTTTTCGACGTTCAGAA	16
HIF1A	4	TCTCGTGTTTTTCTTGTTGT	TTTTCTTTTCGACGTTCAGA	17
HIF1A	5	TTCTTGTTGTTGTTAAGTAG	TCGAGACTTTTCTTTTCGAC	14
HSPA4	1	TGGTGGGCATAGACCTGGGC	TGCCGCCGGCGCGGGCCACA	20
HSPA4	2	TGGGCATAGACCTGGGCTTC	TGCCGCCGGCGCGGGCCACA	17
HSPA4	3	TAGACCTGGGCTTCCAGAGC	TCGATGCCGCCGGCGCGGGC	15
HSPA4	4	TAGACCTGGGCTTCCAGAGC	TCTCGATGCCGCCGGCGCGG	17
HSPA4	5	TAGACCTGGGCTTCCAGAGC	TAGTCTCGATGCCGCCGGCG	20
HSPA4	1	TCTTAAGTGCTTTTTTTGTC	TGAACGATTCTTAGGACCAA	20
HSPA4	2	TTAAGTGCTTTTTTTGTCTT	TGAACGATTCTTAGGACCAA	18
HSPA4	3	TTAAGTGCTTTTTTTGTCTT	TTGAACGATTCTTAGGACCA	19
HSPA4	4	TAAGTGCTTTTTTTGTCTTC	TGAACGATTCTTAGGACCAA	17
HSPA4	5	TAAGTGCTTTTTTTGTCTTC	TTGAACGATTCTTAGGACCA	18
HSP90AA1	1	TGCCCCCGTGTTCGGGCGGG	TCCCGAAGGGAGGGCCCAGG	15
HSP90AA1	2	TGCCCCCGTGTTCGGGCGGG	TGTCCCGAAGGGAGGGCCCA	17
HSP90AA1	3	TCCTGGGCCCTCCCTTCGGG	TCGCGCGGGTATTCAGCACT	20
HSP90AA1	4	TGGGCCCTCCCTTCGGGACA	TCGCGCGGGTATTCAGCACT	17
HSP90AA1	5	TCCCTTCGGGACAGGGACTG	TCCAGACGGTCGCGCGGGTA	19
HSP90AA1	1	TCCAGAAGATTGTGTTTATG	TCTTGGTACCAGTTAACAGG	14
HSP90AA1	2	TGTGTTTATGTTCCCAGCAG	TTGGGCCTTTTCTTGGTACC	14
HSP90AA1	3	TCCCAGCAGGGCACCTGTTA	TGCCAGAGAAACACTTGGGC	17
HSP90AA1	4	TAACTGGTACCAAGAAAAGG	TCCAGACACCATCAGATGCC	15

HSP90AA1	5	TAACTGGTACCAAGAAAAGG	TGGATCCAGACACCATCAGA	19
MYC	1	TCCAGCAGCCTCCCGCGACG	TAGTTCCTGTTGGTGAAGCT	15
MYC	2	TCCAGCAGCCTCCCGCGACG	TCATAGTTCCTGTTGGTGAA	18
MYC	3	TCCCGCGACGATGCCCCTCA	TCGAGGTCATAGTTCCTGTT	14
MYC	4	TCCCGCGACGATGCCCCTCA	TAGTCGAGGTCATAGTTCCT	17
MYC	5	TCCCGCGACGATGCCCCTCA	TCGTAGTCGAGGTCATAGTT	20
PKN3	1	TGCAGCCTGGGCCGAGCCAG	TGGCCCGGCGGATCACCTCC	20
PKN3	2	TGGGCCGAGCCAGTGGCCCC	TGGATGGCCCGGCGGATCAC	17
PKN3	3	TGGGCCGAGCCAGTGGCCCC	TCTGGATGGCCCGGCGGATC	19
PKN3	4	TGGGCCGAGCCAGTGGCCCC	TTCTGGATGGCCCGGCGGAT	20
PKN3	5	TGGCCCCCAGAGGATGAGAA	TCAGCTCTTTCTGGATGGCC	15
RRM2	1	TGGGAAGGGTCGGAGGCATG	TGGCTTTGGTGCCCCGGCCC	16
RRM2	2	TGGGAAGGGTCGGAGGCATG	TTGGCTTTGGTGCCCCGGCC	17
RRM2	3	TCGGAGGCATGGCACAGCCA	TTCCCATTGGCTTTGGTGCC	14
RRM2	4	TGGCACAGCCAATGGGAAGG	TCCCGGCCCTTCCCATTGGC	14
RRM2	5	TGCACCCTGTCCCAGCCGTC	TGGAGGCGCAGCGAAGCAGA	17
APC	1	TATGTACGCCTCCCTGGGCT	TGGTACAGAAGCGGGCAAAG	15
APC	2	TGTACGCCTCCCTGGGCTCG	TGAGGGTGGTACAGAAGCGG	19
APC	3	TACGCCTCCCTGGGCTCGGG	TGAGGGTGGTACAGAAGCGG	17
APC	4	TCGGGTCCGGTCGCCCCTTT	TCCAGGACCCGAGAACTGAG	18
APC	5	TCCGGTCGCCCCTTTGCCCG	TGCTCCAGGACCCGAGAACT	16
APC	1	TTAAACAACTACAAGGAAGT	TCAATCTGTCCAGAAGAAGC	18
APC	2	TAAACAACTACAAGGAAGTA	TCAATCTGTCCAGAAGAAGC	17
APC	3	TACAAGGAAGTATTGAAGAT	TAATAAATCAATCTGTCCAG	16
APC	4	TATTGAAGATGAAGCTATGG	TAAGACGCTCTAATAAATCA	16
APC	5	TATTGAAGATGAAGCTATGG	TTAAGACGCTCTAATAAATC	17
BRCA1	1	TGGATTTATCTGCTCTTCGC	TGCATAGCATTAATGACATT	15
BRCA1	2	TGGATTTATCTGCTCTTCGC	TCTGCATAGCATTAATGACA	17
BRCA1	3	TTATCTGCTCTTCGCGTTGA	TAAGATTTTCTGCATAGCAT	20
BRCA1	4	TATCTGCTCTTCGCGTTGAA	TAAGATTTTCTGCATAGCAT	19
BRCA1	5	TCTGCTCTTCGCGTTGAAGA	TAAGATTTTCTGCATAGCAT	17
BRCA1	1	TGCTAGTCTGGAGTTGATCA	TGCAAAATATGTGGTCACAC	19
BRCA1	2	TGCTAGTCTGGAGTTGATCA	TTGCAAAATATGTGGTCACA	20
BRCA1	3	TAGTCTGGAGTTGATCAAGG	TGCAAAATATGTGGTCACAC	16
BRCA1	4	TAGTCTGGAGTTGATCAAGG	TTGCAAAATATGTGGTCACA	17
BRCA1	5	TAGTCTGGAGTTGATCAAGG	TACTTGCAAAATATGTGGTC	20
BRCA2	1	TGCCTATTGGATCCAAAGAG	TGCAGCGTGTCTTAAAAATT	17
BRCA2	2	TGCCTATTGGATCCAAAGAG	TTGCAGCGTGTCTTAAAAAT	18
BRCA2	3	TGCCTATTGGATCCAAAGAG	TGTTGCAGCGTGTCTTAAAA	20
BRCA2	4	TATTGGATCCAAAGAGAGGC	TTGCAGCGTGTCTTAAAAAT	14
BRCA2	5	TATTGGATCCAAAGAGAGGC	TGTTGCAGCGTGTCTTAAAA	16
BRCA2	1	TAGATTTAGGACCAATAAGT	TGGAGCTTCTGAAGAAAGTT	16

BRCA2	2	TTAGGACCAATAAGTCTTAA	TAGGGTGGAGCTTCTGAAGA	16
BRCA2	3	TTAGGACCAATAAGTCTTAA	TATAGGGTGGAGCTTCTGAA	18
BRCA2	4	TTAGGACCAATAAGTCTTAA	TTATAGGGTGGAGCTTCTGA	19
BRCA2	5	TAGGACCAATAAGTCTTAAT	TATAGGGTGGAGCTTCTGAA	17
TP53	1	TCACTGCCATGGAGGAGCCG	TGACTCAGAGGGGGCTCGAC	15
TP53	2	TCACTGCCATGGAGGAGCCG	TCCTGACTCAGAGGGGGCTC	18
TP53	3	TCACTGCCATGGAGGAGCCG	TTCCTGACTCAGAGGGGGCT	19
TP53	4	TCACTGCCATGGAGGAGCCG	TTTCCTGACTCAGAGGGGGC	20
TP53	5	TGCCATGGAGGAGCCGCAGT	TCCTGACTCAGAGGGGGCTC	14
APP	1	TTCTTTCAGGTACCCACTGA	TGGCAATCTGGGGTTCAGCC	18
APP	2	TCTTTCAGGTACCCACTGAT	TGGCAATCTGGGGTTCAGCC	17
APP	3	TTTCAGGTACCCACTGATGG	TGGCAATCTGGGGTTCAGCC	15
APP	4	TTCAGGTACCCACTGATGGT	TGGCAATCTGGGGTTCAGCC	14
APP	5	TACCCACTGATGGTAATGCT	TGCCACAGAACATGGCAATC	20
IAPP	1	TGGGCATCCTGAAGCTGCAA	TGGTTCAATGCAACAGAGAG	15
IAPP	2	TGGGCATCCTGAAGCTGCAA	TCAGATGGTTCAATGCAACA	20
IAPP	3	TGCAAGTATTTCTCATTGTG	TGGGTGTAGCTTTCAGATGG	17
IAPP	4	TGCTCTCTGTTGCATTGAAC	TTACCAACCTTTCAATGGGT	14
IAPP	1	TGTTACCAGTCATCAGGTGG	TGCGTTGCACATGTGGCAGT	17
IAPP	2	TTACCAGTCATCAGGTGGAA	TGCGTTGCACATGTGGCAGT	15
IAPP	3	TACCAGTCATCAGGTGGAAA	TGCGTTGCACATGTGGCAGT	14
IAPP	4	TCATCAGGTGGAAAAGCGGA	TGCCAGGCGCTGCGTTGCAC	18
IAPP	5	TCATCAGGTGGAAAAGCGGA	TTGCCAGGCGCTGCGTTGCA	19
SNCA	1	TTTTGTAGGCTCCAAAACCA	TTACCTGTTGCCACACCATG	14
SNCA	2	TTTTGTAGGCTCCAAAACCA	TGGAGCTTACCTGTTGCCAC	20
SNCA	3	TTTGTAGGCTCCAAAACCAA	TGGAGCTTACCTGTTGCCAC	19
SNCA	4	TTGTAGGCTCCAAAACCAAG	TGGAGCTTACCTGTTGCCAC	18
SNCA	5	TGTAGGCTCCAAAACCAAGG	TGGAGCTTACCTGTTGCCAC	17
SOD1	1	TAGCGAGTTATGGCGACGAA	TGCACTGGGCCGTCGCCCTT	16
SOD1	2	TTATGGCGACGAAGGCCGTG	TGCCCTGCACTGGGCCGTCG	14
SOD1	3	TTATGGCGACGAAGGCCGTG	TGATGCCCTGCACTGGGCCG	17
SOD1	4	TTATGGCGACGAAGGCCGTG	TGATGATGCCCTGCACTGGG	20
SOD1	5	TATGGCGACGAAGGCCGTGT	TGATGCCCTGCACTGGGCCG	16
SOD1	1	TAATGGACCAGTGAAGGTGT	TGCAGGCCTTCAGTCAGTCC	14
SOD1	2	TAATGGACCAGTGAAGGTGT	TCCATGCAGGCCTTCAGTCA	18
SOD1	3	TGGACCAGTGAAGGTGTGGG	TCCATGCAGGCCTTCAGTCA	15
SOD1	4	TGGACCAGTGAAGGTGTGGG	TGGAATCCATGCAGGCCTTC	20
SOD1	5	TGTGGGGAAGCATTAAAGGA	TCATGAACATGGAATCCATG	15

In some embodiments, the target DNA molecule comprises a gene that is overexpressed in cancer. Example genes that are overexpressed in cancer include, but are not limited to: ABL1, BIRC5, BLK,

BTK, CDK family members, EGFR, ERBB2, FAS, FGR, FLT4, FRK, FYN, HCK, HIF1A, HRAS, HSP90AA1, HSP90AA1, HSPA4, KDR, KIF11, KIF11, KIF20A, KIF21A, KIF25, KIT, KRAS, LCK, LYN, MAPK1, MET, MYC, MYH1, MYO1G, NRAS, NTRK1, PDGFB, PDGFRA, PDGFRB, PKN3, PLK1, RAF1, RB1, RET, RRM1, RRM2, SRC, TNF, TPM2, TYRO3, VEGFA, VEGFB, VEGFC, YES1, and ZAP70. In some embodiments, the target DNA molecule comprises a gene selected from: ABL1, BIRC5, BLK, BTK, a CDK family member, EGFR, ERBB2, FAS, FGR, FLT4, FRK, FYN, HCK, HIF1A, HRAS, HSP90AA1, HSP90AA1, HSPA4, KDR, KIF11, KIF11, KIF20A, KIF21A, KIF25, KIT, KRAS, LCK, LYN, MAPK1, MET, MYC, MYH1, MYO1G, NRAS, NTRK1, PDGFB, PDGFRA, PDGFRB, PKN3, PLK1, RAF1, RB1, RET, RRM1, RRM2, SRC, TNF, TPM2, TYRO3, VEGFA, VEGFB, VEGFC, YES1, and ZAP70 or a fragment or variant thereof. In other embodiments, the target DNA molecule comprises a gene that is mutated in cancer. Example genes that are mutated in cancer include, but are not limited to: AIM1, APC, BRCA1, BRCA2, CDKN1B, CDKN2A, FAS, FZD family members, HNF1A, HOPX, KLF6, MEN1, MLH1, NTRK1, PTEN, RARRES1, RB1, SDHB, SDHD, SFRP1, ST family members, TNF, TP53, TP63, TP73, VBP1, VHL, WNT family members, BRAF, CTNNB1, PIK3CA, PIK3R1, SMAD4, and YPEL3. In some embodiments, the target DNA molecule comprises a gene selected from: AIM1, APC, BRCA1, BRCA2, CDKN1B, CDKN2A, FAS, a FZD family member, HNF1A, HOPX, KLF6, MEN1, MLH1, NTRK1, PTEN, RARRES1, RB1, SDHB, SDHD, SFRP1, a ST family member, TNF, TP53, TP63, TP73, VBP1, VHL, a WNT family member, BRAF, CTNNB1, PIK3CA, PIK3R1, SMAD4, and YPEL3 or a fragment or variant thereof. In one embodiment, the method further comprises administering to a patient a therapeutically effective amount of a repair template.

Mutations in certain genes can increase the likelihood of a cell becoming cancerous. In certain situations, however, it can be detrimental to inactivate a cancer-associated gene in non-cancerous cells, for example, if the non-mutated form of the cancer-associated gene is beneficial. It has now been discovered that gene-editing proteins can be used to specifically inactivate, partially or completely, mutated forms of genes. Examples of cancer-associated mutations include, but are not limited to: ALK (F1174, R1275), APC (R876, Q1378, R1450), BRAF (V600), CDKN2A (R58, R80, H83, D84, E88, D108G, W110, P114), CTNNB1 (D32, S33, G34, S37, T41, or S45), EGFR (G719, T790, L858), EZH2 (Y646), FGFR3 (S249, Y373), FLT3 (D835), GNAS (R201), HRAS (G12, G13, Q61), IDH1 (R132), JAK2 (V617), KIT (D816), KRAS (G12, G13), NRAS (G12, G13, Q61), PDGFRA (D842), PIK3CA (E542, E545, H1047), PTEN (R130), and TP53 (R175, H179, G245, R248, R249, R273, W282). Certain embodiments are therefore directed to a gene-editing protein that binds to a disease-associated mutation. In one embodiment, the gene-editing protein binds to DNA containing a specific mutation with greater affinity than DNA that does not contain the mutation. In another embodiment, the disease is cancer.

Neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and dementia with Lewy bodies, are characterized by the progressive loss of function and/or death of cells of the central and/or peripheral nervous systems. Disease progression can be accompanied by the accumulation of protein-rich plaques that can comprise the protein α-synuclein (encoded, in humans, by the SNCA gene). As a result, researchers have sought to develop therapeutics that can break up these plaques, for example, by means of an antibody that binds to the plaque and tags it for destruction by the immune system. However, in many cases, breaking up plaques has little or no effect on patient symptoms or the progression of the disease. It has now been discovered that the failure of existing therapies that target neurodegenerative disease-associated plaques is due in part to the inability of the nervous system to repair the damage to cells that occurs during the early stages of plaque formation. It has been further discovered that inducing a cell to express one or more gene-editing proteins that target the SNCA gene can result in disruption of the SNCA gene, can induce the cell to express a plaque-resistant variant of α-synuclein protein, can slow or halt the growth of neurodegenerative disease-associated plaques, can protect the cell and nearby cells from the damaging effects of neurodegenerative disease-associated plaques, can slow and/or halt the progression of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and dementia with Lewy bodies, and can result in a reduction of symptoms and/or gain of function in patients with neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and dementia with Lewy bodies. Other neurodegenerative diseases include, for example, vision loss, including blindness, hearing loss, including deafness, balance disorders, loss of taste and/or smell, and other sensory disorders. Certain embodiments are therefore directed to a gene-editing protein that targets the SNCA gene. In one embodiment, the gene-editing protein binds to one or more regions in the SNCA gene. In another embodiment, the gene-editing protein binds to one or more nucleic-acid sequences that encode SEQ ID NO: 51 or a biologically active fragment, variant or analogue thereof. Other embodiments are directed to a method for treating a neurodegenerative disease comprising administering to a patient a therapeutically effective amount of a gene-editing protein or a nucleic acid encoding a gene-editing protein, wherein the gene-editing protein is capable of binding to a nucleotide sequence that encodes a protein that forms disease-associated plaques, and resulting in a reduction of disease-associated plaques in the patient and/or delayed or halted progression of the disease. In one embodiment, the nucleotide sequence comprises the SNCA gene. In another embodiment, the nucleotide sequence encodes α-synuclein. In a further embodiment, the neurodegenerative disease is selected from: Parkinson's disease, Alzheimer's disease, and dementia.

Certain embodiments are directed to a method for identifying a disease-causing toxicant comprising transfecting a cell with a gene-editing protein or a nucleic acid encoding a gene-editing protein to alter the DNA sequence of the cell, wherein the altered DNA sequence confers susceptibility to a disease, contacting the cell with a suspected disease-causing toxicant, and assessing the degree to which the

cell exhibits a phenotype associated with the disease. In one embodiment, the disease is a neurodegenerative disease, autoimmune disease, respiratory disease, reproductive disorder or cancer. Other embodiments are directed to a method for assessing the safety of a therapeutic substance comprising transfecting a cell with a gene-editing protein or a nucleic acid encoding a gene-editing protein to alter the DNA sequence of the cell, wherein the altered DNA sequence confers susceptibility to one or more toxic effects of the therapeutic substance, contacting the cell with the therapeutic substance, and measuring one or more toxic effects of the therapeutic substance on the cell. Still other embodiments are directed to a method for assessing the effectiveness of a therapeutic substance comprising transfecting a cell with a gene-editing protein or a nucleic acid encoding a gene-editing protein to alter the DNA sequence of the cell, wherein the altered DNA sequence causes the cell to exhibit one or more disease-associated phenotypes, contacting the cell with the therapeutic substance, and measuring the degree to which the one or more disease-associated phenotypes are reduced.

In some embodiments, the patient is diagnosed with a proteopathy. Example proteopathies and proteopathy-associated genes are given in Table 6, and are included by way of example, and not by way of limitation. In one embodiment, the proteopathy is selected from: AA (secondary) amyloidosis, Alexander disease, Alzheimer's disease, amyotrophic lateral sclerosis, aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, bibrinogen amyloidosis, cardiac atrial amyloidosis, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, cerebral β-amyloid angiopathy, dialysis amyloidosis, familial amyloid cardiomyopathy, familial amyloid polyneuropathy, familial amyloidosis (Finnish type), familial British dementia, familial Danish dementia, frontotemporal lobar degeneration, hereditary cerebral amyloid angiopathy, hereditary lattice corneal dystrophy, Huntington's disease, inclusion body myositis/myopathy, lysozyme amyloidosis, medullary thyroid carcinoma, odontogenic (Pindborg) tumor amyloid, Parkinson's disease, pituitary prolactinoma, prion diseases, pulmonary alveolar proteinosis, retinal ganglion cell degeneration in glaucoma, retinitis pigmentosa with rhodopsin mutations, senile systemic amyloidosis, serpinopathies, synucleinopathies, tauopathies, type II diabetes, dementia pugilistica (chronic traumatic encephalopathy), frontotemporal dementia, frontotemporal lobar degeneration, gangliocytoma, ganglioglioma, Hallervorden-Spatz disease, lead encephalopathy, lipofuscinosis, Lytico-Bodig disease, meningioangiomatosis, progressive supranuclear palsy, subacute sclerosing panencephalitis, tangle-predominant dementia, and tuberous sclerosis. In another embodiment, the target DNA molecule comprises a gene selected from: APOA1, APOA2, APOA4, APP, B2M, CALCA, CST3, FGA, FGB, FGG, FUS, GFAP, GSN, HTT, IAPP, ITM2B, LYZ, MAPT, MFGE8, NOTCH3, NPPA, ODAM, PRL, PRNP, RHO, a SAA family member, a SERPIN family member, SFTPC, SNCA, a SOD family member, TARDBP, TGFBI, and TRR or a fragment or variant thereof. In a further embodiment, the target DNA molecule encodes a

gene selected from Table 6 or a fragment thereof, and the patient is diagnosed with the corresponding disease listed in Table 6.

Table 6. Exemplary Proteopathies and Proteopathy-Associated Genes

Gene / Family	Disease / Condition
APOA1	ApoAI amyloidosis
APOA2	ApoAII amyloidosis
APOA4	ApoAIV amyloidosis
APP	Cerebral β-amyloid angiopathy
APP	Retinal ganglion cell degeneration in glaucoma
APP	Inclusion body myositis/myopathy
APP, MAPT	Alzheimer's disease
B2M	Dialysis amyloidosis
CALCA	Medullary thyroid carcinoma
CST3	Hereditary cerebral amyloid angiopathy (Icelandic)
FGA, FGB, FGG	Fibrinogen amyloidosis
GFAP	Alexander disease
GSN	Familial amyloidosis, Finnish type
HTT	Huntington's disease
IAPP	Type II diabetes
ITM2B	Familial British dementia
ITM2B	Familial Danish dementia
LYZ	Lysozyme amyloidosis
MAPT	Tauopathies (multiple)
MFGE8	Aortic medial amyloidosis
NOTCH3	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)
NPPA	Cardiac atrial amyloidosis
ODAM	Odontogenic (Pindborg) tumor amyloid
PRL	Pituitary prolactinoma
PRNP	Prion diseases (multiple)
RHO	Retinitis pigmentosa with rhodopsin mutations
SAA family genes	AA (secondary) amyloidosis
SERPIN family genes	Serpinopathies (multiple)
SFTPC	Pulmonary alveolar proteinosis
SNCA	Parkinson's disease and other synucleinopathies (multiple)
SNCA	Other synucleinopathies
SOD family genes, TARDBP, FUS	Amyotrophic lateral sclerosis (ALS)
TARDBP, FUS	Frontotemporal lobar degeneration (FTLD)
TGFBI	Hereditary lattice corneal dystrophy
LMNA	Hutchinson–Gilford Progeria Syndrome

TRR	Senile systemic amyloidosis (SSA), familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC)

Example tauopathies include, but are not limited to Alzheimer's disease, Parkinson's disease, and Huntington's disease. Other example tauopathies include: dementia pugilistica (chronic traumatic encephalopathy), frontotemporal dementia, frontotemporal lobar degeneration, gangliocytoma, ganglioglioma, Hallervorden-Spatz disease, lead encephalopathy, lipofuscinosis, Lytico-Bodig disease, meningioangiomatosis, progressive supranuclear palsy, subacute sclerosing panencephalitis, tangle-predominant dementia, and tuberous sclerosis. In some embodiments, the patient is diagnosed with a tauopathy. In one embodiment, the tauopathy is selected from: Alzheimer's disease, Parkinson's disease, and Huntington's disease. In another embodiment, the tauopathy is selected from: dementia pugilistica (chronic traumatic encephalopathy), frontotemporal dementia, frontotemporal lobar degeneration, gangliocytoma, ganglioglioma, Hallervorden-Spatz disease, lead encephalopathy, lipofuscinosis, Lytico-Bodig disease, meningioangiomatosis, progressive supranuclear palsy, subacute sclerosing panencephalitis, tangle-predominant dementia, and tuberous sclerosis.

Autoimmune diseases, including but not limited to lupus, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and transplant rejection, are characterized by symptoms caused in part by one or more elements of the immune system attacking uninfected and non-cancerous isogenic cells and/or tissues. Certain embodiments are therefore directed to a method for treating an autoimmune disease. In one embodiment, the autoimmune disease is selected from: lupus, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and transplant rejection. In another embodiment, the target DNA molecule encodes a polypeptide sequence that can be recognized by the host immune system.

Infectious agents can contain nucleic acid sequences that are not present in the host organism. It has now been discovered that gene-editing proteins can be used to eliminate, reduce or otherwise alter, in whole or in part, infectious agents and/or the effects of infection, and that when used in this manner, gene-editing proteins and nucleic acids encoding gene-editing proteins, can constitute potent anti-infection therapeutics. Infectious agents that can be treated in such a manner include, but are not limited to: viruses, bacteria, fungi, yeast, and parasites. Certain embodiments are therefore directed to a method for inducing a cell to express a gene-editing protein that targets one or more infectious agent-associated sequences. In one embodiment, the cell is one of: a bacterial cell, a fungal cell, a yeast cell, and a parasite cell. In another embodiment, the cell is a mammalian cell. In a further embodiment, the cell is a human cell. Other embodiments are directed to a therapeutic composition comprising a nucleic acid that encodes one or more gene-editing proteins that targets one or more infectious agent-associated sequences. Certain embodiments are directed to a method for inducing a cell to express a gene-editing protein that targets one or more sequences associated with susceptibility or resistance to infection. Other embodiments are directed to a therapeutic composition comprising a

nucleic acid that encodes one or more gene-editing proteins that targets one or more sequences associated with susceptibility or resistance to infection. In one embodiment, the cell is transfected with a nucleic acid encoding one or more gene-editing proteins and a nucleic acid encoding one or more repair templates. In another embodiment, the repair template contains a resistance gene or a biologically active fragment or variant thereof. In a further embodiment, the repair template contains an RNAi sequence. In a still further embodiment, the RNAi sequence is a shRNA. Other embodiments are directed to a method for treating an infectious disease comprising administering to a patient a therapeutically effective amount of a gene-editing protein or a nucleic acid encoding a gene-editing protein, wherein the gene-editing protein is capable of binding to one or more nucleotide sequences that are present in the infectious agent.

It has now been discovered that the ratio of non-homologous end joining events to homologous recombination events can be altered by altering the expression and/or function of one or more components of a DNA-repair pathway. Non-limiting examples of genes that encode components of a DNA-repair pathway include, but are not limited to: Artemis, BLM, CtIP, DNA-PK, DNA-PKcs, EXOl, FEN1, Ku70, Ku86, LIGIII, LIGIV, MRE11, NBS1, PARP1, RAD50, RAD54B, XLF, XRCC1, XRCC3, and XRCC4. Certain embodiments are therefore directed to a method for altering the expression and/or function of one or more components of a DNA-repair pathway. In certain embodiments, the expression and/or function is increased. In other embodiments, the expression and/or function is decreased. DNA-dependent protein kinase (DNA-PK) is a component of the non-homologous end-joining DNA-repair pathway. It has now been discovered that repair via homologous recombination can be increased by altering the expression of DNA-PK. In one embodiment, a cell is contacted with a DNA-PK inhibitor. Example DNA-PK inhibitors include, but are not limited to: Compound 401 (2-(4-Morpholinyl)-4H-pyrimido[2,1-a]isoquinolin-4-one), DMNB, IC87361, LY294002, NU7026, NU7441, OK-1035, PI 103 hydrochloride, vanillin, and wortmannin.

Genetic mutations can affect the length of a protein product, for example, by introducing a stop codon and/or disrupting an open reading frame. Certain diseases, including Duchenne muscular dystrophy, can be caused by the production of truncated and/or frameshifted proteins. It has now been discovered that gene-editing proteins can be used to treat diseases that are associated with the production of one or more truncated and/or frameshifted proteins. In one embodiment, the gene-editing protein creates a double strand break within about 1kb or about 0.5kb or about 0.1kb of an exon containing a disease-contributing mutation. In another embodiment, the gene-editing protein is co-expressed with a DNA sequence comprising one or more wild-type sequences. In certain embodiments, the DNA is single-stranded. In other embodiments, the DNA is double-stranded. Diseases caused by the expression of truncated proteins can be treated by exon skipping. It has now been discovered that gene-editing proteins can be used to induce exon skipping. In one embodiment, the gene-editing protein creates a double-strand break within about 1kb or about 0.5kb or about 0.1kb of the exon to be skipped. In

another embodiment, the gene-editing protein creates a double-strand break within about 1kb or about 0.5kb or about 0.1kb of an intron upstream of the exon to be skipped. In another embodiment, the gene-editing protein creates a double-strand break within about 1kb or about 0.5kb or about 0.1kb of the splice-acceptor site of an intron upstream of the exon to be skipped.

Nucleic acids, including liposomal formulations containing nucleic acids, when delivered *in vivo*, can accumulate in the liver and/or spleen. It has now been discovered that nucleic acids encoding gene-editing proteins can modulate gene expression in the liver and spleen, and that nucleic acids used in this manner can constitute potent therapeutics for the treatment of liver and spleen diseases. Certain embodiments are therefore directed to a method for treating liver and/or spleen disease by delivering to a patient a nucleic acid encoding one or more gene-editing proteins. Other embodiments are directed to a therapeutic composition comprising a nucleic acid encoding one or more gene-editing proteins, for the treatment of liver and/or spleen disease. Diseases and conditions of the liver and/or spleen that can be treated include, but are not limited to: hepatitis, alcohol-induced liver disease, drug-induced liver disease, Epstein Barr virus infection, adenovirus infection, cytomegalovirus infection, toxoplasmosis, Rocky Mountain spotted fever, non-alcoholic fatty liver disease, hemochromatosis, Wilson's Disease, Gilbert's Disease, and cancer of the liver and/or spleen. Other examples of sequences (including genes, gene families, and loci) that can be targeted by gene-editing proteins using the methods of the present invention are set forth in Table 7, and are given by way of example, and not by way of limitation.

Table 7. Exemplary Gene Editing-Protein Targets

Disease/Condition	Gene/Family/Locus
Age-related macular degeneration	VEGF family
Alzheimer's disease	APP, PSEN1, PSEN2, APOE, CR1, CLU, PICALM, BIN1, MS4A4, MS4A6E, CD2AP, CD33, EPHA1
Amyotrophic lateral sclerosis	SOD1
Cancer	BRCA1, EGFR, MYC family, TP53, PKN3, RAS family, BIRC5, PTEN, RET, KIT, MET, APC, RB1, BRCA2, VEGF family, TNF, HNPCC1, HNPCC2, HNPCC5
Cystic fibrosis	CFTR
Diabetes	GCK, HNF1A, HNF4A, HNF1B
Duchenne muscular dystrophy	DMD
Fanconi anemia	BRCA2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN, FANCP, RAD51C
Hemochromatosis	HFE, HJV, HAMP, TFR2, SLC40A1
Hemophilia	F8, F9, F11
HIV/AIDS	CCR5, CXCR4
Huntington's disease	HTT

Marfan's syndrome	FBN1
Neurofibromatosis	NF1, NF2
Parkinson's disease	SNCA, PRKN, LRRK2, PINK1, PARK7, ATP13A2
Safe-harbor locus in humans	AAVS1
Safe-harbor locus in mice and rats	Rosa26
Sickle-cell anemia	HBB
Tay-Sachs disease	HEXA
Xeroderma pigmentosum	XPA, XPB, XPC, XPD, DDB2, ERCC4, ERCC5, ERCC6, RAD2, POLH
Psoriasis, Rheumatoid arthritis, Ankylosing spondylitis, Crohn's disease, Hidradenitis suppurativa, Refractory asthma	TNF
Psoriasis, Rheumatoid arthritis, Polycythemia vera, Essential thrombocythemia, Myeloid metaplasia with myelofibrosis	JAK family

Certain embodiments are directed to a combination therapy comprising one or more of the therapeutic compositions of the present invention and one or more adjuvant therapies. Example adjuvant therapies are set forth in Table 8 and Table 5 of U.S. Provisional Application No. 61/721,302, the contents of which are hereby incorporated by reference, and are given by way of example, and not by way of limitation.

Table 8. Exemplary Adjuvant Therapies

Therapy Class	Disease/Condition	Example Therapy
Acetylcholinesterase inhibitors	Myasthenia gravis, Glaucoma, Alzheimer's disease, Lewy body dementia, Postural tachycardia syndrome	Edrophonium
Angiotensin-converting-enzyme inhibitor	Hypertension, Congestive heart failure	Perindopril
Alkylating agents	Cancer	Cisplatin
Angiogenesis inhibitors	Cancer, Macular degeneration	Bevacizumab
Angiotensin II receptor antagonists	Hypertension, Diabetic nephropathy, Congestive heart failure	Valsartan
Antibiotics	Bacterial infection	Amoxicillin
Antidiabetic drugs	Diabetes	Metformin
Antimetabolites	Cancer, Infection	5-fluorouracil (5FU)
Antisense oligonucleotides	Cancer, Diabetes, Amyotrophic lateral sclerosis (ALS), Hypercholesterolemia	Mipomersen
Cytotoxic antibiotics	Cancer	Doxorubicin
Deep-brain stimulation	Chronic pain, Parkinson's disease, Tremor, Dystonia	N/A
Dopamine agonists	Parkinson’s disease, Type II diabetes, Pituitary tumors	Bromocriptine
Entry/Fusion inhibitors	HIV/AIDS	Maraviroc

Glucagon-like peptide-1 agonists	Diabetes	Exenatide
Glucocorticoids	Asthma, Adrenal insufficiency, Inflammatory diseases, Immune diseases, Bacterial meningitis	Dexamethasone
Immunosuppressive drugs	Organ transplantation, Inflammatory diseases, Immune diseases	Azathioprine
Insulin/Insulin analogs	Diabetes	NPH insulin
Integrase inhibitors	HIV/AIDS	Raltegravir
MAO-B inhibitors	Parkinson's disease, Depression, Dementia	Selegiline
Maturation inhibitors	HIV/AIDS	Bevirimat
Nucleoside analog reverse-transcriptase inhibitors	HIV/AIDS, Hepatitis B	Lamivudine
Nucleotide analog reverse-transcriptase inhibitors	HIV/AIDS, Hepatitis B	Tenofovir
Non-nucleoside reverse-transcriptase inhibitors	HIV/AIDS	Rilpivirine
Pegylated interferon	Hepatitis B/C, Multiple sclerosis	Interferon beta-1a
Plant alkaloids/terpenoids	Cancer	Paclitaxel
Protease inhibitors	HIV/AIDS, Hepatitis C, Other viral infections	Telaprevir
Radiotherapy	Cancer	Brachytherapy
Renin inhibitors	Hypertension	Aliskiren
Statins	Hypercholesterolemia	Atorvastatin
Topoisomerase inhibitors	Cancer	Topotecan
Vasopressin receptor antagonist	Hyponatremia, Kidney disease	Tolvaptan

Pharmaceutical preparations may additionally comprise delivery reagents (a.k.a. “transfection reagents”) and/or excipients. Pharmaceutically acceptable delivery reagents, excipients, and methods of preparation and use thereof, including methods for preparing and administering pharmaceutical preparations to patients (a.k.a. “subjects”) are well known in the art, and are set forth in numerous publications, including, for example, in US Patent Appl. Pub. No. US 2008/0213377, the entirety of which is hereby incorporated by reference.

For example, the present compositions can be in the form pharmaceutically acceptable salts. Such salts include those listed in, for example, *J. Pharma. Sci.* 66, 2-19 (1977) and *The Handbook of Pharmaceutical Salts; Properties, Selection, and Use*. P. H. Stahl and C. G. Wermuth (eds.), Verlag, Zurich (Switzerland) 2002, which are hereby incorporated by reference in their entirety. Non-limiting examples of pharmaceutically acceptable salts include: sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, camphorsulfonate, pamoate, phenylacetate,

trifluoroacetate, acrylate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, isobutyrate, phenylbutyrate, α-hydroxybutyrate, butyne-1,4-dicarboxylate, hexyne-1,4-dicarboxylate, caprate, caprylate, cinnamate, glycollate, heptanoate, hippurate, malate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, phthalate, teraphthalate, propiolate, propionate, phenylpropionate, sebacate, suberate, p-bromobenzenesulfonate, chlorobenzenesulfonate, ethylsulfonate, 2-hydroxyethylsulfonate, methylsulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, naphthalene-1,5-sulfonate, xylenesulfonate, tartarate salts, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or tri-alkylamines, dicyclohexylamine; tributyl amine; pyridine; N-methyl, N-ethylamine; diethylamine; triethylamine; mono-, bis-, or tris-(2-OH-lower alkylamines), such as mono-; bis-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N,N-di-lower alkyl-N-(hydroxyl-lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)amine or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like.

The present pharmaceutical compositions can comprises excipients, including liquids such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical excipients can be, for example, saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating, and coloring agents can be used. In one embodiment, the pharmaceutically acceptable excipients are sterile when administered to a subject. Suitable pharmaceutical excipients also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Any agent described herein, if desired, can also comprise minor amounts of wetting or emulsifying agents, or pH buffering agents.

In various embodiments, the compositions described herein can administered in an effective dose of, for example, from about 1 mg/kg to about 100 mg/kg, about 2.5 mg/kg to about 50 mg/kg, or about 5 mg/kg to about 25 mg/kg. The precise determination of what would be considered an effective dose may be based on factors individual to each patient, including their size, age, and type of disease. Dosages can be readily ascertained by those of ordinary skill in the art from this disclosure and the knowledge in the art. For example, doses may be determined with reference *Physicians' Desk Reference*, 66th Edition, PDR Network; 2012 Edition (December 27, 2011), the contents of which are incorporated by reference in its entirety.

The active compositions of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present invention may be via any common

route so long as the target tissue is available via that route. This includes oral, nasal, or buccal. Alternatively, administration may be by intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or by direct injection into cancer tissue. The agents disclosed herein may also be administered by catheter systems. Such compositions would normally be administered as pharmaceutically acceptable compositions as described herein.

Upon formulation, solutions may be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations may easily be administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution generally is suitably buffered and the liquid diluent first rendered isotonic with, for example, sufficient saline or glucose. Such aqueous solutions may be used, for example, for intravenous, intramuscular, subcutaneous and intraperitoneal administration. Preferably, sterile aqueous media are employed as is known to those of skill in the art, particularly in light of the present disclosure.

Exemplary subjects or patients refers to any vertebrate including, without limitation, humans and other primates (*e.g.*, chimpanzees and other apes and monkey species), farm animals (*e.g.*, cattle, sheep, pigs, goats, and horses), domestic mammals (*e.g.*, dogs and cats), laboratory animals (*e.g.*, rodents such as mice, rats, and guinea pigs), and birds (*e.g.*, domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like). In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

This invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1 RNA Synthesis

RNA encoding the human proteins Oct4, Sox2, Klf4, c-Myc-2 (T58A), and Lin28 or TALENs targeting the human genes XPA, CCR5, TERT, MYC, and BIRC5, and comprising various combinations of canonical and non-canonical nucleotides, was synthesized from DNA templates using the T7 High Yield RNA Synthesis Kit and the Vaccinia Capping System kit with mRNA Cap 2′-O-Methyltransferase (all from New England Biolabs, Inc.), according to the manufacturer's instructions and the present inventors' previously disclosed inventions (U.S. Application No. 13/465,490 (now U.S. Patent No. 8,497,124), U.S. Provisional Application No. 61/637,570, U.S. Provisional Application No. 61/664,494, International Application No. PCT/US12/67966, U.S. Provisional Application No. 61/785,404, U.S. Application No. 13/931,251, and U.S. Provisional Application No. 61/842,874, the contents of all of which are hereby incorporated by reference in their entirety) (Table 9, **FIG. 1A**, **FIG. 1B**, and **FIG. 15**). The RNA was then diluted with nuclease-free water to between 100ng/μL and 200ng/μL. For certain experiments, an RNase inhibitor (Superase·In,

Life Technologies Corporation) was added at a concentration of 1μL/100μg of RNA. RNA solutions were stored at 4°C. For reprogramming experiments, RNA encoding Oct4, Sox2, Klf4, c-Myc-2 (T58A), and Lin28 was mixed at a molar ratio of 3:1:1:1:1.

Table 9. RNA Synthesis

Template	Nucleotides	Reaction Volume/μL	ivT Yield/μg
Oct4	A, G, U, C	10	64.9
Oct4	A, G, 0.25 4sU, C	10	64.3
Oct4	A, G, 0.5 4sU, C	10	62.8
Oct4	A, G, 0.75 4sU, C	10	51.9
Oct4	A, G, 4sU, C	10	0
Oct4	A, 0.5 7dG, 0.75 4sU, 0.25 piC	20	70.1
Sox2	A, 0.5 7dG, 0.75 4sU, 0.25 piC	10	29.6
Klf4	A, 0.5 7dG, 0.75 4sU, 0.25 piC	10	29.5
c-Myc-2 (T58A)	A, 0.5 7dG, 0.75 4sU, 0.25 piC	10	25.9
Lin28	A, 0.5 7dG, 0.75 4sU, 0.25 piC	10	36.7
Oct4	A, 0.5 7dG, 0.75 4sU, 0.5 piC	20	51.7
Sox2	A, 0.5 7dG, 0.75 4sU, 0.5 piC	10	23.0
Klf4	A, 0.5 7dG, 0.75 4sU, 0.5 piC	10	22.3
c-Myc-2 (T58A)	A, 0.5 7dG, 0.75 4sU, 0.5 piC	10	21.4
Lin28	A, 0.5 7dG, 0.75 4sU, 0.5 piC	10	23.3
Oct4	A, 0.5 7dG, 0.8 4sU, 0.2 5mU, 0.5 piC	20	50.8
Oct4	A, 0.5 7dG, 0.7 4sU, 0.3 5mU, 0.5 piC	20	58.3
Oct4	A, 0.5 7dG, 0.6 4sU, 0.4 5mU, 0.5 piC	20	58.3
Oct4	A, 0.5 7dG, 0.5 4sU, 0.5 5mU, 0.5 piC	20	68.2
Oct4	A, 0.5 7dG, 0.4 4sU, 0.6 5mU, 0.5 piC	20	78.7
Oct4	A, G, psU, 5mC	10	110.4
Oct4	A, G, psU, 0.5 piC	10	85.0
Oct4	A, 0.5 7dG, psU, 0.5 piC	10	58.3
Oct4	A, 0.5 7dG, psU, 5mC	10	27.0
Oct4	A, 0.5 7dG, 0.5 5mU, 0.5 piC	20	109.0
Oct4	A, 0.5 7dG, 0.6 5mU, 0.5 piC	20	114.8
Oct4	A, 0.5 7dG, 0.7 5mU, 0.5 piC	20	107.2
Oct4	A, 0.5 7dG, 0.8 5mU, 0.5 piC	20	110.9
Oct4	A, 0.5 7dG, 0.9 5mU, 0.5 piC	20	103.4
Oct4	A, 0.5 7dG, 5mU, 0.5 piC	20	97.8
Oct4	A, 0.5 7dG, psU, 0.5 piC	20	124.5
Sox2	A, 0.5 7dG, psU, 0.5 piC	20	109.0
Klf4	A, 0.5 7dG, psU, 0.5 piC	20	112.8
c-Myc-2 (T58A)	A, 0.5 7dG, psU, 0.5 piC	20	112.8

Lin28	A, 0.5 7dG, psU, 0.5 piC	20	126.5
Oct4	A, G, psU, 5mC	20	213.4
Sox2	A, G, psU, 5mC	10	107.2
Klf4	A, G, psU, 5mC	10	106.1
c-Myc-2 (T58A)	A, G, psU, 5mC	10	97.8
Lin28	A, G, psU, 5mC	10	95.9
Oct4	A, 0.5 7dG, psU, 0.5 piC	20	124.2
Sox2	A, 0.5 7dG, psU, 0.5 piC	10	57.3
Klf4	A, 0.5 7dG, psU, 0.5 piC	10	59.6
c-Myc-2 (T58A)	A, 0.5 7dG, psU, 0.5 piC	10	66.7
Lin28	A, 0.5 7dG, psU, 0.5 piC	10	65.2
Oct4	A, 0.5 7dG, psU, 0.3 piC	10	60.5
Sox2	A, 0.5 7dG, psU, 0.3 piC	10	58.8
Klf4	A, 0.5 7dG, psU, 0.3 piC	10	57.9
c-Myc-2 (T58A)	A, 0.5 7dG, psU, 0.3 piC	10	62.0
Lin28	A, 0.5 7dG, psU, 0.3 piC	10	64.3
Oct4	A, 0.5 7dG, 0.5 5mU, 5mC	10	64.7
Sox2	A, 0.5 7dG, 0.5 5mU, 5mC	10	62.4
Klf4	A, 0.5 7dG, 0.5 5mU, 5mC	10	75.6
c-Myc-2 (T58A)	A, 0.5 7dG, 0.5 5mU, 5mC	10	69.4
Lin28	A, 0.5 7dG, 0.5 5mU, 5mC	10	60.7
Oct4	A, 0.5 7dG, 0.5 4sU, 0.5 5mU, 5mC	10	48.3
Sox2	A, 0.5 7dG, 0.5 4sU, 0.5 5mU, 5mC	10	54.0
Klf4	A, 0.5 7dG, 0.5 4sU, 0.5 5mU, 5mC	10	58.7
c-Myc-2 (T58A)	A, 0.5 7dG, 0.5 4sU, 0.5 5mU, 5mC	10	54.7
Lin28	A, 0.5 7dG, 0.5 4sU, 0.5 5mU, 5mC	10	54.1
Oct4	A, 0.5 7dG, 0.3 5mU, 5mC	10	69.6
Sox2	A, 0.5 7dG, 0.3 5mU, 5mC	10	69.6
Klf4	A, 0.5 7dG, 0.3 5mU, 5mC	10	87.4
c-Myc-2 (T58A)	A, 0.5 7dG, 0.3 5mU, 5mC	10	68.1
Lin28	A, 0.5 7dG, 0.3 5mU, 5mC	10	74.3
Oct4	A, 0.5 7dG, 0.4 5mU, 5mC	10	71.3
Sox2	A, 0.5 7dG, 0.4 5mU, 5mC	10	69.7
Klf4	A, 0.5 7dG, 0.4 5mU, 5mC	10	74.8
c-Myc-2 (T58A)	A, 0.5 7dG, 0.4 5mU, 5mC	10	83.7
Lin28	A, 0.5 7dG, 0.4 5mU, 5mC	10	69.9
XPA-L1	A, G, psU, 5mC	20	120.0
XPA-L2	A, G, psU, 5mC	20	114.0

XPA-R1	A, G, psU, 5mC	20	159.6
CCR5-L1	A, G, psU, 5mC	20	170.4
CCR5-L2	A, G, psU, 5mC	20	142.8
CCR5-R1	A, G, psU, 5mC	20	132.0
CCR5-R2	A, G, psU, 5mC	20	154.8
CCR5-L1	A, G, psU, 5mC	10	56.6
CCR5-L2	A, G, psU, 5mC	10	58.5
CCR5-R1	A, G, psU, 5mC	10	56.8
CCR5-R2	A, G, psU, 5mC	10	58.7
TERT-L	A, G, U, C	10	49.4
TERT-R	A, G, U, C	10	37.6
MYC-L	A, G, U, C	10	39.6
MYC-R	A, G, U, C	10	33.7
BIRC5-L	A, G, U, C	10	63.0
BIRC5-R	A, G, U, C	10	44.5
TERT-L	A, 0.5 7dG, 0.4 5mU, 5mC	10	50.8
TERT-R	A, 0.5 7dG, 0.4 5mU, 5mC	10	58.3
MYC-L	A, 0.5 7dG, 0.4 5mU, 5mC	10	40.8
MYC-R	A, 0.5 7dG, 0.4 5mU, 5mC	10	41.4
BIRC5-L	A, 0.5 7dG, 0.4 5mU, 5mC	10	35.8
BIRC5-R	A, 0.5 7dG, 0.4 5mU, 5mC	10	41.5
Oct4 (SEQ ID NO: 8)	A, 0.5 7dG, 0.4 5mU, 5mC	300	2752.0
Sox2 (SEQ ID NO: 9)	A, 0.5 7dG, 0.4 5mU, 5mC	100	965.0
Klf4 (SEQ ID NO: 10)	A, 0.5 7dG, 0.4 5mU, 5mC	100	1093.8
c-Myc-2 (T58A)	A, 0.5 7dG, 0.4 5mU, 5mC	100	1265.6
Lin28	A, 0.5 7dG, 0.4 5mU, 5mC	100	1197.8
Oct4	A, 0.5 7dG, 0.35 5mU, 5mC	30	155.7
Sox2	A, 0.5 7dG, 0.35 5mU, 5mC	15	79.8
Klf4	A, 0.5 7dG, 0.35 5mU, 5mC	15	90.0
c-Myc-2 (T58A)	A, 0.5 7dG, 0.35 5mU, 5mC	15	83.2
Lin28	A, 0.5 7dG, 0.35 5mU, 5mC	15	74.0
APP UTR_L (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	37.9
APP UTR_R (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	40.0
APP Exon2L (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	38.6
APP Exon2R (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	37.9
APP 6L (Human)	A, 0.5 7dG, 0.4 5mU, 5mC	20	43.1
APP 6R (Human)	A, 0.5 7dG, 0.4 5mU, 5mC	20	43.7

APP 7L (Human)	A, 0.5 7dG, 0.4 5mU, 5mC	20	42.1
APP 7R (Human)	A, 0.5 7dG, 0.4 5mU, 5mC	20	36.2
APP 670L (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	27.0
APP 670R (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	28.3
APP 678L (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	30.1
APP 678R (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	26.2
APP 680L (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	8.1
APP 680R (Rat)	A, 0.5 7dG, 0.4 5mU, 5mC	20	25.4
APP 6L (Human)	A, 0.5 7dG, 0.4 5mU, 5mC	40	48.6
APP 6R (Human)	A, 0.5 7dG, 0.4 5mU, 5mC	40	48.6
APP 6L (Human)	A,G,U,C	10	54.0
APP 6R (Human)	A,G,U,C	10	61.0
APP 6L (Human)	A, 0.5 7dG, 0.4 5mU, 5mC	10	35.4
APP 6R (Human)	A, 0.5 7dG, 0.4 5mU, 5mC	10	48.0

Example 2 Transfection of Cells with Synthetic RNA

For transfection in 6-well plates, 2μg RNA and 6μL transfection reagent (Lipofectamine RNAiMAX, Life Technologies Corporation) were first diluted separately in complexation medium (Opti-MEM, Life Technologies Corporation or DMEM/F12 + 10μg/mL insulin + 5.5μg/mL transferrin + 6.7ng/mL sodium selenite + 2μg/mL ethanolamine) to a total volume of 60μL each. Diluted RNA and transfection reagent were then mixed and incubated for 15min at room temperature, according to the transfection reagent-manufacturer's instructions. Complexes were then added to cells in culture. Between 30μL and 240μL of complexes were added to each well of a 6-well plate, which already contained 2mL of transfection medium per well. Plates were shaken gently to distribute the complexes throughout the well. Cells were incubated with complexes for 4 hours to overnight, before replacing the medium with fresh transfection medium (2mL/well). Volumes were scaled for transfection in 24-well and 96-well plates. Alternatively, between 0.5μg and 5μg of RNA and between 2-3μL of transfection reagent (Lipofectamine 2000, Life Technologies Corporation) per μg of RNA were first diluted separately in complexation medium (Opti-MEM, Life Technologies Corporation or DMEM/F12 + 10μg/mL insulin + 5.5μg/mL transferrin + 6.7ng/mL sodium selenite + 2μg/mL ethanolamine) to a total volume of between 5μL and 100μL each. Diluted RNA and transfection reagent were then mixed and incubated for 10min at room temperature. Complexes were then added to cells in culture. Between 10μL and 200μL of complexes were added to each well of a 6-well plate, which already contained 2mL of transfection medium per well. In certain experiments, DMEM + 10% FBS or DMEM + 50% FBS was used in place of transfection medium. Plates were shaken gently to distribute the complexes throughout the well. Cells were incubated with complexes for 4 hours to

overnight. In certain experiments, the medium was replaced with fresh transfection medium (2mL/well) 4h or 24h after transfection.

Example 3 Toxicity of and Protein Translation from Synthetic RNA Containing Non-Canonical Nucleotides

Primary human fibroblasts were transfected according to Example 2, using RNA synthesized according to Example 1. Cells were fixed and stained 20-24h after transfection using an antibody against Oct4. The relative toxicity of the RNA was determined by assessing cell density at the time of fixation.

Example 4 Transfection Medium Formulation

A cell-culture medium was developed to support efficient transfection of cells with nucleic acids and efficient reprogramming ("transfection medium"):

DMEM/F12 + 15mM HEPES + 2mM L-alanyl-L-glutamine + 10µg/mL insulin + 5.5µg/mL transferrin + 6.7ng/mL sodium selenite + 2µg/mL ethanolamine + 50µg/mL L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate + 4µg/mL cholesterol + 1µM hydrocortisone + 25µg/mL polyoxyethylenesorbitan monooleate + 2µg/mL D-alpha-tocopherol acetate + 20ng/mL bFGF + 5mg/mL treated human serum albumin.

A variant of this medium was developed to support robust, long-term culture of a variety of cell types, including pluripotent stem cells ("maintenance medium"):

DMEM/F12 + 2mM L-alanyl-L-glutamine + 10µg/mL insulin + 5.5µg/mL transferrin + 6.7ng/mL sodium selenite + 2µg/mL ethanolamine + 50µg/mL L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate + 20ng/mL bFGF + 2ng/mL TGF-β1.

Transfection medium, in which the treated human serum albumin was treated by addition of 32mM sodium octanoate, followed by heating at 60°C for 4h, followed by treatment with ion-exchange resin (AG501-X8(D), Bio-Rad Laboratories, Inc.) for 6h at room temperature, followed by treatment with dextran-coated activated charcoal (C6241, Sigma-Aldrich Co. LLC.) overnight at room temperature, followed by centrifugation, filtering, adjustment to a 10% solution with nuclease-free water, followed by addition to the other components of the medium, was used as the transfection medium in all Examples described herein, unless otherwise noted. For reprogramming experiments, cells were plated either on uncoated plates in DMEM + 10%-20% serum or on fibronectin and vitronectin-coated plates in transfection medium, unless otherwise noted. The transfection medium was not conditioned, unless otherwise noted. It is recognized that the formulation of the transfection medium can be adjusted to meet the needs of the specific cell types being cultured. It is further recognized that treated human serum albumin can be replaced with other treated albumin, for example, treated bovine serum albumin, without negatively affecting the performance of the medium. It is further recognized that

other glutamine sources can be used instead of or in addition to L-alanyl-L-glutamine, for example, L-glutamine, that other buffering systems can be used instead of or in addition to HEPES, for example, phosphate, bicarbonate, etc., that selenium can be provided in other forms instead of or in addition to sodium selenite, for example, selenous acid, that other antioxidants can be used instead of or in addition to L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate and/or D-alpha-tocopherol acetate, for example, L-ascorbic acid, that other surfactants can be used instead of or in addition to polyoxyethylenesorbitan monooleate, for example, Pluronic F-68 and/or Pluronic F-127, that other basal media can be used instead of or in addition to DMEM/F12, for example, MEM, DMEM, etc., and that the components of the culture medium can be varied with time, for example, by using a medium without TGF-β from day 0 to day 5, and then using a medium containing 2ng/mL TGF-β after day 5, without negatively affecting the performance of the medium. It is further recognized that other ingredients can be added, for example, fatty acids, lysophosphatidic acid, lysosphingomyelin, sphingosine-1-phosphate, other sphingolipids, ROCK inhibitors, including Y-27632 and thiazovivin, members of the TGF-β/NODAL family of proteins, IL-6, members of the Wnt family of proteins, etc., at appropriate concentrations, without negatively affecting the performance of the medium, and that ingredients that are known to promote or inhibit the growth of specific cell types and/or agonists and/or antagonists of proteins or other molecules that are known to promote or inhibit the growth of specific cell types can be added to the medium at appropriate concentrations when it is used with those cell types without negatively affecting the performance of the medium, for example, sphingosine-1-phosphate and pluripotent stem cells. The present invention relates equally to ingredients that are added as purified compounds, to ingredients that are added as parts of well-defined mixtures, to ingredients that are added as parts of complex or undefined mixtures, for example, animal or plant oils, and to ingredients that are added by biological processes, for example, conditioning. The concentrations of the components can be varied from the listed values within ranges that will be obvious to persons skilled in the art without negatively affecting the performance of the medium. An animal component-free version of the medium was produced by using recombinant versions of all protein ingredients, and non-animal-derived versions of all other components, including semi-synthetic plant-derived cholesterol (Avanti Polar Lipids, Inc.).

Example 5 Reprogramming Human Fibroblasts Using Synthetic RNA Containing Non-Canonical Nucleotides

Primary human neonatal fibroblasts were plated in 6-well plates coated with recombinant human fibronectin and recombinant human vitronectin (each diluted in DMEM/F12 to a concentration of 1μg/mL, 1mL/well, and incubated at room temperature for 1h) at a density of 10,000 cells/well in transfection medium. The following day, the cells were transfected as in Example 2, using RNA containing A, 0.5 7dG, 0.5 5mU, and 5mC, and an RNA dose of 0.5μg/well on day 1, 0.5μg/well on day 2, 2μg/well on day 3, 2μg/well on day 4, and 4μg/well on day 5. Small colonies of cells

exhibiting morphology consistent with reprogramming became visible as early as day 5. The medium was replaced with maintenance medium on day 6. Cells were stained using an antibody against Oct4. Oct4-positive colonies of cells exhibiting a morphology consistent with reprogramming were visible throughout the well (**FIG. 2**).

Example 6 Feeder-Free, Passage-Free, Immunosuppressant-Free, Conditioning-Free Reprograming of Primary Adult Human Fibroblasts Using Synthetic RNA

Wells of a 6-well plate were coated with a mixture of recombinant human fibronectin and recombinant human vitronectin (1µg/mL in DMEM/F12, 1mL/well) for 1h at room temperature. Primary adult human fibroblasts were plated in the coated wells in transfection medium at a density of 10,000 cells/well. Cells were maintained at 37°C, 5% CO_2, and 5% O_2. Beginning the following day, cells were transfected according to Example 2 daily for 5 days with RNA synthesized according to Example 1. The total amount of RNA transfected on each of the 5 days was 0.5µg, 0.5µg, 2µg, 2µg, and 4µg, respectively. Beginning with the fourth transfection, the medium was replaced twice a day. On the day following the final transfection, the medium was replaced with transfection medium, supplemented with 10µM Y-27632. Compact colonies of cells with a reprogrammed morphology were visible in each transfected well by day 4 (**FIG. 8**).

Example 7 Efficient, Rapid Derivation and Reprogramming of Cells from Adult Human Skin Biopsy Tissue

A full-thickness dermal punch biopsy was performed on a healthy, 31 year-old volunteer, according to an approved protocol. Briefly, an area of skin on the left, upper arm was anesthetized by topical application of 2.5% lidocaine. The field was disinfected with 70% isopropanol, and a full-thickness dermal biopsy was performed using a 1.5 mm-diameter punch. The tissue was rinsed in phosphate-buffered saline (PBS), was placed in a 1.5mL tube containing 250µL of TrypLE Select CTS (Life Technologies Corporation), and was incubated at 37°C for 30min. The tissue was then transferred to a 1.5mL tube containing 250µL of DMEM/F12-CTS (Life Technologies Corporation) + 5mg/mL collagenase, and was incubated at 37°C for 2h. The epidermis was removed using forceps, and the tissue was mechanically dissociated. Cells were rinsed twice in DMEM/F12-CTS. Phlebotomy was also performed on the same volunteer, and venous blood was collected in Vacutainer SST tubes (Becton, Dickinson and Company). Serum was isolated according to the manufacturer's instructions. Isogenic plating medium was prepared by mixing DMEM/F12-CTS + 2mM L-alanyl-L-glutamine (Sigma-Aldrich Co. LLC.) + 20% human serum. Cells from the dermal tissue sample were plated in a fibronectin-coated well of a 6-well plate in isogenic plating medium. Many cells with a fibroblast morphology attached and began to spread by day 2 (**FIG. 3A**). Cells were expanded and frozen in Synth-a-Freeze (Life Technologies Corporation).

Cells were passaged into 6-well plates at a density of 5,000 cells/well. The following day, the medium was replaced with transfection medium, and the cells were transfected as in Example 2, using RNA containing A, 0.5 7dG, 0.4 5mU, and 5mC, and an RNA dose of 0.5μg/well on day 1, 0.5μg/well on day 2, 2μg/well on day 3, 2μg/well on day 4, and 2μg/well on day 5. Certain wells received additional 2μg/well transfections on day 6 and day 7. In addition, certain wells received 2ng/mL TGF-β1 from day 4 onward. The medium was replaced with maintenance medium on day 6. Colonies of cells exhibiting morphology consistent with reprogramming became visible between day 5 and day 10 (**FIG. 3B**). Colonies grew rapidly, and many exhibited a morphology similar to that of embryonic stem-cell colonies (**FIG. 3C**). Colonies were picked and plated in wells coated with recombinant human fibronectin and recombinant human vitronectin (each diluted in DMEM/F12 to a concentration of 1μg/mL, 1mL/well, incubated at room temperature for 1h). Cells grew rapidly, and were passaged to establish lines.

Example 8 Synthesis of RiboSlice Targeting CCR5

RiboSlice pairs targeting the following sequences: L1: TCATTTTCCATACAGTCAGT, L2: TTTTCCATACAGTCAGTATC, R1: TGACTATCTTTAATGTCTGG, and R2: TATCTTTAATGTCTGGAAAT were synthesized according to Example 1 (**FIG. 4A** and **FIG. 4B**). These pairs target 20-bp sites within the human CCR5 gene on the sense (L1 and L2) or antisense strand (R1 and R2). The following pairs were prepared: L1&R1, L1&R2, L2&R1, and L2&R2.

Example 9 Measurement of CCR5 Gene-Editing Efficiency using a Mismatch-Detecting Nuclease

Primary human fibroblasts were plated in 6-well plates coated with recombinant human fibronectin and recombinant human vitronectin (each diluted in DMEM/F12 to a concentration of 1μg/mL, 1mL/well, and incubated at room temperature for 1h) at a density of 10,000 cells/well in transfection medium. The following day, the cells were transfected as in Example 2 with RNA synthesized according to Example 8. Two days after the transfection, genomic DNA was isolated and purified. A region within the CCR5 gene was amplified by PCR using the primers F: AGCTAGCAGCAAACCTTCCCTTCA and R: AAGGACAATGTTGTAGGGAGCCCA. 150ng of the amplified PCR product was hybridized with 150ng of reference DNA in 10mM Tris-Cl + 50mM KCl + 1.5mM $MgCl_2$. The hybridized DNA was treated with a mismatch-detecting endonuclease (SURVEYOR nuclease, Transgenomic, Inc.) and the resulting products were analyzed by agarose gel electrophoresis (**FIG. 4C** and **FIG. 4D**).

Example 10 High-Efficiency Gene Editing by Repeated Transfection with RiboSlice

Primary human fibroblasts were plated as in Example 9. The following day, the cells were transfected as in Example 2 with RNA synthesized according to Example 8. The following day cells in one of the

wells were transfected a second time. Two days after the second transfection, the efficiency of gene editing was measured as in Example 9 (**FIG. 4E**).

Example 11 Gene-Editing of CCR5 using RiboSlice and DNA-Free, Feeder-Free, Immunosuppressant-Free, Conditioning-Free Reprogramming of Human Fibroblasts

Primary human fibroblasts were plated as in Example 9. The following day, the cells were transfected as in Example 2 with RNA synthesized according to Example 8. Approximately 48h later, the cells were reprogrammed according to Example 5, using RNA synthesized according to Example 1. Large colonies of cells with a morphology characteristic of reprogramming became visible as in Example 5 (**FIG. 4F**). Colonies were picked to establish lines. Cell lines were subjected to direct sequencing to confirm successful gene editing (**FIG. 4G**).

Example 12 Personalized Cell-Replacement Therapy for HIV/AIDS Comprising Gene-Edited Reprogrammed Cells

Patient skin cells are gene-edited and reprogrammed to hematopoietic cells according to the present inventors' previously disclosed inventions (U.S. Application No. 13/465,490, U.S. Provisional Application No. 61/637,570, and U.S. Provisional Application No. 61/664,494) and/or Example 11. Cells are then enzymatically released from the culture vessel, and CD34+/CD90+/Lin- or CD34+/CD49f+/Lin- cells are isolated. Between about 1 X 10^3 and about 1 X 10^5 cells are infused into a main vein of the patient. Hematopoietic cells home to the bone marrow cavity and engraft.

Example 13 Production of APP-Inactivated Rat Embryonic Stem Cells

Rat embryonic stem cells are plated in 6-well plates at a density of 10,000 cells/well in rat stem cell medium. The following day, the cells are transfected as in Example 2 with 0.5µg/well of RiboSlice synthesized according to Example 1 targeting the following sequences: L: TTCTGTGGTAAACTCAACAT and R: TCTGACTCCCATTTTCCATT (0.25µg L and 0.25µg R).

Example 14 Production of APP-Knockout Rats using APP-Inactivated Rat Embryonic Stem Cells

Rat embryonic stem cells are gene-editing according to Example 13 and microinjected into rat blastocysts. The microinjected blastocysts are then transferred to a pseudopregnant female rat.

Example 15 Production of APP-Inactivated Embryos for the Generation of Knockout Rats

A RiboSlice pair targeting the following sequences: L: TTCTGTGGTAAACTCAACAT and R: TCTGACTCCCATTTTCCATT is synthesized according to Example 1. RiboSlice at a concentration of 5µg/µL is injected into the pronucleus or cytoplasm of a 1-cell-stage rat embryo. The embryo is then transferred to a pseudopregnant female rat.

Example 16 Transfection of Cells with Synthetic RNA Containing Non-Canonical Nucleotides and DNA Encoding a Repair Template

For transfection in 6-well plates, 1μg RNA encoding gene-editing proteins targeting exon 16 of the human APP gene, 1μg single-stranded repair template DNA containing a PstI restriction site that was not present in the target cells, and 6μL transfection reagent (Lipofectamine RNAiMAX, Life Technologies Corporation) were first diluted separately in complexation medium (Opti-MEM, Life Technologies Corporation) to a total volume of 120μL. Diluted RNA, repair template, and transfection reagent were then mixed and incubated for 15min at room temperature, according to the transfection reagent-manufacturer's instructions. Complexes were added to cells in culture. Approximately 120μL of complexes were added to each well of a 6-well plate, which already contained 2mL of transfection medium per well. Plates were shaken gently to distribute the complexes throughout the well. Cells were incubated with complexes for 4 hours to overnight, before replacing the medium with fresh transfection medium (2mL/well). The next day, the medium was changed to DMEM + 10% FBS. Two days after transfection, genomic DNA was isolated and purified. A region within the APP gene was amplified by PCR, and the amplified product was digested with PstI and analyzed by gel electrophoresis (**FIG. 16**).

Example 17 Insertion of a Transgene into Rat Embryonic Stem Cells at a Safe Harbor Location

Rat embryonic stem cells are plated in 6-well plates at a density of 10,000 cells/well in rat stem cell medium. The following day, the cells are transfected as in Example 13 with RiboSlice targeting the following sequences: L: TATCTTCCAGAAAGACTCCA and R: TTCCCTTCCCCCTTCTTCCC, synthesized according to Example 1, and a repair template containing a transgene flanked by two regions each containing approximately 400 bases of homology to the region surrounding the rat Rosa26 locus.

Example 18 Humanized LRRK2 Rat

Rat embryonic stem cells are plated and transfected as in Example 13 with RiboSlice targeting the following sequences: L: TTGAAGGCAAAAATGTCCAC and R: TCTCATGTAGGAGTCCAGGA, synthesized according to Example 1. Two days after transfection, the cells are transfected according Example 17, wherein the transgene contains the human LRRK2 gene, and, optionally, part or all of the human LRRK2 promoter region.

Example 19 Insertion of a Transgene into Human Fibroblasts at a Safe Harbor Location

Primary human fibroblasts are plated as in Example 9. The following day, the cells are transfected as in Example 2 with RiboSlice targeting the following sequences: L: TTATCTGTCCCCTCCACCCC and R: TTTTCTGTCACCAATCCTGT, synthesized according to Example 1, and a repair template containing a transgene flanked by two regions each containing approximately 400 bases of homology to the region surrounding the human AAVS1 locus.

Example 20 Inserting an RNAi Sequence into a Safe Harbor Location

Primary human fibroblasts are plated and transfected according to Example 19, wherein the transgene contains a sequence encoding an shRNA, preceded by the PolIII promoter.

Example 21 Gene Editing of Myc using RiboSlice

Primary human fibroblasts were plated in 6-well plates at a density of 50,000 cells/well in DMEM + 10% FBS. Two days later, the medium was changed to transfection medium. Four hours later, the cells were transfected as in Example 2 with 1μg/well of RiboSlice targeting the following sequences: L: TCGGCCGCCGCCAAGCTCGT and R: TGCGCGCAGCCTGGTAGGAG, synthesized according to Example 1. The following day gene-editing efficiency was measured as in Example 9 using the following primers: F: TAACTCAAGACTGCCTCCCGCTTT and R: AGCCCAAGGTTTCAGAGGTGATGA (**FIG. 5**).

Example 22 Cancer Therapy Comprising RiboSlice Targeting Myc

HeLa cervical carcinoma cells were plated in 6-well plates at a density of 50,000 cells/well in folate-free DMEM + 2mM L-alanyl-L-glutamine + 10% FBS. The following day, the medium was changed to transfection medium. The following day, the cells were transfected as in Example 21.

Example 23 Gene Editing of BIRC5 using RiboSlice

Primary human fibroblasts were plated in 6-well plates at a density of 50,000 cells/well in DMEM + 10% FBS. Two days later, the medium was changed to transfection medium. Four hours later, the cells were transfected as in Example 2 with 1μg/well of RiboSlice targeting the following sequences: L: TTGCCCCCTGCCTGGCAGCC and R: TTCTTGAATGTAGAGATGCG, synthesized according to Example 1. The following day gene-editing efficiency was measured as in Example 9 using the following primers: F: GCGCCATTAACCGCCAGATTTGAA and R: TGGGAGTTCACAACAACAGGGTCT (**FIG. 6**).

Example 24 Cancer Therapy Comprising RiboSlice Targeting BIRC5

HeLa cervical carcinoma cells were plated in 6-well plates at a density of 50,000 cells/well in folate-free DMEM + 2mM L-alanyl-L-glutamine + 10% FBS. The following day, the medium was changed to transfection medium. The following day, the cells were transfected as in Example 23 (**FIG. 7A** and **FIG. 7B**).

Example 25 Culture of Cancer-Cell Lines

The cancer cell lines HeLa (cervical carcinoma), MDA-MB-231 (breast), HCT 116 (colon), U87 MG (glioma), and U-251 (glioma) were propagated in culture. Cells were cultured in DMEM + 10% FBS or DMEM + 50% FBS and maintained at 37°C, 5%CO_2, and either ambient O_2 or 5% O_2. Cells grew rapidly under all conditions, and were routinely passaged every 2-5 days using a solution of trypsin in HBSS.

Example 26 RiboSlice Gene-Editing RNA Design Process and Algorithm

The annotated DNA sequence of the BIRC5 gene was retrieved from NCBI using the eFetch utility and a python script. The same python script was used to identify the DNA sequences encoding the protein within each of the four exons of the BIRC5 gene. The script then searched these sequences, and the 40 bases flanking each side, for sequence elements satisfying the following conditions: (i) one element exists on the primary strand, the other on the complementary strand, (ii) each element begins with a T, and (iii) the elements are separated by no fewer than 12 bases and no more than 20 bases. Each element was then assigned a score representing its likelihood of binding to other elements within the human genome using Qblast (NCBI). This score was computed as the sum of the inverse of the nine lowest E-values, excluding the match to the target sequence. Pair scores were computed by adding the scores for the individual elements.

Example 27 Synthesis of RNA Encoding Gene-Editing Proteins (RiboSlice)

RNA encoding gene-editing proteins was designed according to Example 26, and synthesized according to Example 1 (Table 10, **FIG. 9**). The RNA was diluted with nuclease-free water to between 200ng/μL and 500ng/μL, and was stored at 4°C.

Table 10. RiboSlice Synthesis

Template (SEQ ID of Binding Site)	Nucleotides	Reaction Volume/μL	ivT Yield/μg
BIRC5-1.1L (SEQ ID NO: 16)	A, 0.5 7dG, 0.4 5mU, 5mC	20	124.1
BIRC5-1.1R (SEQ ID NO: 17)	A, 0.5 7dG, 0.4 5mU, 5mC	20	115.6
BIRC5-1.2L (SEQ ID NO: 18)	A, 0.5 7dG, 0.4 5mU, 5mC	20	120.3
BIRC5-1.2R (SEQ ID NO: 19)	A, 0.5 7dG, 0.4 5mU, 5mC	20	121.3
BIRC5-1.3L (SEQ ID NO: 20)	A, 0.5 7dG, 0.4 5mU, 5mC	20	120.3
BIRC5-1.3R (SEQ ID NO: 21)	A, 0.5 7dG, 0.4 5mU, 5mC	20	113.7
BIRC5-2.1L (SEQ ID NO: 22)	A, 0.5 7dG, 0.4 5mU, 5mC	20	105.3
BIRC5-2.1R (SEQ ID NO: 23)	A, 0.5 7dG, 0.4 5mU, 5mC	20	120.3
BIRC5-2.2L (SEQ ID NO: 24)	A, 0.5 7dG, 0.4 5mU, 5mC	20	101.5
BIRC5-2.2R (SEQ ID NO: 25)	A, 0.5 7dG, 0.4 5mU, 5mC	20	111.9
BIRC5-3.1L (SEQ ID NO: 26)	A, 0.5 7dG, 0.4 5mU, 5mC	20	107.2
BIRC5-3.1R (SEQ ID NO: 27)	A, 0.5 7dG, 0.4 5mU, 5mC	20	113.7

BIRC5-2.1L (SEQ ID NO: 22)	A, 0.5 7dG, 0.35 5mU, 5mC	300	577.9
BIRC5-2.1R (SEQ ID NO: 23)	A, 0.5 7dG, 0.35 5mU, 5mC	300	653.6

Example 28 Activity Analysis of RiboSlice targeting BIRC5

Primary adult human fibroblasts were transfected according to Example 2 with 6 RiboSlice pairs targeting BIRC5, designed according to Example 26, and synthesized according to Example 27. Two days after transfection, genomic DNA was isolated and purified. To measure gene-editing efficiency, 150ng of the amplified PCR product was hybridized with 150ng of reference DNA in 10mM Tris-Cl + 50mM KCl + 1.5mM $MgCl_2$. The hybridized DNA was treated with the SURVEYOR mismatch-specific endonuclease (Transgenomic, Inc.), and the resulting products were analyzed by agarose gel electrophoresis (**FIG. 10A**). All six of the tested RiboSlice pairs efficiently edited the BIRC5 gene, as demonstrated by the appearance of bands of the expected sizes (asterisks in **FIG. 10A**).

Example 29 Mitosis-Inhibition Analysis of RiboSlice targeting BIRC5

Primary adult human fibroblasts were gene edited according to Example 28, and were then propagated in culture. After 11 days, genomic DNA was isolated and purified, and gene-editing efficiency was measured as in Example 28 (**FIG. 10B**). None of the tested RiboSlice pairs inhibited the proliferation of the fibroblasts, as shown by the appearance of bands of the expected sizes (asterisks in **FIG. 10B**) in genomic DNA isolated from the proliferating cells, demonstrating the low toxicity to normal fibroblasts of these RiboSlice pairs.

Example 30 Anti-Cancer-Activity Analysis of RiboSlice targeting BIRC5

Primary adult human fibroblasts and HeLa cervical carcinoma cells, cultured according to Example 25 were transfected with RiboSlice pairs according to Example 28. Proliferation of the fibroblasts slowed briefly due to transfection reagent-associated toxicity, but recovered within 2 days of transfection. In contrast, proliferation of HeLa cells slowed markedly, and many enlarged cells with fragmented nuclei were observed in transfected wells. After 2-3 days, many cells exhibited morphology indicative of apoptosis, demonstrating the potent anti-cancer activity of RiboSlice targeting BIRC5.

Example 31 in vivo RiboSlice Safety Study

40 female NCr nu/nu mice were injected subcutaneously with 5 x 10^6 MDA-MB-231 tumor cells in 50% Matrigel (BD Biosciences). Cell injection volume was 0.2mL/mouse. The age of the mice at the start of the study was 8 to 12 weeks. A pair match was conducted, and animals were divided into 4 groups of 10 animals each when the tumors reached an average size of 100-150mm^3, and treatment was begun. Body weight was measured every day for the first 5 days, and then biweekly to the end of

the study. Treatment consisted of RiboSlice BIRC5-1.2 complexed with a vehicle (Lipofectamine 2000, Life Technologies Corporation). To prepare the dosing solution for each group, 308µL of complexation buffer (Opti-MEM, Life Technologies Corporation) was pipetted into each of two sterile, RNase-free 1.5mL tubes. 22µL of RiboSlice BIRC5-1.2 (500ng/µL) was added to one of the two tubes, and the contents of the tube were mixed by pipetting. 22µL of vehicle was added to the second tube. The contents of the second tube were mixed, and then transferred to the first tube, and mixed with the contents of the first tube by pipetting to form complexes. Complexes were incubated at room temperature for 10min. During the incubation, syringes were loaded. Animals were injected either intravenously or intratumorally with a total dose of 1µg RNA/animal in 60µL total volume/animal. A total of 5 treatments were given, with injections performed every other day. Doses were not adjusted for body weight. Animals were followed for 17 days. No significant reduction in mean body weight was observed (**FIG. 11**; RiboSlice BIRC5-1.2 is labeled "ZK1"), demonstrating the *in vivo* safety of RiboSlice gene-editing RNA.

Example 32 Anti-Cancer-Activity Analysis of RiboSlice targeting BIRC5 in a Glioma Model

The U-251 glioma cell line, cultured according to Example 25, was transfected with RiboSlice pairs according to Example 28. Glioma cells responded to treatment similarly to HeLa cells: proliferation slowed markedly, and many enlarged cells with fragmented nuclei were observed in transfected wells. After 2-3 days, many cells exhibited morphology indicative of apoptosis, demonstrating the potent anti-cancer activity of RiboSlice targeting BIRC5 in a glioma model.

Example 33 Screening of Reagents for Delivery of Nucleic Acids to Cells

Delivery reagents including polyethyleneimine (PEI), various commercial lipid-based transfection reagents, a peptide-based transfection reagent (N-TER, Sigma-Aldrich Co. LLC.), and several lipid-based and sterol-based delivery reagents were screened for transfection efficiency and toxicity *in vitro*. Delivery reagents were complexed with RiboSlice BIRC5-1.2, and complexes were delivered to HeLa cells, cultured according to Example 25. Toxicity was assessed by analyzing cell density 24h after transfection. Transfection efficiency was assessed by analyzing morphological changes, as described in Example 30. The tested reagents exhibited a wide range of toxicities and transfection efficiencies. Reagents containing a higher proportion of ester bonds exhibited lower toxicities than reagents containing a lower proportion of ester bonds or no ester bonds.

Example 34 High-Concentration Liposomal RiboSlice

High-Concentration Liposomal RiboSlice was prepared by mixing 1µg RNA at 500ng/µL with 3µL of complexation medium (Opti-MEM, Life Technologies Corporation), and 2.5µL of transfection reagent (Lipofectamine 2000, Life Technologies Corporation) per µg of RNA with 2.5µL of complexation medium. Diluted RNA and transfection reagent were then mixed and incubated for

10min at room temperature to form High-Concentration Liposomal RiboSlice. Alternatively, a transfection reagent containing DOSPA or DOSPER is used.

Example 35 In Vivo RiboSlice Efficacy Study – Subcutaneous Glioma Model

40 female NCr nu/nu mice were injected subcutaneously with 1 x 10^7 U-251 tumor cells. Cell injection volume was 0.2mL/mouse. The age of the mice at the start of the study was 8 to 12 weeks. A pair match was conducted, and animals were divided into 4 groups of 10 animals each when the tumors reached an average size of 35-50mm^3, and treatment was begun. Body weight was measured every day for the first 5 days, and then biweekly to the end of the study. Caliper measurements were made biweekly, and tumor size was calculated. Treatment consisted of RiboSlice BIRC5-2.1 complexed with a vehicle (Lipofectamine 2000, Life Technologies Corporation). To prepare the dosing solution, 294µL of complexation buffer (Opti-MEM, Life Technologies Corporation) was pipetted into a tube containing 196µL of RiboSlice BIRC5-1.2 (500ng/µL), and the contents of the tube were mixed by pipetting. 245µL of complexation buffer was pipetted into a tube containing 245µL of vehicle. The contents of the second tube were mixed, and then transferred to the first tube, and mixed with the contents of the first tube by pipetting to form complexes. Complexes were incubated at room temperature for 10min. During the incubation, syringes were loaded. Animals were injected intratumorally with a total dose of either 2µg or 5µg RNA/animal in either 20µL or 50µL total volume/animal. A total of 5 treatments were given, with injections performed every other day. Doses were not adjusted for body weight. Animals were followed for 25 days.

Example 36 Synthesis of High-Activity/High-Fidelity RiboSlice In Vitro-Transcription Template

An *in vitro*-transcription template encoding a T7 bacteriophage RNA-polymerase promoter, 5′-untranslated region, strong Kozak sequence, TALE N-terminal domain, 18 repeat sequences designed according to Example 26, TALE C-terminal domain, and nuclease domain comprising the StsI sequence (SEQ ID NO: 1), StsI-HA sequence (SEQ ID NO: 2), StsI-HA2 sequence (SEQ ID NO: 3), StsI-UHA sequence (SEQ ID NO: 4), StsI-UHA2 sequence (SEQ ID NO: 5), StsI-HF sequence (SEQ ID NO: 6) or StsI-HF2 sequence (SEQ ID NO: 7) is synthesized using standard cloning and molecular biology techniques, or alternatively, is synthesized by direct chemical synthesis, for example using a gene fragment assembly technique (e.g., gBlocks, Integrated DNA Technologies, Inc.).

Example 37 Synthesis of High-Activity/High-Fidelity RiboSlice Gene-Editing RNA

High-Activity RiboSlice and High-Fidelity RiboSlice are synthesized according to Example 27, using *in vitro*-transcription templates synthesized according to Example 36.

Example 38 Generation of RiboSlice-encoding Replication-Incompetent Virus for Treatment of Proteopathy

A nucleotide sequence comprising RiboSlice targeting a DNA sequence that encodes a plaque-forming protein sequence is incorporated into a mammalian expression vector comprising a replication-incompetent viral genome, and transfected into a packaging cell line to produce replication-incompetent virus. The culture supernatant is collected, and filtered using a 0.45μm filter to remove debris.

Example 39 Generation of RiboSlice-encoding Replication-Competent Oncolytic Virus for Treatment of Cancer

A nucleotide sequence comprising RiboSlice targeting the BIRC5 gene, is incorporated into a mammalian expression vector comprising a replication-competent viral genome, and transfected into a packaging cell line to produce replication-competent virus. The culture supernatant is collected and filtered, according to Example 38.

Example 40 in vivo RiboSlice Efficacy Study – Orthotopic Glioma Model, Intrathecal Route of Administration

40 female NCr nu/nu mice are injected intracranially with 1 x 10^5 U-251 tumor cells. Cell injection volume is 0.02mL/mouse. The age of the mice at the start of the study is 8 to 12 weeks. After 10 days, animals are divided into 4 groups of 10 animals each, and treatment is begun. Body weight is measured every day for the first 5 days, and then biweekly to the end of the study. Treatment consists of RiboSlice BIRC5-2.1 complexed with a vehicle (Lipofectamine 2000, Life Technologies Corporation). To prepare the dosing solution, 294μL of complexation buffer (Opti-MEM, Life Technologies Corporation) is pipetted into a tube containing 196μL of RiboSlice BIRC5-1.2 (500ng/μL), and the contents of the tube are mixed by pipetting. 245μL of complexation buffer is pipetted into a tube containing 245μL of vehicle. The contents of the second tube are mixed, and then transferred to the first tube, and mixed with the contents of the first tube by pipetting to form complexes. Complexes are incubated at room temperature for 10min. During the incubation, syringes are loaded. Animals are injected intrarthecally with a total dose of 1-2μg RNA/animal in 10-20μL total volume/animal. A total of 5 treatments are given, with injections performed every other day. Doses are not adjusted for body weight. Animals are followed for 60 days.

Example 41 Treatment of Glioma with RiboSlice – IV Perfusion

A patient with a diagnosis of glioma is administered 1mg of High-Concentration Liposomal RiboSlice BIRC5-2.1, prepared according to Example 34 by IV infusion over the course of 1h, 3 times a week for 4 weeks. For an initial tumor volume of greater than 500mm^3, the tumor is debulked surgically and optionally by radiation therapy and/or chemotherapy before RiboSlice treatment is begun. The patient is optionally administered TNF-α and/or 5-FU using a standard dosing regimen as a combination therapy.

Example 42 Treatment of Glioma with RiboSlice – Replication-Competent Oncolytic Virus

A patient is administered 1mL of replicating virus particles (1000CFU/mL), prepared according to Example 39, by intrathecal or intracranial injection.

Example 43 Treatment of Parkinson's Disease with RiboSlice Targeting SNCA

A patient with a diagnosis of Parkinson's disease is administered 50μg of RiboSlice targeting the SNCA gene by intrathecal or intracranial injection.

Example 44 Treatment of Alzheimer's Disease with RiboSlice Targeting APP

A patient with a diagnosis of Alzheimer's disease is administered 50μg of RiboSlice targeting the APP gene by intrathecal or intracranial injection.

Example 45 Treatment of Type II Diabetes with RiboSlice Targeting IAPP

A patient with a diagnosis of type II diabetes is administered 5mg of RiboSlice targeting the IAPP gene by intravenous, intraperitoneal or intraportal injection.

Example 46 iRiboSlice Personalized Cancer Therapy

A biopsy is taken from a patient with a diagnosis of cancer. Genomic DNA is isolated and purified from the biopsy, and the sequence of the DNA (either the whole-genome sequence, exome sequence or the sequence of one or more cancer-associated genes) is determined. A RiboSlice pair targeting the patient's individual cancer sequence (iRiboSlice) is designed according to Example 26 and synthesized according to Example 27. The patient is administered the personalized iRiboSlice using a route of administration appropriate for the location and type of cancer.

Example 47 RiboSlice Mixtures for Genetically Diverse/Treatment-Resistant Cancer

A patient with a diagnosis of genetically diverse and/or treatment-resistant cancer is administered a mixture of RiboSlice pairs targeting multiple cancer-associated genes and/or multiple sequences in one or more cancer-associated genes.

Example 48 Mito-RiboSlice for mitochondrial disease

A patient with a diagnosis of a mitochondrial disease is administered 2mg of RiboSlice targeting the disease-associated sequence and containing a mitochondrial localization sequence by intramuscular injection.

Example 49 Treatment of Eye Disease with RiboSlice Eye Drops

A patient with a diagnosis of a corneal or conjunctival disease is administered RiboSlice formulated as a 0.5% isotonic solution.

Example 50 Treatment of Skin Disease with RiboSlice Topical Formulation

A patient with a diagnosis of a skin disease is administered RiboSlice formulated as a 1% topical cream/ointment containing one or more stabilizers that prevent degradation of the RNA.

Example 51 Treatment of Lung or Respiratory Disease with RiboSlice Aerosol Formulation

A patient with a diagnosis of a lung or respiratory disease is administered RiboSlice formulated as a 0.5% aerosol spray.

Example 52 Treatment of Infectious Disease with RiboSlice Targeting a DNA Sequence Present in the Infectious Agent

A patient with a diagnosis of an infectious disease is administered RiboSlice targeting a sequence present in the specific infectious agent with which the patient is infected using a route of administration appropriate to the location and type of infection, and a dose appropriate for the route of administration and severity of the infection.

Example 53 Gene-Edited Human Zygotes for in vitro Fertilization

A human germ cell, zygote or early-stage blastocyst is transfected with RiboSlice targeting a gene that encodes a disease-associated mutation or mutation associated with an undesired trait. The genome is characterized, and the cell is prepared for *in vitro* fertilization.

Example 54 Cleavage-Domain Screen for Activity, Fidelity Enhancement of Gene-Editing Proteins

A panel of RiboSlice pairs, each comprising a different cleavage domain, are designed according to Example 26 and synthesized according to Example 27. The activity of the RiboSlice pairs is determined as in Example 28.

Example 55 Gene-Edited Cells for Screening Parkinson's Disease-Causing Toxicants

Primary human adult fibroblasts are gene edited according to Example 28 using RiboSlice targeting SNCA (Table 11) and repair templates to generate cells with the SNCA A30P, E46K, and A53T mutations. Cells are reprogrammed and differentiated to dopaminergic neurons. The neurons are used in a high-throughput α-synuclein-aggregation toxicant-screening assay to identify toxicants that can contribute to Parkinson's disease.

Table 11. RiboSlice Pairs for Generation of SNCA A30P, E46K, and A53T.

Exon	Target Amino Acid	Left RiboSlice Binding Site	Right RiboSlice Binding Site	Spacing
1	A30	TGAGAAAACCAAACAGGGTG	TAGAGAACACCCTCTTTTGT	20
2	E46	TGTTTTTGTAGGCTCCAAAA	TACCTGTTGCCACACCATGC	16
2	A53	TCCAAAACCAAGGAGGGAGT	TAAGCACAATGGAGCTTACC	19

Example 56 Gene-Edited Cells for Screening Cancer-Causing Toxicants

Primary human adult fibroblasts are gene edited according to Example 28 using RiboSlice targeting TP53 (Table 12) and repair templates to generate cells with the TP53 P47S, R72P, and V217M mutations. Cells are reprogrammed and differentiated to hepatocytes. The hepatocytes are used in a high-throughput *in vitro*-transformation toxicant-screening assay to identify toxicants that can contribute to cancer.

Table 12. RiboSlice Pairs for Generation of TP53 P47S, R72P, and V217M.

Exon	Target Amino Acid	Left RiboSlice Binding Site	Right RiboSlice Binding Site	Spacing
4	P47	TCCCAAGCAATGGATGATTT	TGAACCATTGTTCAATATCG	15
4	R72	TGAAGCTCCCAGAATGCCAG	TAGGAGCTGCTGGTGCAGGG	19
6	V217	TGGATGACAGAAACACTTTT	TCAGGCGGCTCATAGGGCAC	15

Example 57 Design and Synthesis of RNA Encoding Engineered Gene-Editing Proteins (RiboSlice)

RNA encoding gene-editing proteins designed according to Example 26 was synthesized according to Example 27 (Table 13). Each gene-editing protein comprised a DNA-binding domain comprising a transcription activator-like (TAL) effector repeat domain comprising 35-36 amino acid-long repeat sequences, as indicated in Table 13. Sequence ID numbers are given for the 36 amino acid-long repeat sequences. The label "18" in the template name indicates that the 18th repeat sequence was 36 amino acids long. The label "EO" in the template name indicates that every other repeat sequence was 36 amino acids long. The amino acids following the label "18" or "EO" indicate the amino acids at the C-terminus of the 36 amino acid-long repeat sequence(s). The label "StsI" indicates that the nuclease domain contained the StsI cleavage domain. Templates without the "StsI" label contained the FokI cleavage domain.

Table 13. RiboSlice Encoding Engineered Gene-Editing Proteins.

Template (SEQ ID of Repeat Sequence)	Nucleotides	Reaction Volume/µL	ivT Yield/µg
BIRC5-2.1L-18-AHGGG (SEQ ID NO: 54)	A, 0.5 7dG, 0.4 5mU, 5mC	20	11.9
BIRC5-2.1R-18-AHGGG (SEQ ID NO: 54)	A, 0.5 7dG, 0.4 5mU, 5mC	20	11.9
BIRC5-2.1L-18-AGHGG (SEQ ID NO: 55)	A, 0.5 7dG, 0.4 5mU, 5mC	20	10.7
BIRC5-2.1R-18-AGHGG (SEQ ID NO: 55)	A, 0.5 7dG, 0.4 5mU, 5mC	20	10.9
BIRC5-2.1L-18-AHGSG (SEQ ID NO: 56)	A, 0.5 7dG, 0.4 5mU, 5mC	20	11.9
BIRC5-2.1R-18-AHGSG (SEQ ID NO: 56)	A, 0.5 7dG, 0.4 5mU, 5mC	20	12.7

BIRC5-2.1L-18-AHGGG (SEQ ID NO: 54)	A, 0.5 7dG, 0.4 5mU, 5mC	20	34.5
BIRC5-2.1R-18-AHGGG (SEQ ID NO: 54)	A, 0.5 7dG, 0.4 5mU, 5mC	20	34.8
BIRC5-2.1L-18-AGHGG (SEQ ID NO: 55)	A, 0.5 7dG, 0.4 5mU, 5mC	20	32.7
BIRC5-2.1R-18-AGHGG (SEQ ID NO: 55)	A, 0.5 7dG, 0.4 5mU, 5mC	20	37.4
BIRC5-2.1L-18-AHGSG (SEQ ID NO: 56)	A, 0.5 7dG, 0.4 5mU, 5mC	20	31.5
BIRC5-2.1R-18-AHGSG (SEQ ID NO: 56)	A, 0.5 7dG, 0.4 5mU, 5mC	20	34.1
BIRC5-2.1L	A, 0.5 7dG, 0.4 5mU, 5mC	20	34.9
BIRC5-2.1R	A, 0.5 7dG, 0.4 5mU, 5mC	20	25.9
BIRC5-2.1L	A, 0.5 7dG, 0.4 5mU, 5mC	20	41.5
BIRC5-2.1R	A, 0.5 7dG, 0.4 5mU, 5mC	20	38.8
BIRC5-2.1L-StsI	A, 0.5 7dG, 0.4 5mU, 5mC	20	22.2
BIRC5-2.1R-StsI	A, 0.5 7dG, 0.4 5mU, 5mC	20	18.4
BIRC5-2.1L-EO-AGHGG (SEQ ID NO: 55)	A, 0.5 7dG, 0.4 5mU, 5mC	20	21.6
BIRC5-2.1L	A, 0.5 7dG, 0.4 5mU, 5mC	20	17.3
BIRC5-2.1L-StsI	A, G, U, C	10	71.3
BIRC5-2.1R-StsI	A, G, U, C	10	75.1
BIRC5-2.1L-EO-AGHGG (SEQ ID NO: 55)	A, G, U, C	10	66.4
BIRC5-2.1R-EO-AGHGG (SEQ ID NO: 55)	A, G, U, C	10	52.4

Example 58 Activity Analysis of RiboSlice targeting BIRC5

The activity of RiboSlice molecules synthesized according to Example 57 was analyzed according to Example 28 (**FIG. 12A**, **FIG. 12B**, and **FIG. 14**). High-efficiency gene editing was observed in cells expressing gene-editing proteins containing one or more 36 amino acid-long repeat sequences. Gene-editing efficiency was highest in cells expressing gene-editing proteins containing one or more repeat sequences containing the amino-acid sequence: GHGG.

Example 59 in vivo RiboSlice AAV Safety and Efficacy Study – Subcutaneous Glioma Model, Intratumoral Route of Delivery

Animals were set up with tumors comprising U-251 human glioma cells according to Example 35. AAV serotype 2 encoding GFP, BIRC5-2.1L RiboSlice, and BIRC5-2.1R RiboSlice was prepared according to standard techniques (AAV-2 Helper Free Expression System, Cell Biolabs, Inc.). Viral stocks were stored at 4°C (short term) or -80°C (long term). Animals received intratumoral injections

of either 160μL GFP AAV on day 1 or 80μL BIRC5-2.1L RiboSlice AAV + 80μL BIRC5-2.1R RiboSlice AAV on day 1 and day 15. Animals were followed for 25 days. No significant reduction in mean body weight was observed (**FIG. 13A**), demonstrating the *in vivo* safety of RiboSlice AAV. Tumor growth was inhibited in the RiboSlice AAV group (**FIG. 13B**), demonstrating the *in vivo* efficacy of RiboSlice AAV.

Example 60 Treatment of Cancer with RiboSlice AAV

A patient is administered 1mL of RiboSlice AAV virus particles, prepared according to Example 59, by intrathecal or intracranial injection. Dosing is repeated as necessary. For a patient with an initial tumor volume of greater than $500mm^3$, the tumor is debulked surgically and optionally by radiation therapy and/or chemotherapy before RiboSlice AAV treatment is begun. The patient is optionally administered TNF-α and/or 5-FU using a standard dosing regimen as a combination therapy.

Example 61 iRiboSlice AAV Personalized Cancer Therapy

A biopsy is taken from a patient with a diagnosis of cancer. Genomic DNA is isolated and purified from the biopsy, and the sequence of the DNA (either the whole-genome sequence, exome sequence or sequence of one or more cancer-associated genes) is determined. A RiboSlice pair targeting the patient's individual cancer sequence (iRiboSlice) is designed according to Example 26 and synthesized according to Example 59. The patient is administered the personalized iRiboSlice AAV using a route of administration appropriate for the location and type of cancer.

Example 62 Liposome Formulation and Nucleic-Acid Encapsulation

Liposomes are prepared using the following formulation: 3.2mg/mL N-(carbonyl-ethoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (MPEG2000-DSPE), 9.6mg/mL fully hydrogenated phosphatidylcholine, 3.2mg/mL cholesterol, 2mg/mL ammonium sulfate, and histidine as a buffer. pH is controlled using sodium hydroxide and isotonicity is maintained using sucrose. To form liposomes, lipids are mixed in an organic solvent, dried, hydrated with agitation, and sized by extrusion through a polycarbonate filter with a mean pore size of 800nm. Nucleic acids are encapsulated by combining 10μg of the liposome formulation per 1μg of nucleic acid and incubating at room temperature for 5 minutes.

Example 63 Folate-Targeted Liposome Formulation

Liposomes are prepared according to Example 62, except that 0.27mg/mL 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-5000] (FA-MPEG5000-DSPE) is added to the lipid mixture.

Example 64 Cancer Therapy Comprising Liposomal RiboSlice Targeting BIRC5

Liposomes encapsulating RiboSlice pairs synthesized according to Example 23 are prepared according to Example 62 or Example 63. The liposomes are administered by injection or intravenous infusion, and tumor response and interferon plasma levels are monitored daily.

Example 65 Cancer Therapy Comprising Liposomal RiboSlice Targeting a Cancer-Associated Gene

Liposomes encapsulating RiboSlice targeting a cancer-associated gene, synthesized according to Example 1, are prepared according to Example 62 or Example 63. The liposomes are administered by injection or intravenous infusion, and tumor response and interferon plasma levels are monitored daily.

Example 66 Therapy Comprising Liposomal Protein-Encoding RNA

Liposomes encapsulating synthetic RNA encoding a therapeutic protein, synthesized according to Example 1, are prepared according to Example 62 or Example 63. The liposomes are administered by injection or intravenous infusion.

Example 67 Combination Cancer Therapy Comprising RiboSlice Targeting BIRC5 and TNF-α

Patients are administered isolated limb perfusion (ILP) with tumor necrosis factor alpha (TNF-α) and liposomes encapsulating RiboSlice targeting BIRC5 (see Example 64). Following warming of the limb, liposomes are injected into the arterial line of the extracorporeal ILP circuit over approximately 5 minutes, and perfusion proceeds for another 85 minutes. After 1-2 days, ILP is repeated with TNF-α injected into the arterial line of the extracorporeal ILP circuit over 3-5 minutes and perfusion continues for an additional 60 minutes. Tumor response and interferon plasma levels are monitored daily.

Example 68 Combination Cancer Therapy Comprising RiboSlice Targeting BIRC5 and Fluorouracil (5-FU)

On day 1 patients receive a 60-minute intravenous infusion of liposomes encapsulating RiboSlice targeting BIRC5 (see Example 64), followed by a 46-hour intravenous infusion of 5-FU on days 2 and 3. Tumor response and interferon plasma levels are monitored daily.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

INCORPORATION BY REFERENCE

All patents and publications referenced herein are hereby incorporated by reference in their entireties.

CLAIMS

What is claimed is:

1. A synthetic RNA molecule comprising at least two of: 5-methyluridine, 5-methylcytidine, and 7-deazaguanosine.

2. The synthetic RNA molecule of claim 1, further comprising at least one residue of each of 5-methyluridine, 5-methylcytidine, and 7-deazaguanosine.

3. The synthetic RNA molecule of claim 1, wherein the synthetic RNA molecule comprises uridine moieties and between about 20% and about 80% of the uridine moieties are 5-methyluridines.

4. The synthetic RNA molecule of claim 1, wherein the synthetic RNA molecule comprises cytidine moieties and between about 50% and about 100% of the cytidine moieties are 5-methylcytidines.

5. The synthetic RNA molecule of claim 1, wherein the synthetic RNA molecule comprises guanosine moieties and between about 20% and about 80% of the guanosine moieties are 7-deazaguanosines.

6. The synthetic RNA molecule of claim 2, wherein the synthetic RNA molecule comprises uridine, cytidine, and guanosine moieties and between about 20% and about 80% of the uridines are 5-methyluridines, between about 50% and about 100% of the cytidines are 5-methylcytidines, and between about 20% and about 80% of the guanosines are 7-deazaguanosines.

7. The synthetic RNA molecule of claim 1, further comprising a 5′-cap structure.

8. The synthetic RNA molecule of claim 7, wherein the 5′-cap structure is the Cap 1 structure.

9. The synthetic RNA molecule of claim 1, further comprising a 3′-poly(A) tail.

10. The synthetic RNA molecule of claim 1, further comprising a UTR.

11. The synthetic RNA molecule of claim 1, further comprising a strong Kozak sequence.

12. The synthetic RNA molecule of claim 1, wherein the synthetic RNA molecule encodes a reprogramming protein.

13. The synthetic RNA molecule of claim 12, wherein the reprogramming protein is selected from Oct4 protein, Sox2 protein, Klf4 protein, c-Myc protein, or Lin28 protein.

14. The synthetic RNA molecule of claim 1, wherein the synthetic RNA molecule encodes a gene-editing protein.

15. The synthetic RNA molecule of claim 14, wherein the gene-editing protein comprises a DNA binding domain and the catalytic domain of a DNA endonuclease or a biologically active fragment thereof.

16. The synthetic RNA molecule of claim 14, wherein the gene-editing protein comprises at least one of: a zinc-finger nuclease, a transcription activator-like effector nuclease, a nuclease, a meganuclease, a nickase, and a clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein or a biologically active fragment or variant thereof.

17. A nucleic acid encoding a gene-editing protein, wherein the gene-editing protein targets a member of: TERT, TERC, HBB, BRCA1, BRCA2, CCR5, CFTR, CXCR4, DDB2, DMD, EGFR, ERCC4, ERCC5, ERCC6, F9, F8, F11, SLC40A1, HAMP, HEXA, HFE, HJV, the JAK family, the MYC family, NF1, NF2, TP53, POLH, RAD2, PKN3, the RAS family, BIRC5, TFR2, XPA, XPB, XPC, XPD, Rosa26, AAVS1, FBN1, HTT, APP, PSEN1, PSEN2, APOE, SNCA, PRKN, LRRK2, CR1, CLU, PICALM, BIN1, MS4A4, MS4A6E, CD2AP, CD33, EPHA1, PINK1, PARK7, ATP13A2, HNPCC1, HNPCC2, HNPCC5, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN, FANCP, RAD51C, the VEGF family, GCK, HNF1A, HNF4A, HNF1B, SOD1, PTEN, RET, KIT, MET, APC, RB1, and TNF or a variant thereof.

18. The synthetic RNA molecule of claim 14, wherein the gene-editing protein reduces the expression of one or more proteins.

19. A nucleic acid encoding a gene-editing protein, wherein the gene-editing protein produces a gene that encodes a non-functional protein.

20. A nucleic acid encoding a gene-editing protein, wherein the gene-editing protein produces a gene that encodes a dominant-negative protein.

21. A nucleic acid encoding a gene-editing protein, wherein the gene-editing protein targets a sequence that is not present in the non-cancer human genome.

22. A nucleic acid encoding a gene-editing protein, wherein the gene-editing protein targets a member of: a viral sequence, a bacterial sequence, a fungal sequence, a parasite sequence, and a cancer-genome sequence.

23. The nucleic acid of claim 17, wherein the gene-editing protein targets at least one of: TTGCCCCCTGCCTGGCAGCC and TTCTTGAATGTAGAGATGCG.

24. A synthetic RNA molecule encoding a gene-editing protein comprising at least one of: pseudouridine, 5-methylpseudouridine, 5-methyluridine, 5-methylcytidine, 5-

hydroxymethylcytidine, N4-methylcytidine, N4-acetylcytidine, and 7-deazaguanosine or a derivative thereof.

25. A nucleic acid encoding a gene-editing protein, wherein the gene-editing protein reduces the expression of a survivin protein.

26. A nucleic acid encoding a gene-editing protein, wherein the gene-editing protein produces a gene that encodes a non-functional variant of a survivin protein.

27. A nucleic acid encoding a gene-editing protein, wherein the gene-editing protein produces a gene that encodes a dominant-negative variant of a survivin protein.

28. A therapeutic composition comprising the synthetic RNA molecule of claim 1 or the nucleic acid of claim 17.

29. The therapeutic composition of claim 28, further comprising a delivery reagent.

30. The therapeutic composition of claim 29, wherein the delivery reagent comprises a lipid.

31. The therapeutic composition of claim 29, wherein the delivery reagent comprises a polyethylene glycol or a derivative thereof.

32. The therapeutic composition of claim 28, wherein the therapeutic composition is prepared as a sterile, aqueous suspension of particles.

33. The therapeutic composition of claim 32, wherein the particles are liposomes.

34. The therapeutic composition of claim 28, further comprising a second synthetic RNA molecule or nucleic acid.

35. The therapeutic composition of claim 28, further comprising a repair template.

36. A method of treating cancer, comprising administering an effective amount of the therapeutic composition of claim 28 to a patient in need thereof.

37. The method of claim 36, wherein the cancer includes at least one of: prostate cancer, colon cancer, breast cancer, lung cancer, skin cancer, brain cancer, leukemia, pancreatic cancer, liver cancer, bladder cancer, stomach cancer, bone cancer, testicular cancer, sarcoma, carcinoma, throat cancer, renal cancer, lymphoma, heart cancer, cervical cancer, ovarian cancer, eye cancer, uterine cancer, ductal cancer, gall bladder cancer, oral cancer, neck cancer, mesothelioma, nasal cancer, sinus cancer, penile cancer, anal cancer, salivary gland cancer, small-intestine cancer, thyroid cancer, urethral cancer, vaginal cancer, and vulvar cancer.

38. A method for inducing a cell to express a protein of interest, comprising contacting the cell with the synthetic RNA molecule of claim 1 or the nucleic acid of claim 17.

39. A cell produced by the method of claim 38.

40. An organism produced by implanting the cell of claim 39 into a blastocyst or a uterus.

41. The organism of claim 40, wherein the organism is selected from rat, mouse, rabbit, guinea pig, primate, pig, cow, chicken, goat, donkey, cat, dog, and zebrafish.

42. The organism of claim 40, wherein the cell is further contacted with a nucleic acid encoding a human gene or fragment thereof.

43. The organism of claim 42, wherein the human gene is inserted into the genome of the organism.

44. The organism of claim 40, wherein the gene-editing protein targets one or more endogenous orthologues of the human gene.

45. The organism of claim 44, wherein the one or more endogenous orthologues is inactivated.

46. The cell of claim 39, wherein the cell is further differentiated into a member of: a skin cell, a glucose-responsive insulin-producing cell, a hematopoietic cell, a cardiac cell, a retinal cell, a renal cell, a neural cell, a stromal cell, a fat cell, a bone cell, a muscle cell, an oocyte, and a sperm cell.

47. The cell of claim 39, wherein the cell is cultured in a high-throughput screening-compatible format.

48. The cell of claim 47, wherein the format is a multi-well plate.

49. A therapeutic composition comprising the cell of claim 39.

50. A composition for altering the DNA sequence of a living cell, comprising a nucleic acid, encoding a gene-editing protein, wherein the gene-editing protein comprises:

a. a DNA-binding domain, and

b. a nuclease domain,

wherein the DNA-binding domain comprises a plurality of repeat sequences, at least two of the repeat sequences having at least 50% homology to each other, and at least one of the repeat sequences containing one or more regions capable of binding to a binding site in a target DNA molecule, the binding site containing a defined sequence of between 1 and 5 bases in length, and the nuclease domain comprises the catalytic domain of a protein selected from StsI, StsI-HA, StsI-HA2, StsI-UHA, StsI-UHA2, StsI-HF, StsI-UHF or a biologically active fragment thereof.

51. A composition for altering the DNA sequence of a living cell comprising a nucleic-acid mixture comprising:

a. a first nucleic acid that encodes a first gene-editing protein, and

b. a second nucleic acid that encodes a second gene-editing protein,

wherein the first gene-editing protein or the second gene-editing protein or both the first gene-editing protein and the second gene-editing protein comprises:

i. a DNA-binding domain, and

ii. a nuclease domain,

wherein the DNA-binding domain comprises a plurality of repeat sequences, at least two of the repeat sequences having at least 50% homology to each other, and at least one of the repeat sequences containing one or more regions capable of binding to a binding site in a target DNA molecule, the binding site containing a defined sequence of between 1 and 5 bases in length, and the nuclease domain comprises the catalytic domain of a protein selected from FokI, StsI, StsI-HA, StsI-HA2, StsI-UHA, StsI-UHA2, StsI-HF, StsI-UHF or a biologically active fragment thereof.

52. A method for modifying the genome of a cell, comprising introducing into the cell a nucleic acid molecule encoding a non-naturally occurring fusion protein comprising an artificial transcription activator-like (TAL) effector repeat domain comprising one or more repeat units 36 amino acids in length and an endonuclease domain, wherein the repeat domain is engineered for recognition of a predetermined nucleotide sequence, wherein the fusion protein recognizes the predetermined nucleotide sequence.

53. The method of claim 52, wherein the cell is a eukaryotic cell.

54. The method of claim 52, wherein the cell is an animal cell.

55. The method of claim 52, wherein the cell is a mammalian cell.

56. The method of claim 52, wherein the cell is a human cell.

57. The method of claim 52, wherein the cell is a plant cell.

58. The method of claim 52, wherein the cell is a prokaryotic cell.

59. The method of claim 52, wherein the fusion protein introduces an endonucleolytic cleavage in a nucleic acid of the cell, whereby the genome of the cell is modified.

60. A nucleic acid molecule encoding a non-naturally occurring fusion protein, comprising an artificial transcription activator-like (TAL) effector repeat domain comprising one or more repeat units 36 amino acids in length and restriction endonuclease activity, wherein the repeat domain is engineered for recognition of a predetermined nucleotide sequence and wherein the fusion protein recognizes the predetermined nucleotide sequence.

61. The nucleic acid molecule of claim 60, wherein each of the repeat units differ by no more than seven amino acids.

62. The nucleic acid molecule of claim 60, wherein each of the repeat units contain the amino acid sequence: LTPXQVVAIAS where X can be either E or Q, and wherein the amino acid

sequence LTPXQVVAIAS is followed on the carboxyl terminus by either one or two amino acids that determine recognition for one of adenine, cytosine, guanine or thymine.

63. The nucleic acid of claim 60, encoding about 1.5 to about 28.5 repeat units.

64. The nucleic acid molecule of claim 60, encoding about 11.5, about 14.5, about 17.5 or about 18.5 repeat units.

65. The nucleic acid molecule of claim 60, wherein the predetermined nucleotide sequence is a promoter region.

66. A vector containing the nucleic acid molecule of claim 60.

67. The vector of claim 66, wherein the vector is a viral vector.

68. The viral vector of claim 67, wherein the vector comprises one or more of an adenovirus, a retrovirus, a lentivirus, a herpes virus, an adeno-associated virus, and an engineered virus.

69. A nucleic acid molecule encoding a non-naturally occurring fusion protein, comprising a first region that recognizes a predetermined nucleotide sequence and a second region with endonuclease activity, wherein the first region contains an artificial TAL effector repeat domain comprising one or more repeat units 36 acids in length which differ from each other by no more than seven amino acids, wherein the repeat domain is engineered for recognition of the predetermined nucleotide sequence.

70. The nucleic acid molecule of claim 69, wherein the first region contains the amino acid sequence: LTPXQVVAIAS where X can be either E or Q.

71. The nucleic acid molecule of claim 70, wherein the amino acid sequence LTPXQVVAIAS of the encoded non-naturally occurring fusion protein is immediately followed by an amino acid sequence selected from the group consisting of: HD, NG, NS, NI, NN, and N.

72. The nucleic acid molecule of claim 69, wherein the fusion protein comprises restriction endonuclease activity.

73. A vector containing the nucleic acid molecule of claim 69.

74. The vector of claim 73, wherein the vector is a viral vector.

75. The viral vector of claim 74, wherein the vector comprises one or more of an adenovirus, a retrovirus, a lentivirus, a herpes virus, an adeno-associated virus, and an engineered virus.

76. A nucleic acid molecule encoding a protein that comprises one or more sequences selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, and SEQ ID NO: 60.

77. The composition of claim 50, wherein the nucleic acid further comprises a nuclear-localization sequence.

78. The composition of claim 77, wherein the nuclear-localization sequence includes the amino-acid sequence PKKKRKV.

79. The composition of claim 50, wherein the nucleic acid further comprises a mitochondrial-localization sequence.

80. The composition of claim 30, wherein the mitochondrial-localization sequence includes the amino-acid sequence LGRVIPRKIASRASLM.

81. The composition of claim 50, wherein the DNA-binding domain and the nuclease domain are separated by a linker.

82. The composition of claim 81, wherein the linker is between about 1 to about 10 amino acids long.

83. A gene-editing protein comprising a plurality of repeat sequences, wherein the plurality of repeat sequences comprises at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is N, H or I, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA.

84. The gene-editing protein of claim 83, wherein the plurality of repeat sequences includes at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is N, H or I, "y" is selected from: D, A, I, N, H, K, S, and G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA.

85. The gene-editing protein of claim 83, wherein the plurality of repeat sequences includes at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is any amino acid other than N, H and I, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA.

86. The gene-editing protein of claim 83, wherein the plurality of repeat sequences includes at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwIyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "y" is any amino acid other than G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA.

87. The gene-editing protein of claim 83, wherein the plurality of repeat sequences includes at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwIAzGHGG, wherein "v" is Q, D or E, "w" is S or N, and "z" is GGRPALE,

GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA.

88. The gene-editing protein of claim 83, wherein the plurality of repeat sequences includes at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is S, T or Q, "y" is any amino acid or no amino acid, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA.

89. The gene-editing protein of claim 83, wherein the plurality of repeat sequences includes at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwxyzGHGG, wherein "v" is Q, D or E, "w" is S or N, "x" is S, T or Q, "y" is selected from: D, A, I, N, H, K, S, and G, and "z" is GGRPALE, GGKQALE, GGKQALETVQRLLPVLCQD, GGKQALETVQRLLPVLCQA, GKQALETVQRLLPVLCQD or GKQALETVQRLLPVLCQA.

90. The gene-editing protein of claim 83, wherein the plurality of repeat sequences includes at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwx, wherein "v" is Q, D or E, "w" is S or N, and "x" is S, T or Q.

91. The gene-editing protein of claim 83, wherein the plurality of repeat sequences includes at least one repeat sequence that comprises the amino acid sequence: LTPvQVVAIAwxy, wherein "v" is Q, D or E, "w" is S or N, "x" is S, T or Q, and "y" is selected from: D, A, I, N, H, K, S, and G.

92. The composition of claim 51, wherein the binding site of the first gene-editing protein and the binding site of the second gene-editing protein are present in the same target DNA molecule.

93. The composition of claim 92, wherein the binding site of the first gene-editing protein and the binding site of the second gene-editing protein are separated by no more than about 50 bases.

94. The composition of claim 50, wherein the gene-editing protein is capable of generating a nick or double-strand break in the target DNA molecule.

95. The composition of claim 51, wherein the nuclease domain of the first gene-editing protein and the nuclease domain of the second gene-editing protein are capable of forming a dimer, and the dimer is capable of generating a nick or double-strand break in the target DNA molecule.

96. The composition of claim 50, wherein the plurality of repeat sequences includes at least one repeat sequence having at least about 50% homology to a transcription activator-like effector monomer.

97. The composition of claim 50, wherein the plurality of repeat sequences includes at least one zinc finger monomer.

98. The composition of claim 50, wherein the nucleic acid is a synthetic RNA molecule.

99. The composition of claim 98, wherein the synthetic RNA molecule comprises at least one of pseudouridine, 5-methylpseudouridine, 5-methyluridine, 5-methylcytidine, 5-hydroxymethylcytidine, N4-methylcytidine, N4-acetylcytidine, and 7-deazaguanosine.

100. An article of manufacture for synthesizing a gene-editing protein or nucleic-acid encoding a gene-editing protein comprising a nucleic acid, wherein the nucleic acid comprises:

a. a nucleotide sequence encoding a DNA-binding domain, and

b. a nucleotide sequence encoding a nuclease domain,

wherein the DNA-binding domain comprises a plurality of repeat sequences, at least two of the repeat sequences having at least about 50% homology to each other, and at least one of the repeat sequences containing one or more regions capable of binding to a binding site in a target DNA molecule, the binding site containing a defined sequence of between about 1 and about 5 bases in length, and the nuclease domain comprises the catalytic domain of a protein selected from: FokI, StsI, StsI-HA, StsI-HA2, StsI-UHA, StsI-UHA2, StsI-HF, StsI-UHF or a biologically active fragment thereof.

101. The article of claim 100, wherein the nucleic acid further comprises an RNA-polymerase promoter.

102. The article of claim 101, wherein the RNA polymerase promoter is selected from a T7 promoter and a SP6 promoter.

103. The article of claim 100, wherein the nucleic acid further comprises a viral promoter.

104. The article of claim 100, wherein the nucleic acid further comprises an untranslated region.

105. The article of claim 100, wherein the nucleic acid is an *in vitro*-transcription template.

106. A method for inducing a living cell to express a gene-editing protein, comprising transfecting the cell with the composition of claim 50.

107. A method for altering the DNA sequence of a living cell, comprising transfecting a cell with the composition of claim 50.

108. A method for reducing the expression of a protein of interest in a living cell comprising transfecting the cell with the composition of claim 50, wherein the target DNA molecule encodes the protein of interest.

109. A method for altering the DNA sequence of a living cell to generate an inactive, reduced-activity or dominant-negative version of a protein of interest comprising transfecting the cell with the composition of claim 50, wherein the target DNA molecule encodes the protein of interest, and resulting in the cell generating an inactive, reduced-activity or dominant-negative version of the protein.

110. The method of claim 109, wherein the protein of interest is survivin.

111. A method for treating a patient comprising administering to the patient a therapeutically effective amount of the composition of claim 50, and resulting in one or more of the patient's symptoms being ameliorated.

112. A method for treating cancer comprising administering to a patient a therapeutically effective amount of the composition of claim 50, and resulting in the growth of cancer cells in the patient being reduced and/or halted.

113. The composition of claim 50, wherein the target DNA molecule comprises the BIRC5 gene.

114. A composition for altering the DNA sequence of a living cell comprising a gene-editing protein, wherein the gene-editing protein is capable of binding to a sequence having at least 50% homology with a sequence selected from: SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15.

115. A composition for altering the DNA sequence of a living cell comprising a gene-editing protein, wherein the gene-editing protein is capable of binding to one or more binding sites, and wherein a plurality of the binding sites are at least 50% homologous to two or more of: SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27.

116. The composition of claim 50, further comprising a repair template.

117. A method for treating a patient comprising:

a. removing a cell from the patient,

b. inducing the cell to express a gene-editing protein by transfecting the cell with the composition of claim 50,

c. reprogramming the cell by transfecting the cell with one or more nucleic acids encoding one or more reprogramming proteins,

d. differentiating the cell into one of a skin cell, a glucose-responsive insulin-producing cell, a hematopoietic cell, a cardiac cell, a retinal cell, a renal cell, a neural cell, a stromal cell, a fat cell, a bone cell, a muscle cell, an oocyte, and a sperm cell, and

e. introducing the cell into the patient.

118. A method for treating a patient comprising:

a. removing a hematopoietic cell or stem cell from the patient,

b. inducing the cell to express a gene-editing protein by transfecting the cell with the composition of claim 50, and

c. introducing the cell into the patient.

119. A method for treating cancer, comprising:

a. removing a biopsy containing one or more cancerous cells from a patient,

b. determining the sequence of a cancer-associated genetic marker in the one or more cancerous cells, and

c. administering to the patient a therapeutically effective amount of the composition of claim 50, wherein the sequence of the target DNA molecule is at least about 50% homologous to the sequence of the cancer-associated genetic marker.

120. The method of claim 119, further comprising comparing the sequence of one or more cancer-associated genetic markers in the one or more cancerous cells to the sequence of the same cancer-associated genetic markers in one or more non-cancerous cells, selecting a cancer-associated genetic marker having a sequence that is different in the one or more cancerous cells and the one or more non-cancerous cells, and wherein the sequence of the target DNA molecule is at least about 50% homologous to the sequence of the selected cancer-associated genetic marker.

121. A method for treating a neurodegenerative disease comprising administering to a patient a therapeutically effective amount of a gene-editing protein or a nucleic acid encoding a gene-editing protein, wherein the gene-editing protein is capable of binding to a nucleotide sequence that encodes a protein that forms disease-associated plaques, and resulting in delayed or halted progression of the disease and/or reduction of disease-associated plaques in the patient.

122. The method of claim 121, wherein the nucleotide sequence comprises the SNCA gene.

123. The method of claim 121, wherein the nucleotide sequence encodes α-synuclein.

124. The method of claim 121, wherein the neurodegenerative disease is selected from Parkinson's disease, Alzheimer's disease, and dementia.

125. A kit for altering the DNA sequence of a living cell comprising the composition of claim 50.

126. A kit for altering the DNA sequence of a living human cell comprising the composition of claim 50, wherein the target DNA molecule comprises a nucleotide sequence that encodes the AAVS1 locus.

127. A kit for altering the DNA sequence of a living rodent cell comprising the composition of claim 50, wherein the target DNA molecule comprises a nucleotide sequence that encodes the Rosa26 locus.

128. A method for identifying a disease-causing toxicant, comprising transfecting a living cell with a gene-editing protein or a nucleic acid encoding a gene-editing protein to alter the DNA sequence of the cell, wherein the altered DNA sequence confers susceptibility to a disease, contacting the cell with a suspected disease-causing toxicant, and assessing the degree to which the cell exhibits a phenotype associated with the disease.

129. The method of claim 128, wherein the disease is a neurodegenerative disease, autoimmune disease, respiratory disease, reproductive disorder, or cancer.

130. A method for assessing the safety of a therapeutic substance comprising transfecting a living cell with a gene-editing protein or a nucleic acid encoding a gene-editing protein to alter the DNA sequence of the cell, wherein the altered DNA sequence confers susceptibility to one or more toxic effects of the therapeutic substance, contacting the cell with the therapeutic substance, and measuring one or more toxic effects of the therapeutic substance on the cell.

131. A method for assessing the effectiveness of a therapeutic substance comprising transfecting a living cell with a gene-editing protein or a nucleic acid encoding a gene-editing protein to alter the DNA sequence of the cell, wherein the altered DNA sequence causes the cell to exhibit one or more disease-associated phenotypes, contacting the cell with the therapeutic substance, and measuring the degree to which the one or more disease-associated phenotypes are reduced.

132. A method for treating an infectious disease comprising administering to a patient a therapeutically effective amount of a gene-editing protein or a nucleic acid encoding a gene-editing protein, wherein the gene-editing protein is capable of binding to one or more nucleotide sequences that are present in the infectious agent.

133. The method of claim 112, wherein the cancer is glioma.

134. The method of claim 112, wherein the patient has previously undergone surgery or radiation therapy for the removal of cancer.

135. The method of claim 112, wherein the administering is by one or more of: intrathecal injection, intracranial injection, intravenous injection, perfusion, subcutaneous injection, intraperitoneal injection, intraportal injection, and topical delivery.

136. The method of claim 111, wherein the patient is diagnosed with a proteopathy.

137. The kit of claim 125, further comprising a repair template.

138. The kit of claim 137, wherein the repair template contains a multiple cloning site.

139. A method for expressing a protein of interest in a cell by contacting the cell with a synthetic RNA molecule comprising 5-methyluridine, 7-deazaguanosine, and at least one of: 5-methylcytidine, 5-hydroxymethylcytidine, N4-methylcytidine, and N4-acetylcytidine.

140. The method of claim 139, wherein the synthetic RNA molecule comprises uridine residues, and about 40% of the uridine residues are 5-methyluridine residues.

141. The method of claim 139, wherein the synthetic RNA molecule comprises cytidine residues, and between about 40% and about 100% of the cytidine residues are selected from: 5-methylcytidine residues, 5-hydroxymethylcytidine residues, N4-methylcytidine residues, and N4-acetylcytidine residues.

142. The method of claim 139, wherein the synthetic RNA molecule comprises guanosine residues, and about 50% of the guanosine residues are 7-deazaguanosine residues.

143. A nucleic acid encoding a protein of interest comprising 5-methyluridine, 7-deazaguanosine, and at least one of: 5-methylcytidine, 5-hydroxymethylcytidine, N4-methylcytidine, and N4-acetylcytidine.

144. A method for increasing homologous recombination in a cell comprising:

a. contacting the cell with a NHEJ inhibitor,

b. transfecting the cell with one or more nucleic acids encoding one or more gene-editing proteins, wherein at least one of the one or more gene-editing proteins binds to a target region, and

c. transfecting the cell with one or more nucleic acids having at least 50% homology to the target region.

145. The method of claim 144, wherein the NHEJ inhibitor is a DNA-PK inhibitor.

146. The method of claim 144, wherein the DNA-PK inhibitor is a member of: Compound 401 (2-(4-Morpholinyl)-4*H*-pyrimido[2,1-*a*]isoquinolin-4-one), DMNB, IC87361, LY294002, NU7026, NU7441, OK-1035, PI 103 hydrochloride, vanillin, and wortmannin or a derivative thereof.

147. A method for treating Duchenne muscular dystrophy, comprising administering to a patient one or more gene-editing proteins or a nucleic acid encoding one or more gene-editing proteins, wherein the one or more gene-editing proteins target a sequence within the DMD gene.

148. The method of claim 147, wherein the treatment results in the production of a truncated form of DMD protein.

149. The method of claim 147, wherein the target sequence is within about 1kb of a splice acceptor site.

FIG. 1A

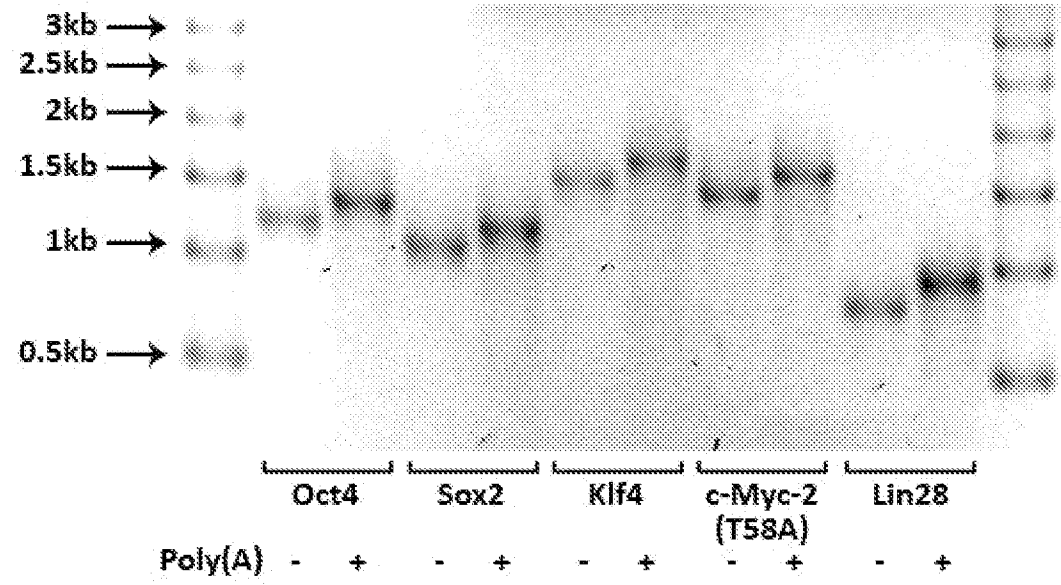


FIG. 1B

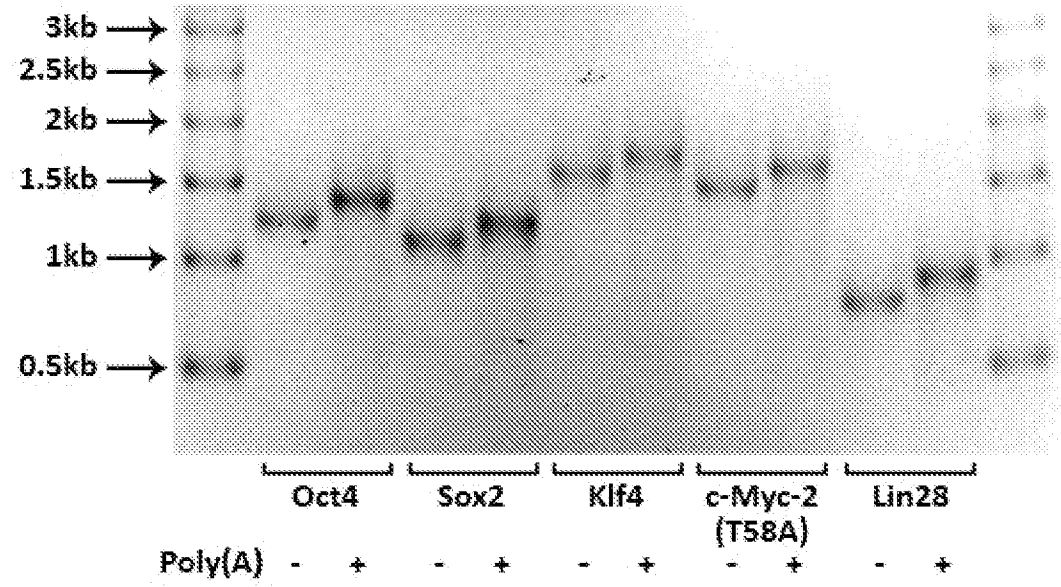


FIG. 2

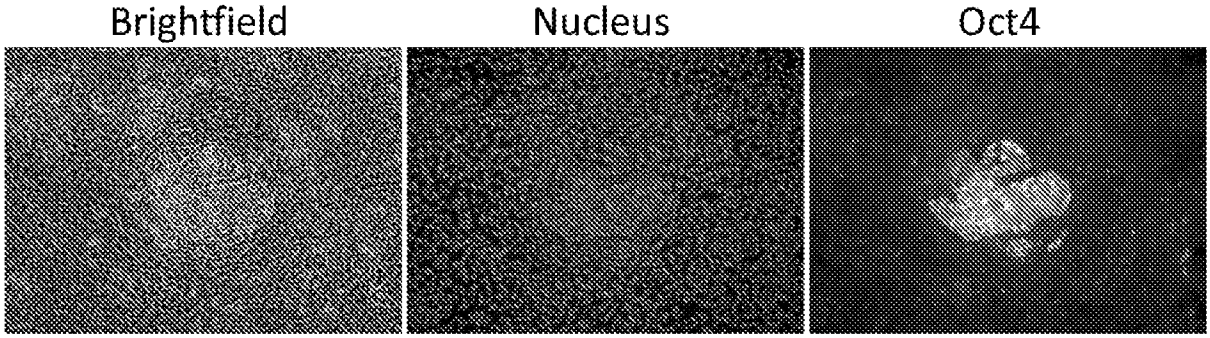


FIG. 3A

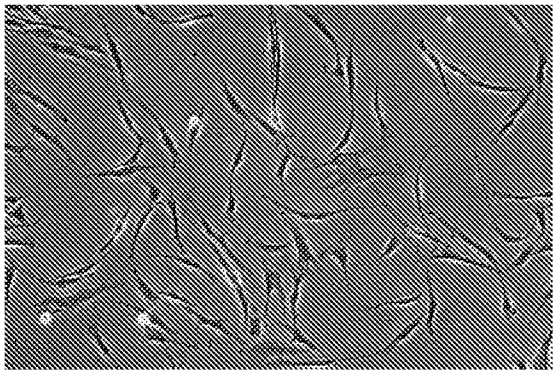
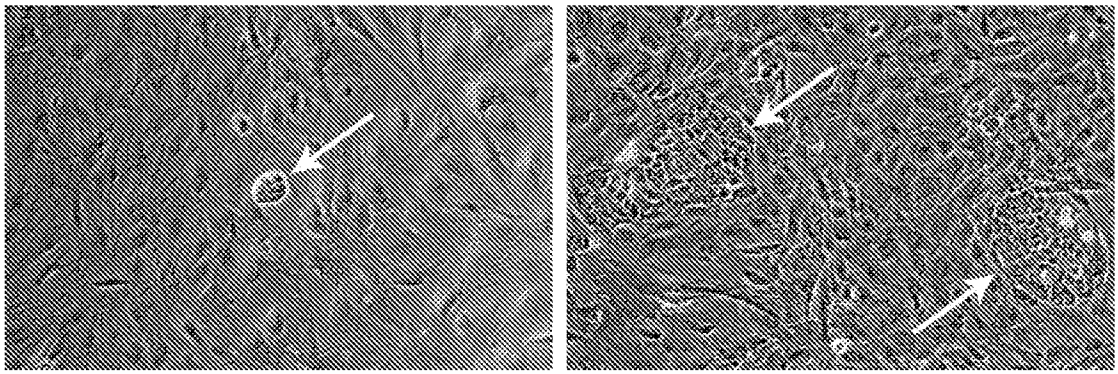


FIG. 3B



FIB. 3C

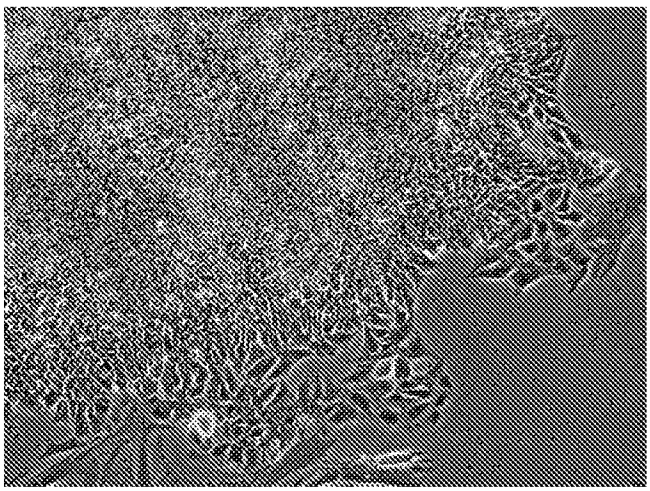


FIG. 4A

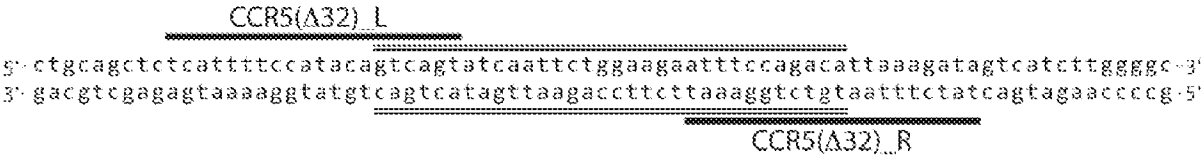


FIG. 4B

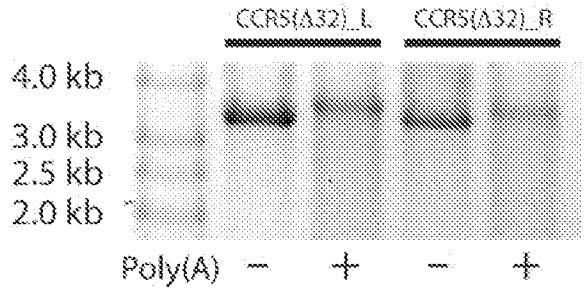


FIG. 4C

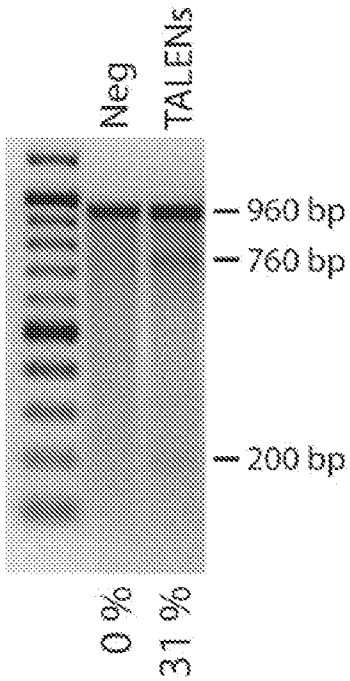


FIG. 4D

FIG. 4E

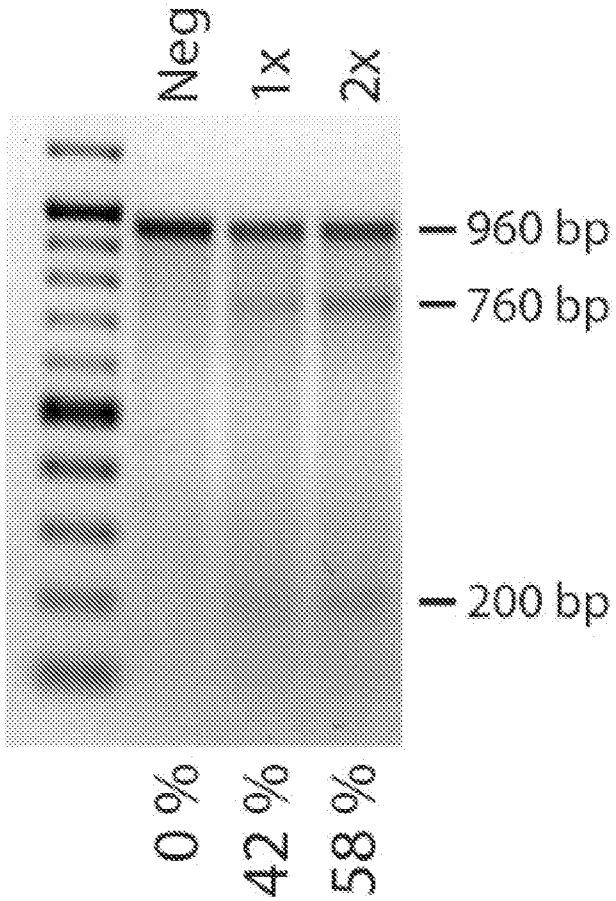


FIG. 4F

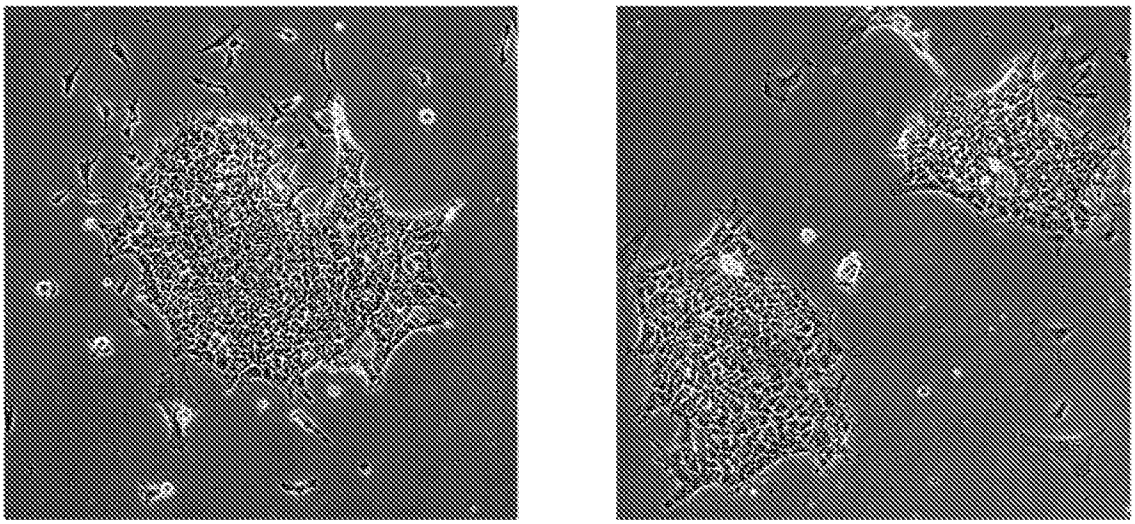


FIG. 4G

CCR5(Δ32)_L
CCR5(Δ32)_R
GM00609
CCR5(Δ32) deletion
GM00609_Control_iPS_1,2
& GM00609_TalCCR5_iPS_5-9
GM00609_TalCCR5_iPS_1 (1 bp deletion)
GM00609_TalCCR5_iPS_2 (9 bp deletion)
GM00609_TalCCR5_iPS_3 (6 bp deletion)
GM00609_TalCCR5_iPS_4 (6 bp deletion)

FIG. 5

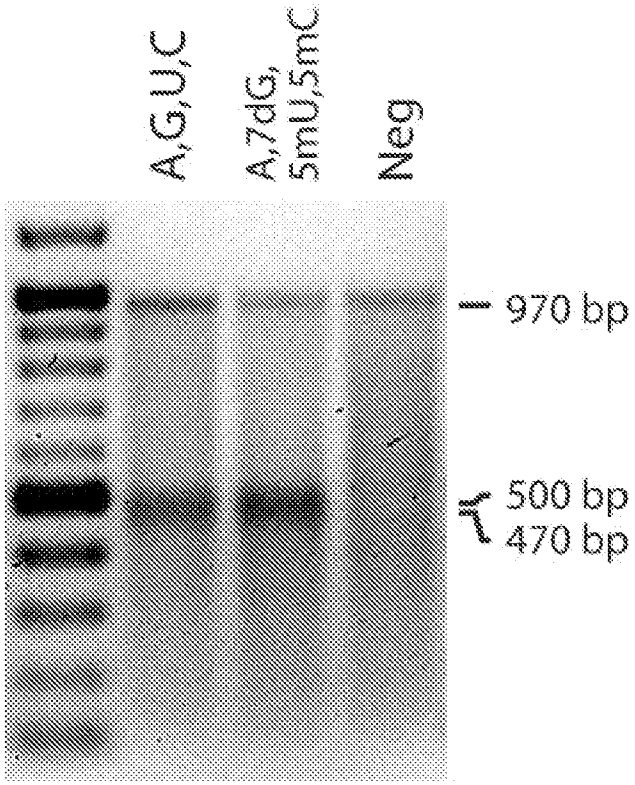

A,G,U,C
A,7dG, 5mU,5mC
Neg
970 bp
500 bp
470 bp

FIG. 6

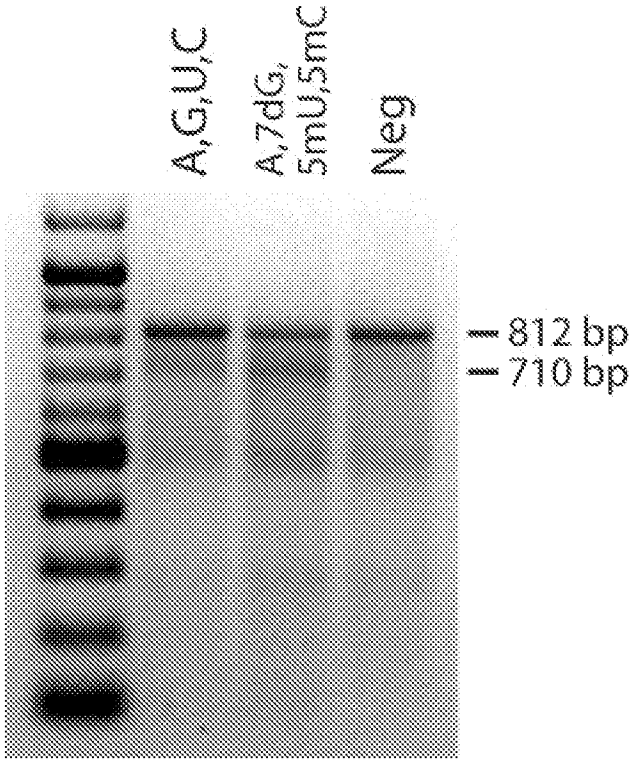

A,G,U,C
A,7dG, 5mU,5mC
Neg
812 bp
710 bp

FIG. 7A

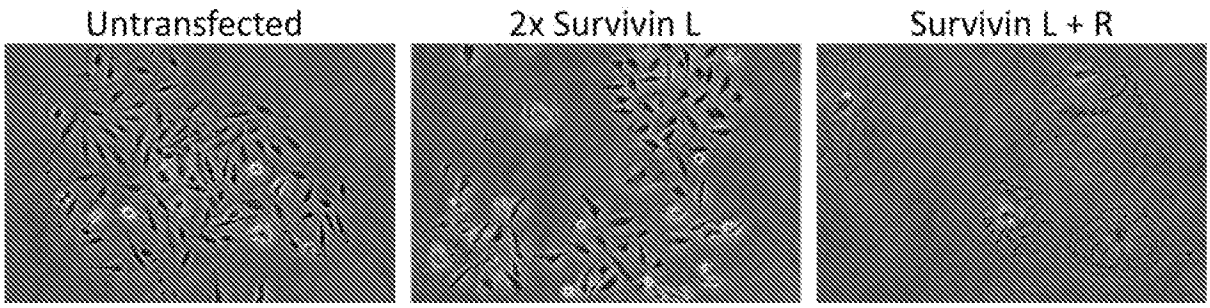

Untransfected
2x Survivin L
Survivin L + R

FIG. 7B

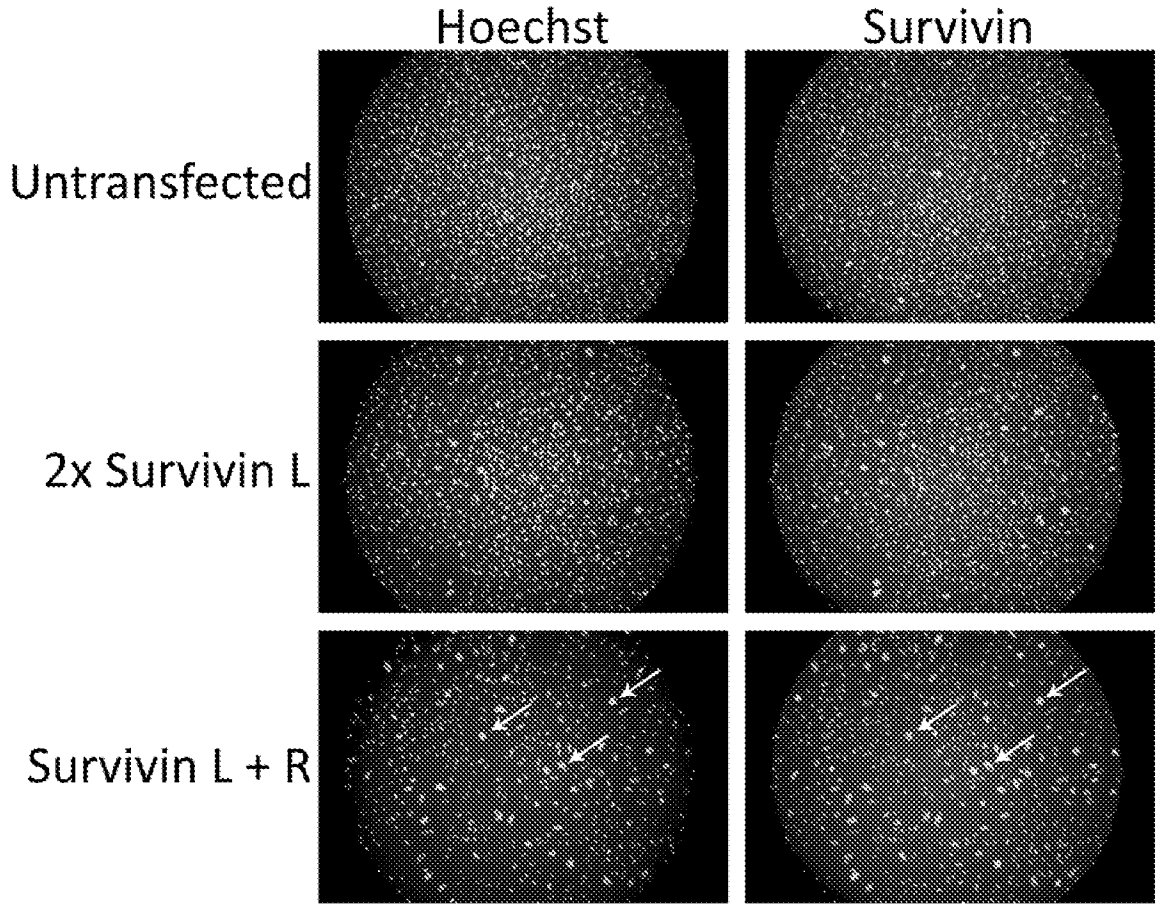

Hoechst
Survivin
Untransfected
2x Survivin L
Survivin L + R

FIG. 8

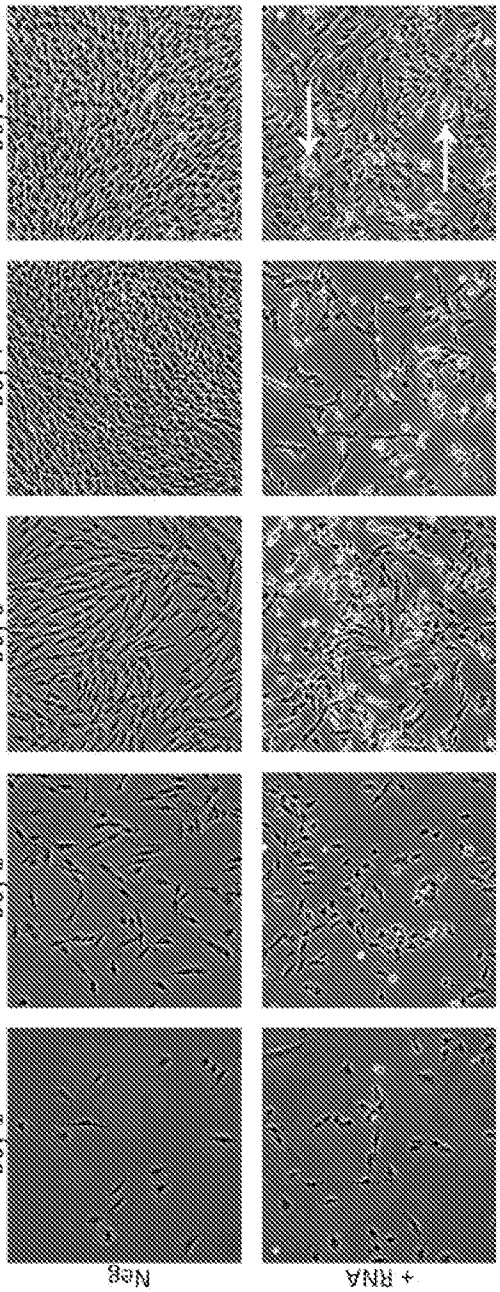

Day 1
Day 2
Day 3
Day 4
Day 5
NE8
+ RNA

FIG. 9

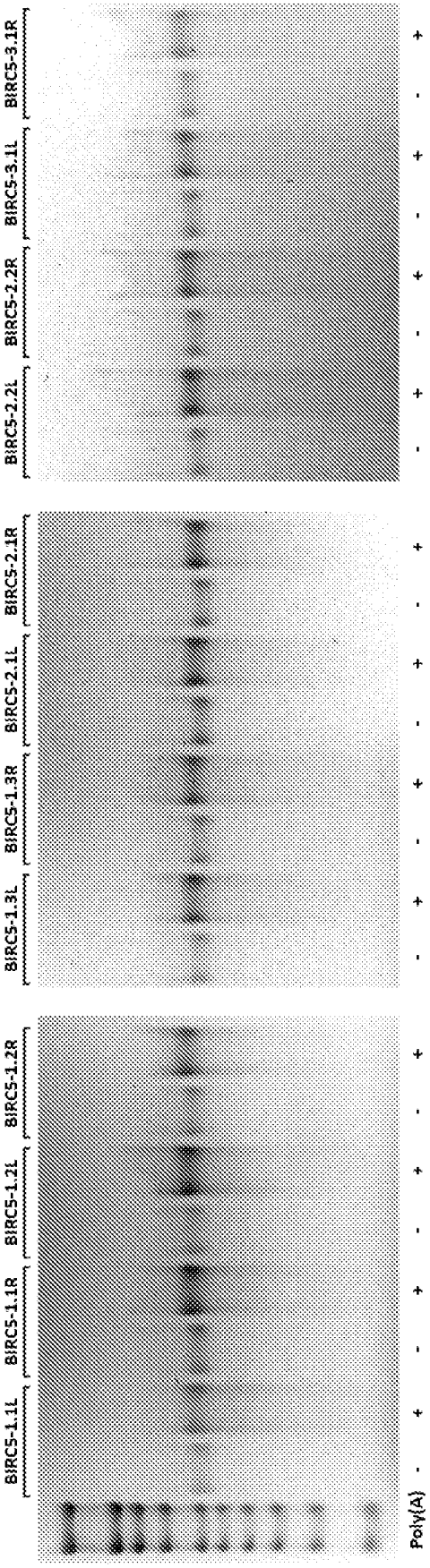

BIRC5-1.1L
BIRC5-1.1R
BIRC5-1.2L
BIRC5-1.2R
BIRC5-1.3L
BIRC5-1.3R
BIRC5-2.1L
BIRC5-2.1R
BIRC5-2.2L
BIRC5-2.2R
BIRC5-3.1L
BIRC5-3.1R
Poly(A)
9kb
6kb
5kb
4kb
3kb
2.5kb
2kb
1.5kb
1kb
0.5kb

FIG. 10A

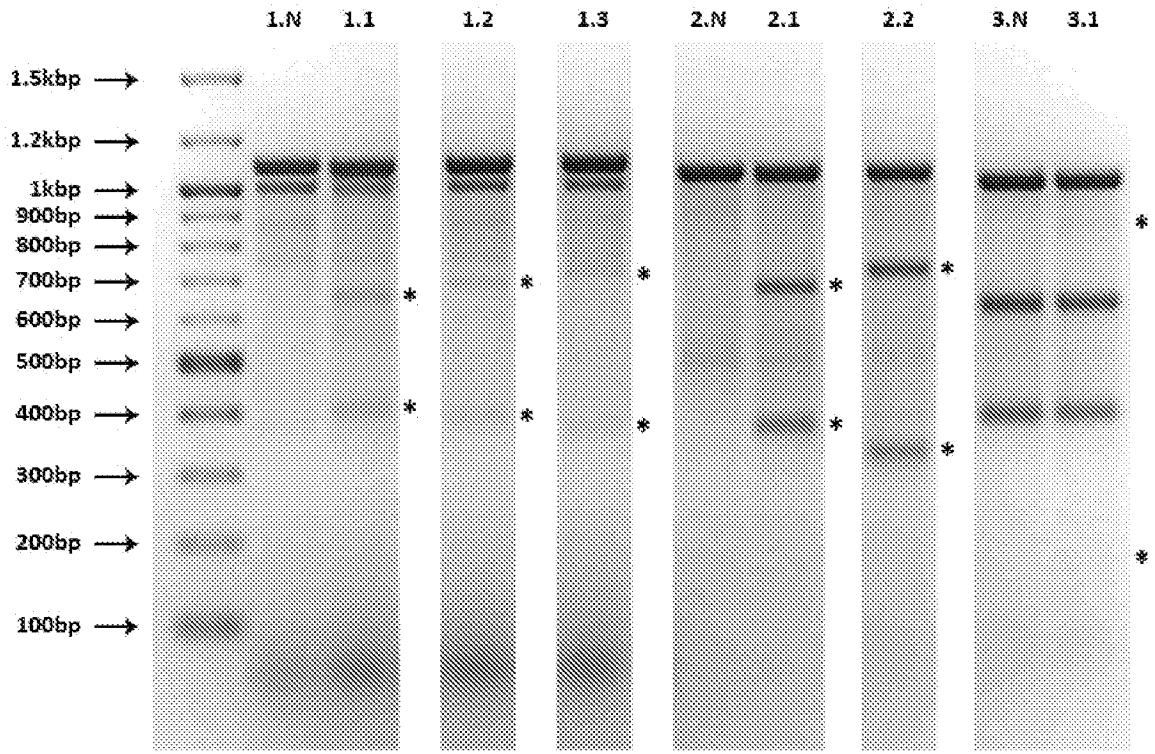


FIG. 10B

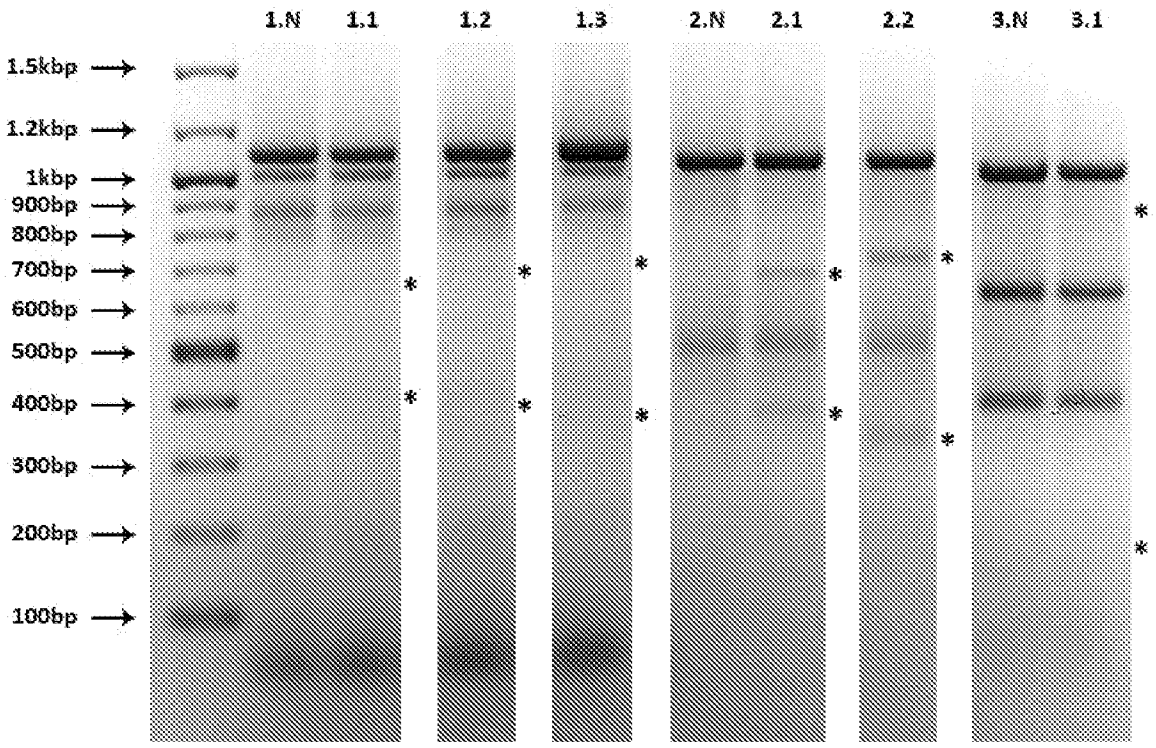


FIG. 11

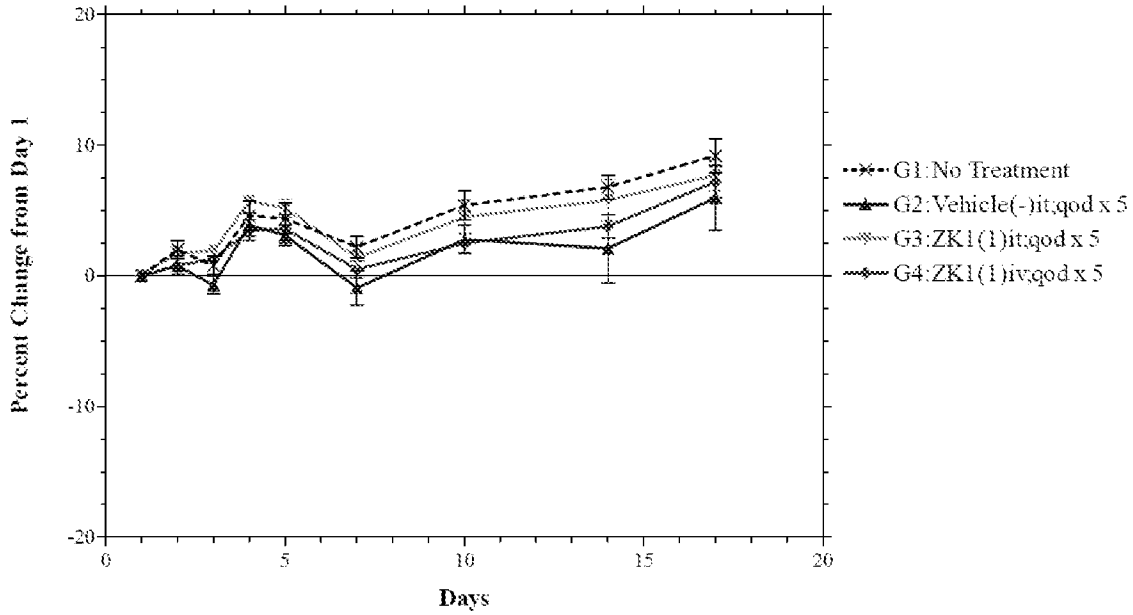


FIG. 12A

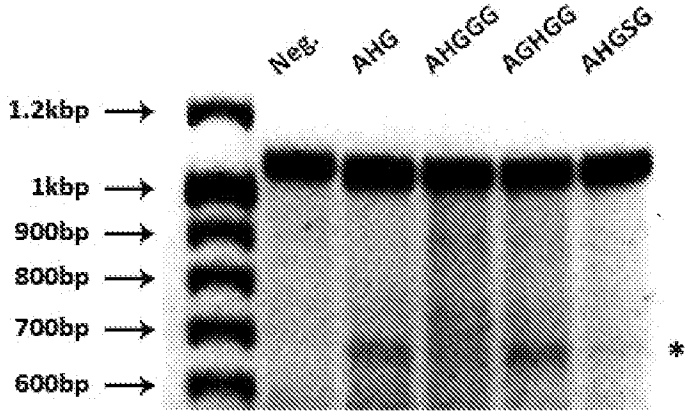


FIG. 12B

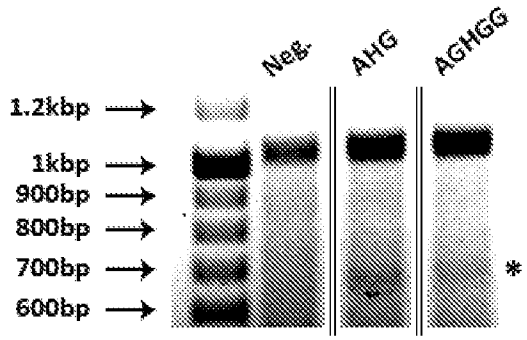


FIG. 13A

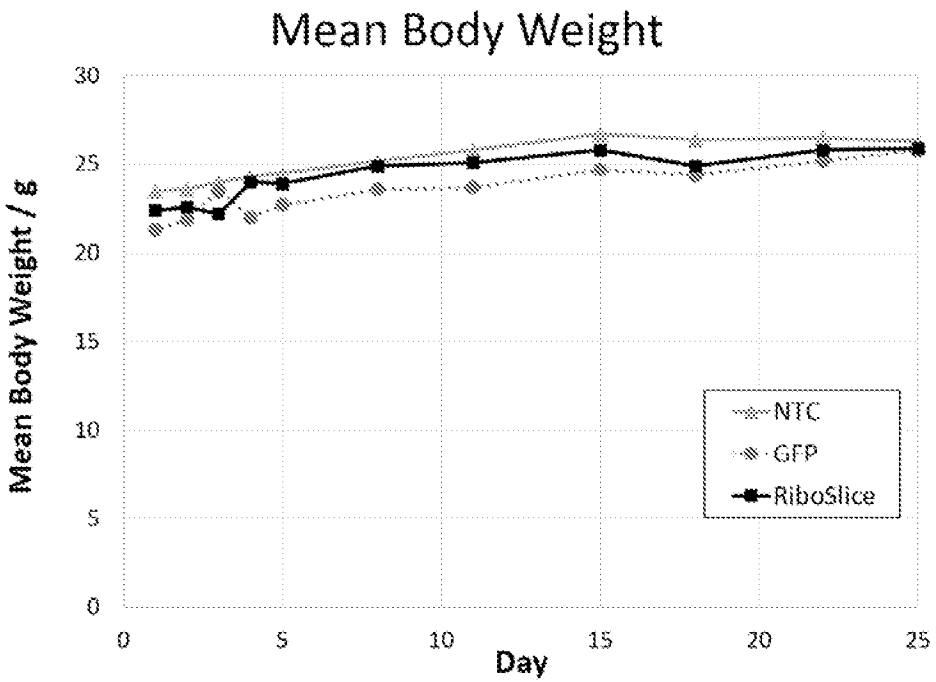


FIG. 13B

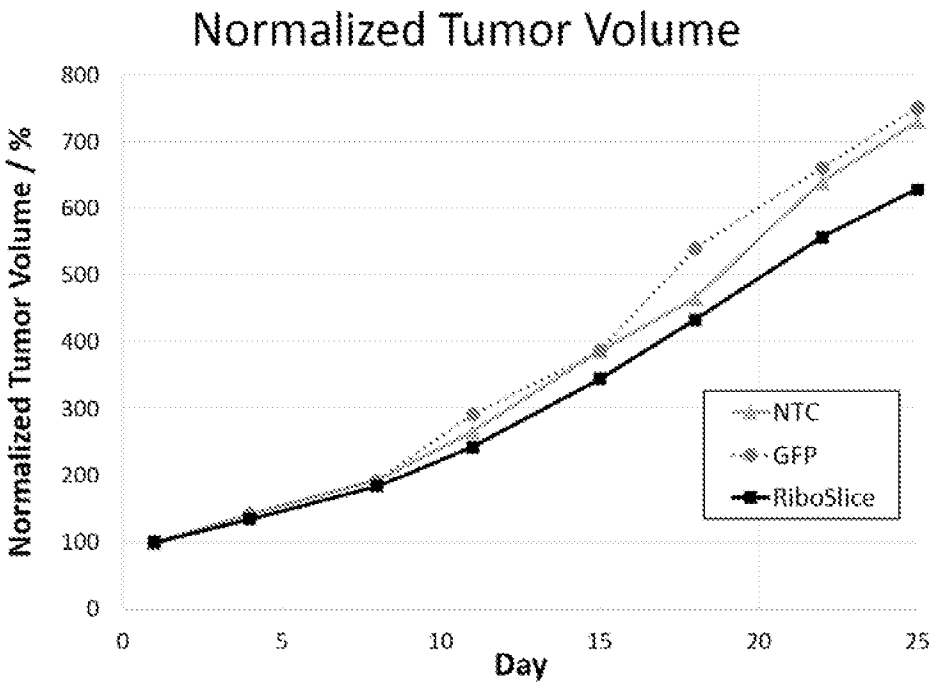


FIG. 14

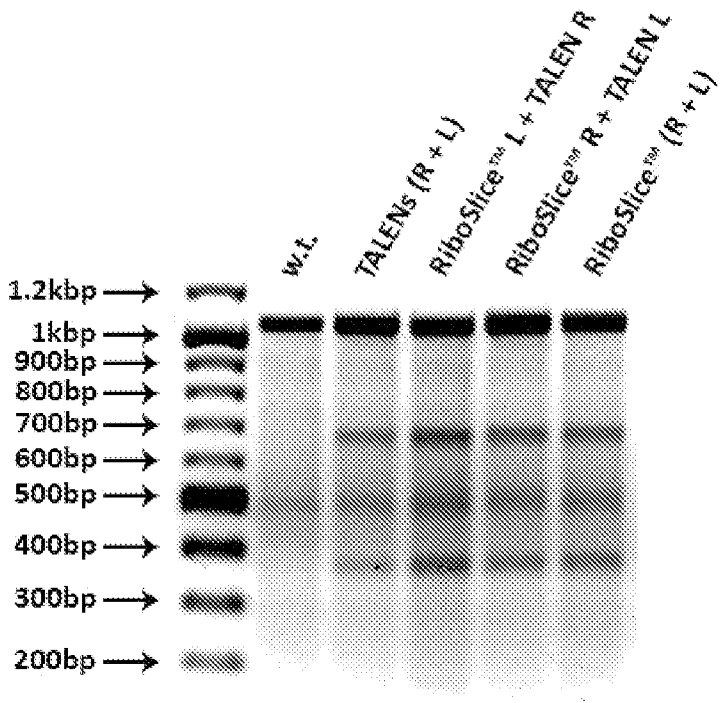


FIG. 15

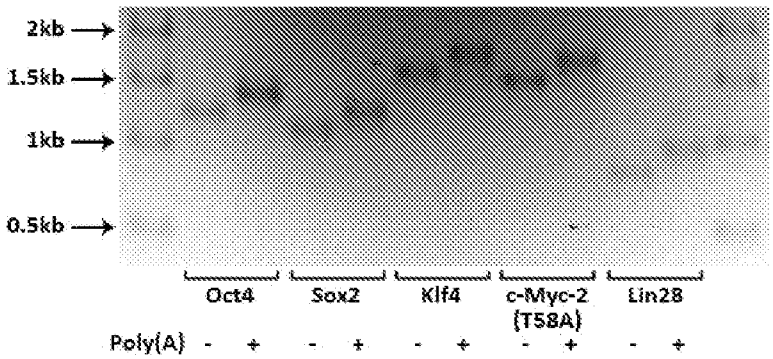
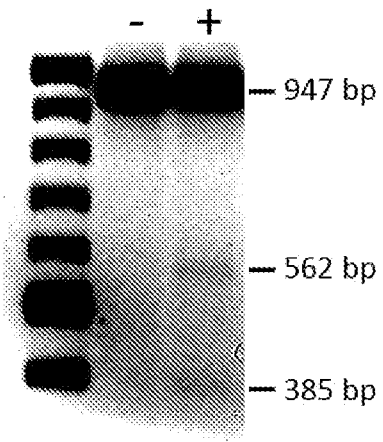


FIG. 16



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2013/068118

A. CLASSIFICATION OF SUBJECT MATTER
(see extra sheet)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C12N 15/31, 5/10, 9/22, C12Q 1/68, C12N 15/63, A61K 31/7088, 31/7115, 48/00, A61P 35/00, 25/28, 25/14, A61K 38/46

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Espacenet, Patentscope, USPTO, Patentscope, RUPTO, EAPATIS, PAJ, KIPRIS, PubMed, NCBI, EBI Fasta

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2241572 A2 (ELI LILLY AND COMPANY) 20.10.2010, paragraphs [0012] - [0015], [0018], [0029], [0032], [0034], [0061], [0106] – [0107], [0118], [0166], [0167], [0169] - [0171], [0177], [0179], [0182] - [0184], examples 1, 9, 11, 18, SEQ ID NO:3	1, 2, 7-10, 19, 20, 28-34, 36-39, 46-49, 139, 143
Y		3-6, 11-18, 21-27, 35, 58, 68, 75, 77, 81, 82, 92-99, 103-105, 110-121, 128-138, 140-142
A		80

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2014 (21.02.2014)	27 March 2014 (27.03.2014)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer T.Babakova Telephone No. (495)531-65-15

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2013/068118

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/154393 A1 (HELMHOLTZ ZENTRUM MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT (GMBH) et al.) 15.12.2011, pp. 6-27, 31-37, fig. 2a-2b	40-45, 51-57, 59-67, 69-74, 76, 83-91, 100-102, 106-109, 125, 127
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