

(10) International Publication Number
WO 2014/081507 A1(43) International Publication Date
30 May 2014 (30.05.2014)

(51) International Patent Classification:

A61K 31/7088 (2006.01) C12N 15/85 (2006.01)
A61K 31/7115 (2006.01) C12N 15/87 (2006.01)

(21) International Application Number:

PCT/US20 13/062943

(22) International Filing Date:

2 October 2013 (02.10.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/729,933	26 November 2012 (26.11.2012)	US
61/737,224	14 December 2012 (14.12.2012)	US
61/758,921	31 January 2013 (31.01.2013)	US
61/775,509	9 March 2013 (09.03.2013)	US
61/781,139	14 March 2013 (14.03.2013)	US
61/829,359	31 May 2013 (31.05.2013)	US
61/829,372	31 May 2013 (31.05.2013)	US
61/839,903	27 June 2013 (27.06.2013)	US
61/842,709	3 July 2013 (03.07.2013)	US
61/857,436	23 July 2013 (23.07.2013)	US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

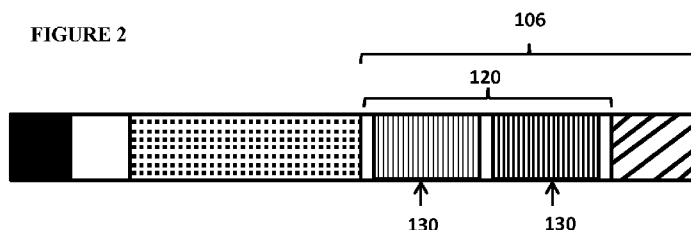
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: TERMINALLY MODIFIED RNA

FIGURE 2



(57) Abstract: The invention relates to compositions and methods for the manufacture and optimization of modified mRNA molecules via optimization of their terminal architecture.

TERMINALLY MODIFIED RNA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Patent Application No 61/729,933, filed November 26, 2012, entitled Terminally Optimized Modified RNAs, US Provisional Patent Application No 61/737,224, filed December 14, 2012, entitled Terminally Optimized Modified RNAs, US Provisional Patent Application No 61/758,921, filed January 31, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No 61/781,139, filed March 14, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No 61/829,359, filed May 31, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No 61/839,903, filed June 27, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No 61/842,709, filed July 3, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No 61/857,436, filed July 23, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No 61/775,509, filed March 9, 2013, entitled Heterologous Untranslated Regions for mRNA and US Provisional Patent Application No 61/829,372, filed May 31, 2013, entitled Heterologous Untranslated Regions for mRNA; the contents of each of which are herein incorporated by reference in their entireties.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled M39PCT2.txt created on October 1, 2013 which is 3,262,934 bytes in size. The information in electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The invention relates to compositions and methods for the manufacture and use of modified and/or optimized mRNA and their use in combination with one or more modified or wild type mRNA encoding an RNA binding protein.

BACKGROUND OF THE INVENTION

[0004] Naturally occurring RNAs are synthesized from four basic ribonucleotides: ATP, CTP, UTP and GTP, but may contain post-transcriptionally modified nucleotides. Further, approximately one hundred different nucleoside modifications have been identified in RNA (Rozenski, J, Crain, P, and McCloskey, J. (1999). The RNA Modification Database: 1999 update. Nucl Acids Res 27: 196-197, herein incorporated by reference in its entirety).

[0005] There are multiple problems with prior methodologies of effecting protein expression. For example, heterologous deoxyribonucleic acid (DNA) introduced into a cell can be inherited by daughter cells (whether or not the heterologous DNA has integrated into the chromosome) or by offspring. Introduced DNA can integrate into host cell genomic DNA at some frequency, resulting in alterations and/or damage to the host cell genomic DNA. In addition, multiple steps must occur before a protein is made. Once inside the cell, DNA must be transported into the nucleus where it is transcribed into RNA. The RNA transcribed from DNA must then enter the cytoplasm where it is translated into protein. This need for multiple processing steps creates lag times before the generation of a protein of interest. Further, it is difficult to obtain DNA expression in cells; frequently DNA enters cells but is not expressed or not expressed at reasonable rates or concentrations. This can be a particular problem when DNA is introduced into cells such as primary cells or modified cell lines. The role of nucleoside modifications on the immuno-stimulatory potential, stability, and on the translation efficiency of RNA, and the consequent benefits to this for enhancing protein expression and producing therapeutics have been previously explored. Such studies are detailed in published co-pending International Publication No WO2012019168 filed August 5, 2011, International Publication No WO2012045082 filed October 3, 2011, International Publication No WO2012045075 filed October 3, 2011, International Publication No WO2013052523 filed October 3, 2012, and International Publication No WO2013090648 filed December 14, 2012 the contents of which are incorporated herein by reference in their entirety.

[0006] The use of modified polynucleotides in the fields of antibodies, viruses, veterinary applications and a variety of in vivo settings have been explored and are disclosed in, for example, co-pending and co-owned U.S. Provisional Patent Application No 61/618,862, filed April 2, 2012, entitled Modified Polynucleotides for the Production

of Biologies; U.S. Provisional Patent Application No 61/681,645, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Biologies; U.S. Provisional Patent Application No 61/737,130, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Biologies; U.S. Provisional Patent Application No 61/618,866, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional Patent Application No 61/681,647, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional Patent Application No 61/737,134, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional Patent Application No 61/618,868, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/681,648, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/737,135, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/618,870, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/681,649, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/737,139, filed December 14, 2012, Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/618,873, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/681,650, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/737,147, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/618,878, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins; U.S. Provisional Patent Application No 61/681,654, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins; U.S. Provisional Patent Application No 61/737,152, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins; U.S. Provisional Patent Application No 61/618,885, filed April 2, 2012, entitled Modified

Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/681,658, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/737,155, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/618,896, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/668,157, filed July 5, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/681,661, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/737,160, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/618,911, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/681,667, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/737,168, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/618,922, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/681,675, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/737,174, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/618,935, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/681,687, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,184, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/618,945, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S.

Provisional Patent Application No 61/681,696, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,191, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/618,953, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/681,704, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,203, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; US Provisional Patent Application No 61/681,720, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; US Provisional Patent Application No 61/737,213, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; US Provisional Patent Application No. 61/681,742, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides; International Application No PCT/US2013/030062, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Biologies and Proteins Associated with Human Disease; US Patent Application No 13/791,922, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Biologies and Proteins Associated with Human Disease; International Application No PCT/US2013/030063, filed March 9, 2013, entitled Modified Polynucleotides; International Application No. PCT/US2013/030064, entitled Modified Polynucleotides for the Production of Secreted Proteins; US Patent Application No 13/791,921, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Secreted Proteins; International Application No PCT/US2013/030059, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Membrane Proteins; International Application No. PCT/US2013/030066, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; International Application No. PCT/US2013/030067, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Nuclear Proteins; International Application No. PCT/US2013/030060, filed March 9, 2013, entitled Modified

Polynucleotides for the Production of Proteins; International Application No. PCT/US20 13/030061, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; US Patent Application No 13/791,910, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; International Application No. PCT/US20 13/030068, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; and International Application No. PCT/US20 13/030070, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides; International Patent Application No. PCT/US20 13/03 1821, filed March 15, 2013, entitled In Vivo Production of Proteins; the contents of each of which are herein incorporated by reference in their entireties.

[0007] Formulations and delivery of modified polynucleotides are described in, for example, co-pending and co-owned International Publication No WO20 13090648, filed December 14, 2012, entitled Modified Nucleoside, Nucleotide, Nucleic Acid Compositions and US Publication No US20130156849, filed December 14, 2012, entitled Modified Nucleoside, Nucleotide, Nucleic Acid Compositions; the contents of each of which are herein incorporated by reference in their entireties.

[0008] There is a need in the art, therefore, for biological modalities to address the modulation of intracellular translation of nucleic acids. The present invention addresses this need by providing methods and compositions for the manufacture and optimization of modified mRNA molecules via alteration of the terminal architecture of the molecules.

SUMMARY OF THE INVENTION

[0009] Disclosed herein are methods of stabilizing or inducing increased protein expression from a modified mRNA. In another method, a cell is contacted with a modified mRNA encoding a polypeptide of interest in combination with a modified mRNA encoding one or more RNA binding proteins.

[0010] In one embodiment, provided herein are terminally optimized mRNA comprising first region of linked nucleosides encoding a polypeptide of interest which is located 5' relative to the first region, a second terminal region located 3' relative to the first terminal region and a 3'tailing region. The first terminal region may comprise at

least one translation enhancer element (TEE) such as, but not limited to, the TEEs described in Table 28 such as, but not limited to, TEE-001 - TEE-705.

[0011] The first terminal region may comprise a 5'untranslated region (UTR) which may be the native 5'UTR of the encoded polypeptide of interest or may be heterologous to the encoded polypeptide of interest. In one aspect, the 5'UTR may comprise at least one translation initiation sequence such as a kozak sequence, an internal ribosome entry site (IRES) and/or a fragment thereof. As a non-limiting example, the 5'UTR may comprise at least one fragment of an IRES. As another non-limiting example, the 5'UTR may comprise at least 5 fragments of an IRES. In another aspect, the 5'UTR may comprise a structured UTR.

[0012] The second terminal region may comprise at least one microRNA binding site, seed sequence or microRNA binding site without a seed sequence. In one aspect, the microRNA is an immune cell specific microRNA such as, but not limited to, mir-122, miR-142-3p, miR-142-5p, miR-146a and miR-146b.

[0013] In one embodiment, the 3'tailing region may comprise a chain terminating nucleoside such as, but not limited to, 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, 2',3'-dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'- dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, and -O- methylnucleoside. In one aspect, the 3' tailing region is a stem loop sequence or a polyA tail.

[0014] In one embodiment, provided herein are terminally optimized mRNA comprising first region of linked nucleosides encoding a polypeptide of interest which is located 5' relative to the first region, a second terminal region located 3' relative to the first terminal region and a 3'tailing region of linked nucleosides and at least one chain terminating nucleoside located 3' relative to the terminally optimized mRNA. In one aspect, the second terminal region may comprise at least one microRNA binding site, seed sequence or microRNA binding site without a seed sequence. In one aspect, the microRNA is an immune cell specific microRNA such as, but not limited to, mir-122, miR-142-3p, miR-142-5p, miR-146a and miR-146b.

[0015] The terminally optimized mRNA described herein may comprise at least one modified nucleoside. In one embodiment, the terminally optimized mRNA comprises a

pseudouridine analog such as, but not limited to, 1-carboxymethyl-pseudouridine, 1-propynyl-pseudouridine, 1-taurinomethyl-pseudouridine, 1-taurinomethyl-4-thio-pseudouridine, 1-methyl-pseudouridine ($m^{\wedge}$), 1-methyl-4-thio-pseudouridine ($m^{\vee}\psi$), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydropseudouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\psi$), and 2'-O-methyl-pseudouridine (ψm). In another embodiment, the terminally optimized mRNA comprises the pseudouridine analog 1-methylpseudouridine. In yet another embodiment, the terminally optimized mRNA comprises the pseudouridine analog 1-methylpseudouridine and comprises the modified nucleoside 5-methylcytidine.

[0016] The terminally optimized mRNA described herein may comprise at least one 5' cap structure such as, but not limited to, CapO, CapI, ARCA, inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azido-guanosine, Cap2, Cap4, and CAP-003 - CAP-225.

[0017] In one aspect, at least one region of the terminally optimized mRNA may be codon optimized. As a non-limiting example, the first region of linked nucleosides may be codon optimized.

[0018] Also provided herein are methods of using the terminally optimized mRNA.

[0019] In one embodiment, provided is a method of reducing antigen-mediated immune response in an organism by contacting the organism with a terminally optimized mRNA. The terminally optimized mRNA may comprise a first region of linked nucleosides encoding a polypeptide of interest which is located 5' relative to the first region, a second terminal region located 3' relative to the first terminal region and a 3'tailing region. The second terminal region may comprise at least one microRNA binding site, seed sequence or microRNA binding site without a seed sequence. In one aspect, the microRNA is an immune cell specific microRNA such as, but not limited to, mir-122, miR-142-3p, miR-142-5p, miR-146a and miR-146b.

[0020] In a another embodiment, terminally optimized mRNA which reduces the antigen-mediated immune response may comprise at least one translation enhancer

element (TEE) sequence such as, but not limited to, TEE-001 - TEE 705, a chain terminating nucleoside and/or a stem loop sequence.

[0021] In yet another embodiment, terminally optimized mRNA which reduces the antigen-mediated immune response may comprise at least one region which is codon optimized. As a non-limiting example, the first region of linked nucleosides may be codon optimized.

[0022] The details of various embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. 1 is a schematic of a primary construct of the present invention.

[0024] FIG. 2 is an expanded schematic of the second flanking region of a primary construct of the present invention illustrating the sensor elements of the polynucleotide.

[0025] FIG. 3 is a clone map useful in the present invention.

[0026] FIG. 4 is a histogram showing the improved protein production from modified mRNAs of the present invention having increasingly longer poly-A tails at two concentrations.

DETAILED DESCRIPTION

[0027] Described herein are compositions and methods for the manufacture and optimization of modified mRNA molecules via alteration of the terminal architecture of the molecules. Specifically disclosed are methods for increasing protein production by altering the terminal regions of the mRNA. Such terminal regions include at least the 5'untranslated region (UTR), and 3'UTR. Other features which may be modified and found to the 5' or 3' of the coding region include the 5'cap and poly-A tail of the modified mRNAs (modified RNAs).

[0028] In general, exogenous nucleic acids, particularly viral nucleic acids, introduced into cells induce an innate immune response, resulting in interferon (IFN) production and cell death. However, it is of great interest for therapeutics, diagnostics, reagents and for biological assays to deliver a nucleic acid, e.g., a ribonucleic acid (RNA) inside a cell, either *in vivo* or *ex vivo*, such as to cause intracellular translation of the nucleic acid and production of the encoded protein. Of particular importance is the

delivery and function of a non-integrative nucleic acid, as nucleic acids characterized by integration into a target cell are generally imprecise in their expression levels, deleteriously transferable to progeny and neighbor cells, and suffer from the substantial risk of mutation.

[0029] The terminal modification described herein may be used in the modified nucleic acids encoding polypeptides of interest, such as, but not limited to, the polypeptides of interest described in, U.S. Provisional Patent Application No 61/618,862, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/681,645, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/737,130, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/618,866, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/681,647, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/737,134, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/618,868, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional Patent Application No 61/681,648, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional Patent Application No 61/737,135, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional Patent Application No 61/618,870, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/681,649, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/737,139, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/618,873, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/681,650, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/737,147,

filed December 14, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/618,878, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins, U.S. Provisional Patent Application No 61/681,654, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins, U.S. Provisional Patent Application No 61/737,152, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins, U.S. Provisional Patent Application No 61/618,885, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/681,658, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/737,155, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/618,896, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/668,157, filed July 5, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/681,661, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/737,160, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/618,911, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/681,667, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/737,168, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/618,922, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins, U.S. Provisional Patent Application No 61/681,675, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins, U.S. Provisional Patent Application No 61/737,174, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins,

U.S. Provisional Patent Application No 61/618,935, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/681,687, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/737,184, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/618,945, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/681,696, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/737,191, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Patent Application No 61/618,953, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Patent Application No 61/681,704, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Patent Application No 61/737,203, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, International Application No PCT/US2013/030062, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Biologies and Proteins Associated with Human Disease; International Application No PCT/US2013/030063, filed March 9, 2013, entitled Modified Polynucleotides; International Application No. PCT/US2013/030064, entitled Modified Polynucleotides for the Production of Secreted Proteins; International Application No PCT/US2013/030059, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Membrane Proteins; International Application No. PCT/US2013/030066, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; International Application No. PCT/US2013/030067, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Nuclear Proteins; International Application No. PCT/US2013/030060, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins; International Application No. PCT/US2013/030061, filed March 9, 2013, entitled

Modified Polynucleotides for the Production of Proteins Associated with Human Disease; International Application No. PCT/US20 13/030068, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; International Application No. PCT/US20 13/030070, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides; and International Application No. PCT/US20 13/03 1821, filed March 15, 2013, entitled In Vivo Production of Proteins, U.S. Provisional Patent Application No. US 61/753,661, filed January 17, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S. Provisional Patent ApphcationNo. US 61/754,159, filed January 18, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S. Provisional Patent Application No. US61/781.097, filed March 14, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S. Provisional Patent Application No. US 61/829,334, filed May 31, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S. Provisional Patent Application No. 61/729,933, filed November 26, 2012, entitled Terminally Optimized Modified RNAs, U.S. Provisional Patent Application No. 61/737,224, filed December 14, 2012, entitled Terminally Optimized Modified RNAs, U.S. Provisional Patent Application No. US 61/758,921, filed January 31, 2013, entitled Differential Targeting Using RNA Constructs, U.S. Provisional Patent Application No. US 61/781,139, filed March 14, 2013, entitled Differential Targeting Using RNA Constructs, U.S. Provisional ApphcationNo. 61/829,359, filed May 31, 2013, entitled Differential Targeting Using RNA Constructs, the contents of each of which are herein incorporated by reference in their entirety.

[0030] Provided herein in part are nucleic acid molecules encoding polypeptides capable of modulating a cell's status, function and/or activity, and methods of making and using these nucleic acids and polypeptides. As described herein and in co-pending and co-owned International Publicaiton No WO2012019168 filed August 5, 201 1, International Publication No WO2012045082 filed October 3, 201 1, International Publication No WO20 12045075 filed October 3, 201 1, International Publication No WO2013052523 filed October 3, 2012, and International Publication No WO2013090648

filed December 14, 2012, the contents of each of which are incorporated by reference herein in their entirety, these modified nucleic acid molecules are capable of reducing the innate immune activity of a population of cells into which they are introduced, thus increasing the efficiency of protein production in that cell population.

[0031] In addition to utilization of non-natural nucleosides and nucleotides, such as those described in US Patent Publication No US201301 15272, filed October 3, 2012 (the contents of which are herein incorporated by reference in its entirety), in the modified RNAs of the present invention, it has now been discovered that concomitant use of altered terminal architecture may also serve to increase protein production from a cell population.

I. Compositions of the Invention

[0032] This invention provides nucleic acid molecules, including RNAs such as mRNAs, which may be synthetic, that contain one or more modified nucleosides (termed "modified nucleic acids" or "modified nucleic acid molecules") and polynucleotides, primary constructs and modified mRNA (mmRNA), which have useful properties including the lack of a substantial induction of the innate immune response of a cell into which the mRNA is introduced. Because these modified nucleic acids enhance the efficiency of protein production, intracellular retention of nucleic acids, and viability of contacted cells, as well as possess reduced immunogenicity, these nucleic acids having these properties are termed "enhanced" nucleic acids or modified RNAs herein.

[0033] In one embodiment, the polynucleotides are nucleic acid transcripts which encode one or more polypeptides of interest that, when translated, deliver a signal to the cell which results in the therapeutic benefit to the organism. The signal polynucleotides may optionally further comprise a sequence (translatable or not) which sense the microenvironment of the polynucleotide and alters (a) the function or phenotype outcome associated with the peptide or protein which is translated, (b) the expression level of the signal polynucleotide, and/or both.

[0034] The term "nucleic acid," in its broadest sense, includes any compound and/or substance that comprise a polymer of nucleotides. These polymers are often referred to as polynucleotides.

[0035] Exemplary nucleic acids include ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs) or hybrids thereof. They may also include RNAi-inducing agents, RNAi agents, siRNAs, shRNAs, miRNAs, antisense RNAs, ribozymes, catalytic DNA, tRNA, RNAs that induce triple helix formation, aptamers, vectors, *etc.* In preferred embodiments, the modified nucleic acid molecule is one or more messenger RNAs (mRNAs).

[0036] In preferred embodiments, the polynucleotide or nucleic acid molecule is a messenger RNA (mRNA). As used herein, the term "messenger RNA" (mRNA) refers to any polynucleotide which encodes a polypeptide of interest and which is capable of being translated to produce the encoded polypeptide of interest *in vitro*, *in vivo*, *in situ* or *ex vivo*. Polynucleotides of the invention may be mRNA or any nucleic acid molecule and may or may not be chemically modified.

[0037] Traditionally, the basic components of an mRNA molecule include at least a coding region, a 5'UTR, a 3'UTR, a 5' cap and a poly-A tail. Building on this wild type modular structure, the present invention expands the scope of functionality of traditional mRNA molecules by providing polynucleotides or primary RNA constructs which maintain a modular organization, but which comprise one or more structural and/or chemical modifications or alterations which impart useful properties to the polynucleotide including, in some embodiments, the lack of a substantial induction of the innate immune response of a cell into which the polynucleotide is introduced. As such, modified mRNA molecules of the present invention, which may be synthetic, are termed "mmRNA." As used herein, a "structural" feature or modification is one in which two or more linked nucleotides are inserted, deleted, duplicated, inverted or randomized in a polynucleotide polynucleotide, primary construct or mmRNA without significant chemical modification to the nucleotides themselves. Because chemical bonds will necessarily be broken and reformed to effect a structural modification, structural modifications are of a chemical nature and hence are chemical modifications. However, structural modifications will result in a different sequence of nucleotides. For example, the polynucleotide "ATCG" may be chemically modified to "AT-5meC-G". The same polynucleotide may be structurally modified from "ATCG" to "ATCCCG". Here, the

dinucleotide "CC" has been inserted, resulting in a structural modification to the polynucleotide.

[0038] Provided are modified nucleic acids containing a translatable region and one, two, or more than two different nucleoside modifications. In some embodiments, the modified nucleic acid exhibits reduced degradation in a cell into which the nucleic acid is introduced, relative to a corresponding unmodified nucleic acid.

[0039] In some embodiments, the chemical modifications can be located on the sugar moiety of the nucleotide

[0040] In some embodiments, the chemical modifications can be located on the phosphate backbone of the nucleotide

[0041] In certain embodiments it is desirable to intracellularly degrade a modified nucleic acid introduced into the cell, for example if precise timing of protein production is desired. Thus, the invention provides a modified nucleic acid containing a degradation domain, which is capable of being acted on in a directed manner within a cell.

Polynucleotide, Primary construct or mmRNA Architecture

[0042] The polynucleotides of the present invention are distinguished from wild type mRNA in their functional and/or structural design features which serve to, as evidenced herein, overcome existing problems of effective polypeptide production using nucleic acid-based therapeutics.

[0043] Figure 1 shows a representative primary construct **100** of the present invention. As used herein, the term "primary construct" or "primary mRNA construct" refers to polynucleotide transcript which encodes one or more polypeptides of interest and which retains sufficient structural and/or chemical features to allow the polypeptide of interest encoded therein to be translated. Primary constructs may be polynucleotides of the invention. When structurally or chemically modified, the primary construct may be referred to as a mmRNA.

[0044] Returning to FIG. 1, the primary construct **100** here contains a first region of linked nucleotides **102** that is flanked by a first flanking region **104** and a second flanking region **106**. As used herein, the "first region" may be referred to as a "coding region" or "region encoding" or simply the "first region." This first region may include, but is not limited to, the encoded polypeptide of interest. The polypeptide of interest may comprise

at its 5' terminus one or more signal peptide sequences encoded by a signal peptide sequence region 103. The flanking region 104 may comprise a region of linked nucleotides comprising one or more complete or incomplete 5' UTRs sequences. The flanking region 104 may also comprise a 5' terminal cap 108. The second flanking region 106 may comprise a region of linked nucleotides comprising one or more complete or incomplete 3' UTRs. The flanking region 106 may also comprise a 3' tailing sequence 110 and a 3'UTR 120.

[0045] Bridging the 5' terminus of the first region 102 and the first flanking region 104 is a first operational region 105. Traditionally this operational region comprises a start codon. The operational region may alternatively comprise any translation initiation sequence or signal including a start codon.

[0046] Bridging the 3' terminus of the first region 102 and the second flanking region 106 is a second operational region 107. Traditionally this operational region comprises a stop codon. The operational region may alternatively comprise any translation initiation sequence or signal including a stop codon. According to the present invention, multiple serial stop codons may also be used. In one embodiment, the operation region of the present invention may comprise two stop codons. The first stop codon may be "TGA" and the second stop codon may be selected from the group consisting of "TAA," "TGA" and "TAG." The operation region may further comprise three stop codons. The third stop codon may be selected from the group consisting of "TAA," "TGA" and "TAG."

[0047] Turning to Figure 2, the 3'UTR 120 of the second flanking region 106 may comprise one or more sensor sequences 130. These sensor sequences as discussed herein operate as pseudo-receptors (or binding sites) for ligands of the local microenvironment of the primary construct or polynucleotide. For example, microRNA binding sites or miRNA seeds may be used as sensors such that they function as pseudoreceptors for any microRNAs present in the environment of the polynucleotide.

[0048] Generally, the shortest length of the first region of the primary construct of the present invention can be the length of a nucleic acid sequence that is sufficient to encode for a dipeptide, a tripeptide, a tetrapeptide, a pentapeptide, a hexapeptide, a heptapeptide, an octapeptide, a nonapeptide, or a decapeptide. In another embodiment, the length may be sufficient to encode a peptide of 2-30 amino acids, e.g. 5-30, 10-30, 2-25, 5-25, 10-25,

or 10-20 amino acids. The length may be sufficient to encode for a peptide of at least 11, 12, 13, 14, 15, 17, 20, 25 or 30 amino acids, or a peptide that is no longer than 40 amino acids, e.g. no longer than 35, 30, 25, 20, 17, 15, 14, 13, 12, 11 or 10 amino acids.

Examples of dipeptides that the polynucleotide sequences can encode or include, but are not limited to, carnosine and anserine.

[0049] Generally, the length of the first region encoding the polypeptide of interest of the present invention is greater than about 30 nucleotides in length (e.g., at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000 or up to and including 100,000 nucleotides). As used herein, the "first region" may be referred to as a "coding region" or "region encoding" or simply the "first region."

[0050] In some embodiments, the polynucleotide polynucleotide, primary construct, or mmRNA includes from about 30 to about 100,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 1,000, from 30 to 1,500, from 30 to 3,000, from 30 to 5,000, from 30 to 7,000, from 30 to 10,000, from 30 to 25,000, from 30 to 50,000, from 30 to 70,000, from 100 to 250, from 100 to 500, from 100 to 1,000, from 100 to 1,500, from 100 to 3,000, from 100 to 5,000, from 100 to 7,000, from 100 to 10,000, from 100 to 25,000, from 100 to 50,000, from 100 to 70,000, from 100 to 100,000, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 3,000, from 500 to 5,000, from 500 to 7,000, from 500 to 10,000, from 500 to 25,000, from 500 to 50,000, from 500 to 70,000, from 500 to 100,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 3,000, from 1,000 to 5,000, from 1,000 to 7,000, from 1,000 to 10,000, from 1,000 to 25,000, from 1,000 to 50,000, from 1,000 to 70,000, from 1,000 to 100,000, from 1,500 to 3,000, from 1,500 to 5,000, from 1,500 to 7,000, from 1,500 to 10,000, from 1,500 to 25,000, from 1,500 to 50,000, from 1,500 to 70,000, from 1,500 to 100,000, from 2,000 to 3,000, from 2,000 to 5,000, from 2,000 to 7,000, from 2,000 to 10,000, from 2,000 to 25,000, from 2,000 to 50,000, from 2,000 to 70,000, and from 2,000 to 100,000).

[0051] According to the present invention, the first and second flanking regions may range independently from 15-1,000 nucleotides in length (e.g., greater than 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, and 900 nucleotides or at least 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, and 1,000 nucleotides).

[0052] According to the present invention, the tailing sequence may range from absent to 500 nucleotides in length (e.g., at least 60, 70, 80, 90, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 nucleotides). Where the tailing region is a polyA tail, the length may be determined in units of or as a function of polyA binding protein binding. In this embodiment, the polyA tail is long enough to bind at least 4 monomers of polyA binding protein. PolyA binding protein monomers bind to stretches of approximately 38 nucleotides. As such, it has been observed that polyA tails of about 80 nucleotides and 160 nucleotides are functional.

[0053] According to the present invention, the capping region may comprise a single cap or a series of nucleotides forming the cap. In this embodiment the capping region may be from 1 to 10, e.g. 2-9, 3-8, 4-7, 1-5, 5-10, or at least 2, or 10 or fewer nucleotides in length. In some embodiments, the cap is absent.

[0054] According to the present invention, the first and second operational regions may range from 3 to 40, e.g., 5-30, 10-20, 15, or at least 4, or 30 or fewer nucleotides in length and may comprise, in addition to a start and/or stop codon, one or more signal and/or restriction sequences.

Cyclic Polynucleotides

[0055] According to the present invention, a nucleic acid, modified RNA or primary construct may be cyclized, or concatemerized, to generate a translation competent molecule to assist interactions between poly-A binding proteins and 5'-end binding proteins. The mechanism of cyclization or concatemerization may occur through at least 3 different routes: 1) chemical, 2) enzymatic, and 3) ribozyme catalyzed. The newly formed 5'-/3'-linkage may be intramolecular or intermolecular.

[0056] In the first route, the 5'-end and the 3'-end of the nucleic acid contain chemically reactive groups that, when close together, form a new covalent linkage between the 5'-end and the 3'-end of the molecule. The 5'-end may contain an NHS-ester

reactive group and the 3'-end may contain a 3'-amino-terminated nucleotide such that in an organic solvent the 3'-amino-terminated nucleotide on the 3'-end of a synthetic mRNA molecule will undergo a nucleophilic attack on the 5'-NHS-ester moiety forming a new 5'-/3'-amide bond.

[0057] In the second route, T4 RNA ligase may be used to enzymatically link a 5'-phosphorylated nucleic acid molecule to the 3'-hydroxyl group of a nucleic acid forming a new phosphodiester linkage. In an example reaction, 1 µg of a nucleic acid molecule is incubated at 37°C for 1 hour with 1-10 units of T4 RNA ligase (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. The ligation reaction may occur in the presence of a split oligonucleotide capable of base-pairing with both the 5'- and 3'-region in juxtaposition to assist the enzymatic ligation reaction.

[0058] In the third route, either the 5'-or 3'-end of the cDNA template encodes a ligase ribozyme sequence such that during *in vitro* transcription, the resultant nucleic acid molecule can contain an active ribozyme sequence capable of ligating the 5'-end of a nucleic acid molecule to the 3'-end of a nucleic acid molecule. The ligase ribozyme may be derived from the Group I Intron, Group I Intron, Hepatitis Delta Virus, Hairpin ribozyme or may be selected by SELEX (systematic evolution of ligands by exponential enrichment). The ribozyme ligase reaction may take 1 to 24 hours at temperatures between 0 and 37°C.

Polynucleotide Multimers

[0059] According to the present invention, multiple distinct nucleic acids, modified RNA or primary constructs may be linked together through the 3'-end using nucleotides which are modified at the 3'-terminus. Chemical conjugation may be used to control the stoichiometry of delivery into cells. For example, the glyoxylate cycle enzymes, isocitrate lyase and malate synthase, may be supplied into HepG2 cells at a 1:1 ratio to alter cellular fatty acid metabolism. This ratio may be controlled by chemically linking nucleic acids or modified RNA using a 3'-azido terminated nucleotide on one nucleic acids or modified RNA species and a C5-ethynyl or alkynyl-containing nucleotide on the opposite nucleic acids or modified RNA species. The modified nucleotide is added post-transcriptionally using terminal transferase (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. After the addition of the 3'-modified

nucleotide, the two nucleic acids or modified RNA species may be combined in an aqueous solution, in the presence or absence of copper, to form a new covalent linkage via a click chemistry mechanism as described in the literature.

[0060] In another example, more than two polynucleotides may be linked together using a functionalized linker molecule. For example, a functionalized saccharide molecule may be chemically modified to contain multiple chemical reactive groups (SH-, NH₂-, N₃, etc. .) to react with the cognate moiety on a 3'-functionalized mRNA molecule (i.e., a 3'-maleimide ester, 3'-NHS-ester, alkynyl). The number of reactive groups on the modified saccharide can be controlled in a stoichiometric fashion to directly control the stoichiometric ratio of conjugated nucleic acid or mRNA.

Modified RNA Conjugates and Combinations

[0061] In order to further enhance protein production, nucleic acids, modified RNA, polynucleotides or primary constructs of the present invention can be designed to be conjugated to other polynucleotides, dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, [MPEG]₂, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases, proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell, hormones and hormone receptors, non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, or a drug.

[0062] Conjugation may result in increased stability and/or half life and may be particularly useful in targeting the nucleic acids, modified RNA, polynucleotides or primary constructs to specific sites in the cell, tissue or organism.

[0063] According to the present invention, the nucleic acids, modified RNA or primary construct may be administered with, or further encode one or more of RNAi agents, siRNAs, shRNAs, miRNAs, miRNA binding sites, antisense RNAs, ribozymes,

catalytic DNA, tRNA, RNAs that induce triple helix formation, aptamers or vectors, and the like.

Bifunctional Polynucleotides

[0064] In one embodiment of the invention are bifunctional polynucleotides (e.g., bifunctional nucleic acids, bifunctional modified RNA or bifunctional primary constructs). As the name implies, bifunctional polynucleotides are those having or capable of at least two functions. These molecules may also by convention be referred to as multi-functional.

[0065] The multiple functionalities of bifunctional polynucleotides may be encoded by the RNA (the function may not manifest until the encoded product is translated) or may be a property of the polynucleotide itself. It may be structural or chemical. Bifunctional modified polynucleotides may comprise a function that is covalently or electrostatically associated with the polynucleotides. Further, the two functions may be provided in the context of a complex of a modified RNA and another molecule.

[0066] Bifunctional polynucleotides may encode peptides which are anti-proliferative. These peptides may be linear, cyclic, constrained or random coil. They may function as aptamers, signaling molecules, ligands or mimics or mimetics thereof. Anti-proliferative peptides may, as translated, be from 3 to 50 amino acids in length. They may be 5-40, 10-30, or approximately 15 amino acids long. They may be single chain, multichain or branched and may form complexes, aggregates or any multi-unit structure once translated.

Noncoding Polynucleotides

[0067] As described herein, provided are nucleic acids, modified RNA, polynucleotides and primary constructs having sequences that are partially or substantially not translatable, e.g., having a noncoding region. Such molecules are generally not translated, but can exert an effect on protein production by one or more of binding to and sequestering one or more translational machinery components such as a ribosomal protein or a transfer RNA (tRNA), thereby effectively reducing protein expression in the cell or modulating one or more pathways or cascades in a cell which in turn alters protein levels. The nucleic acids, polynucleotides, primary constructs or mRNA may contain or encode one or more long noncoding RNA (lncRNA, or lincRNA)

or portion thereof, a small nucleolar RNA (sno-RNA), micro RNA (miRNA), small interfering RNA (siRNA) or Piwi-interacting RNA (piRNA).

Polypeptides of interest

[0068] According to the present invention, the primary construct is designed to encode one or more polypeptides of interest or fragments thereof. A polypeptide of interest may include, but is not limited to, whole polypeptides, a plurality of polypeptides or fragments of polypeptides, which independently may be encoded by one or more nucleic acids, a plurality of nucleic acids, fragments of nucleic acids or variants of any of the aforementioned. As used herein, the term "polypeptides of interest" refers to any polypeptide which is selected to be encoded in the primary construct of the present invention. As used herein, "polypeptide" means a polymer of amino acid residues (natural or unnatural) linked together most often by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. In some instances the polypeptide encoded is smaller than about 50 amino acids and the polypeptide is then termed a peptide. If the polypeptide is a peptide, it will be at least about 2, 3, 4, or at least 5 amino acid residues long. Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. They may also comprise single chain or multichain polypeptides such as antibodies or insulin and may be associated or linked. Most commonly disulfide linkages are found in multichain polypeptides. The term polypeptide may also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid.

[0069] The term "polypeptide variant" refers to molecules which differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants will possess at least about 50% identity (homology) to a native or reference sequence, and preferably, they will be at least about 80%, more preferably at least about 90% identical (homologous) to a native or reference sequence.

[0070] In some embodiments "variant mimics" are provided. As used herein, the term "variant mimic" is one which contains one or more amino acids which would mimic an activated sequence. For example, glutamate may serve as a mimic for phospho-threonine and/or phospho-serine. Alternatively, variant mimics may result in deactivation or in an inactivated product containing the mimic, e.g., phenylalanine may act as an inactivating substitution for tyrosine; or alanine may act as an inactivating substitution for serine.

[0071] "Homology" as it applies to amino acid sequences is defined as the percentage of residues in the candidate amino acid sequence that are identical with the residues in the amino acid sequence of a second sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology. Methods and computer programs for the alignment are well known in the art. It is understood that homology depends on a calculation of percent identity but may differ in value due to gaps and penalties introduced in the calculation.

[0072] By "homologs" as it applies to polypeptide sequences means the corresponding sequence of other species having substantial identity to a second sequence of a second species.

[0073] "Analog" is meant to include polypeptide variants which differ by one or more amino acid alterations, e.g., substitutions, additions or deletions of amino acid residues that still maintain one or more of the properties of the parent or starting polypeptide.

[0074] The present invention contemplates several types of compositions which are polypeptide based including variants and derivatives. These include substitutional, insertional, deletion and covalent variants and derivatives. The term "derivative" is used synonymously with the term "variant" but generally refers to a molecule that has been modified and/or changed in any way relative to a reference molecule or starting molecule.

[0075] As such, polynucleotides encoding polypeptides of interest containing substitutions, insertions and/or additions, deletions and covalent modifications with respect to reference sequences are included within the scope of this invention. For example, sequence tags or amino acids, such as one or more lysines, can be added to the peptide sequences of the invention (e.g., at the N-terminal or C-terminal ends). Sequence

tags can be used for peptide purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the amino acid sequence of a peptide or protein may optionally be deleted providing for truncated sequences. Certain amino acids (e.g., C-terminal or N-terminal residues) may alternatively be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

[0076] "Substitutional variants" when referring to polypeptides are those that have at least one amino acid residue in a native or starting sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

[0077] As used herein the term "conservative amino acid substitution" refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine and leucine for another non-polar residue. Likewise, examples of conservative substitutions include the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, and between glycine and serine. Additionally, the substitution of a basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue such as aspartic acid or glutamic acid for another acidic residue are additional examples of conservative substitutions. Examples of non-conservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, methionine for a polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue.

[0078] "Insertional variants" when referring to polypeptides are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a native or starting sequence. "Immediately adjacent" to an amino acid means connected to either the alpha-carboxy or alpha-amino functional group of the amino acid.

[0079] "Deletional variants" when referring to polypeptides are those with one or more amino acids in the native or starting amino acid sequence removed. Ordinarily, deletional variants will have one or more amino acids deleted in a particular region of the molecule.

[0080] "Covalent derivatives" when referring to polypeptides include modifications of a native or starting protein with an organic proteinaceous or non-proteinaceous derivatizing agent, and/or post-translational modifications. Covalent modifications are traditionally introduced by reacting targeted amino acid residues of the protein with an organic derivatizing agent that is capable of reacting with selected side-chains or terminal residues, or by harnessing mechanisms of post-translational modifications that function in selected recombinant host cells. The resultant covalent derivatives are useful in programs directed at identifying residues important for biological activity, for immunoassays, or for the preparation of anti-protein antibodies for immunoaffinity purification of the recombinant glycoprotein. Such modifications are within the ordinary skill in the art and are performed without undue experimentation.

[0081] Certain post-translational modifications are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues may be present in the polypeptides produced in accordance with the present invention.

[0082] Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)).

[0083] "Features" when referring to polypeptides are defined as distinct amino acid sequence-based components of a molecule. Features of the polypeptides encoded by the mmRNA of the present invention include surface manifestations, local conformational shape, folds, loops, half-loops, domains, half-domains, sites, termini or any combination thereof.

[0084] As used herein when referring to polypeptides the term "surface manifestation" refers to a polypeptide based component of a protein appearing on an outermost surface.

[0085] As used herein when referring to polypeptides the term "local conformational shape" means a polypeptide based structural manifestation of a protein which is located within a definable space of the protein.

[0086] As used herein when referring to polypeptides the term "fold" refers to the resultant conformation of an amino acid sequence upon energy minimization. A fold may occur at the secondary or tertiary level of the folding process. Examples of secondary level folds include beta sheets and alpha helices. Examples of tertiary folds include domains and regions formed due to aggregation or separation of energetic forces. Regions formed in this way include hydrophobic and hydrophilic pockets, and the like.

[0087] As used herein the term "turn" as it relates to protein conformation means a bend which alters the direction of the backbone of a peptide or polypeptide and may involve one, two, three or more amino acid residues.

[0088] As used herein when referring to polypeptides the term "loop" refers to a structural feature of a polypeptide which may serve to reverse the direction of the backbone of a peptide or polypeptide. Where the loop is found in a polypeptide and only alters the direction of the backbone, it may comprise four or more amino acid residues. Oliva et al. have identified at least 5 classes of protein loops (J. Mol Biol 266 (4): 814-830; 1997). Loops may be open or closed. Closed loops or "cyclic" loops may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids between the bridging moieties. Such bridging moieties may comprise a cysteine-cysteine bridge (Cys-Cys) typical in polypeptides having disulfide bridges or alternatively bridging moieties may be non-protein based such as the dibromozilyl agents used herein.

[0089] As used herein when referring to polypeptides the term "half-loop" refers to a portion of an identified loop having at least half the number of amino acid residues as the loop from which it is derived. It is understood that loops may not always contain an even number of amino acid residues. Therefore, in those cases where a loop contains or is identified to comprise an odd number of amino acids, a half-loop of the odd-numbered loop will comprise the whole number portion or next whole number portion of the loop

(number of amino acids of the loop/2 $\pm$ 0.5 amino acids). For example, a loop identified as a 7 amino acid loop could produce half-loops of 3 amino acids or 4 amino acids (7/2=3.5 $\pm$ 0.5 being 3 or 4).

[0090] As used herein when referring to polypeptides the term "domain" refers to a motif of a polypeptide having one or more identifiable structural or functional characteristics or properties (e.g., binding capacity, serving as a site for protein-protein interactions).

[0091] As used herein when referring to polypeptides the term "half-domain" means a portion of an identified domain having at least half the number of amino acid residues as the domain from which it is derived. It is understood that domains may not always contain an even number of amino acid residues. Therefore, in those cases where a domain contains or is identified to comprise an odd number of amino acids, a half-domain of the odd-numbered domain will comprise the whole number portion or next whole number portion of the domain (number of amino acids of the domain/2 $\pm$ 0.5 amino acids). For example, a domain identified as a 7 amino acid domain could produce half-domains of 3 amino acids or 4 amino acids (7/2=3.5 $\pm$ 0.5 being 3 or 4). It is also understood that subdomains may be identified within domains or half-domains, these subdomains possessing less than all of the structural or functional properties identified in the domains or half domains from which they were derived. It is also understood that the amino acids that comprise any of the domain types herein need not be contiguous along the backbone of the polypeptide (i.e., nonadjacent amino acids may fold structurally to produce a domain, half-domain or subdomain).

[0092] As used herein when referring to polypeptides the terms "site" as it pertains to amino acid based embodiments is used synonymously with "amino acid residue" and "amino acid side chain." A site represents a position within a peptide or polypeptide that may be modified, manipulated, altered, derivatized or varied within the polypeptide based molecules of the present invention.

[0093] As used herein the terms "termini" or "terminus" when referring to polypeptides refers to an extremity of a peptide or polypeptide. Such extremity is not limited only to the first or final site of the peptide or polypeptide but may include additional amino acids in the terminal regions. The polypeptide based molecules of the

present invention may be characterized as having both an N-terminus (terminated by an amino acid with a free amino group (NH₂)) and a C-terminus (terminated by an amino acid with a free carboxyl group (COOH)). Proteins of the invention are in some cases made up of multiple polypeptide chains brought together by disulfide bonds or by non-covalent forces (multimers, oligomers). These sorts of proteins will have multiple N- and C-termini. Alternatively, the termini of the polypeptides may be modified such that they begin or end, as the case may be, with a non-polypeptide based moiety such as an organic conjugate.

[0094] Once any of the features have been identified or defined as a desired component of a polypeptide to be encoded by the primary construct or mmRNA of the invention, any of several manipulations and/or modifications of these features may be performed by moving, swapping, inverting, deleting, randomizing or duplicating. Furthermore, it is understood that manipulation of features may result in the same outcome as a modification to the molecules of the invention. For example, a manipulation which involved deleting a domain would result in the alteration of the length of a molecule just as modification of a nucleic acid to encode less than a full length molecule would.

[0095] Modifications and manipulations can be accomplished by methods known in the art such as, but not limited to, site directed mutagenesis. The resulting modified molecules may then be tested for activity using *in vitro* or *in vivo* assays such as those described herein or any other suitable screening assay known in the art.

[0096] According to the present invention, the polypeptides may comprise a consensus sequence which is discovered through rounds of experimentation. As used herein a "consensus" sequence is a single sequence which represents a collective population of sequences allowing for variability at one or more sites.

[0097] As recognized by those skilled in the art, protein fragments, functional protein domains, and homologous proteins are also considered to be within the scope of polypeptides of interest of this invention. For example, provided herein is any protein fragment (meaning an polypeptide sequence at least one amino acid residue shorter than a reference polypeptide sequence but otherwise identical) of a reference protein 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or greater than 100 amino acids in length. In another example,

any protein that includes a stretch of about 20, about 30, about 40, about 50, or about 100 amino acids which are about 40%, about 50%>, about 60%>, about 70%>, about 80%>, about 90%, about 95%~~o~~, or about 100% identical to any of the sequences described herein can be utilized in accordance with the invention. In certain embodiments, a polypeptide to be utilized in accordance with the invention includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations as shown in any of the sequences provided or referenced herein.

Encoded Polypeptides of Interest

[0098] The primary constructs, modified nucleic acids or mmRNA of the present invention may be designed to encode polypeptides of interest such as peptides and proteins.

[0099] In one embodiment, primary constructs, modified nucleic acids or mmRNA of the present invention may encode variant polypeptides which have a certain identity with a reference polypeptide sequence. As used herein, a "reference polypeptide sequence" refers to a starting polypeptide sequence. Reference sequences may be wild type sequences or any sequence to which reference is made in the design of another sequence. A "reference polypeptide sequence" may, e.g., be any one of the protein sequence listed in U.S. Provisional Patent Application No 61/618,862, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/681,645, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/737,130, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/618,866, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/681,647, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/737,134, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/618,868, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional Patent Application No 61/681,648, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional Patent Application No 61/737,135, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional

Patent Application No 61/618,870, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/681,649, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/737,139, filed December 14, 2012, Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/618,873, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/681,650, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/737,147, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/618,878, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins, U.S. Provisional Patent Application No 61/681,654, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins, U.S. Provisional Patent Application No 61/737,152, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins, U.S. Provisional Patent Application No 61/618,885, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/681,658, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/737,155, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/618,896, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/668,157, filed July 5, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/681,661, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/737,160, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/618,911, filed April 2, 2012, entitled

Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/681,667, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/737,168, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/618,922, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins, U.S. Provisional Patent Application No 61/681,675, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins, U.S. Provisional Patent Application No 61/737,174, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins, U.S. Provisional Patent Application No 61/618,935, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/681,687, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/737,184, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/618,945, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/681,696, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/737,191, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Patent Application No 61/618,953, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Patent Application No 61/681,704, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Patent Application No 61/737,203, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, International Application No PCT/US2013/030062, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Biologies and Proteins Associated with Human Disease; International Application No PCT/US2013/030063, filed March 9, 2013, entitled Modified Polynucleotides; International Application No. PCT/US2013/030064,

entitled Modified Polynucleotides for the Production of Secreted Proteins; International Application No PCT/US20 13/030059, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Membrane Proteins; International Application No. PCT/US20 13/030066, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; International Application No. PCT/US20 13/030067, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Nuclear Proteins; International Application No. PCT/US2013/030060, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins; International Application No. PCT/US20 13/030061, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; International Application No. PCT/US20 13/030068, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; International Application No. PCT/US20 13/030070, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides; and International Application No. PCT/US20 13/03 1821, filed March 15, 2013, entitled In Vivo Production of Proteins, the contents of each of which are herein incorporated by reference in their entireties.

[00100] The term "identity" as known in the art, refers to a relationship between the sequences of two or more peptides, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between peptides, as determined by the number of matches between strings of two or more amino acid residues. Identity measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms"). Identity of related peptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M.

and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al, SIAM J. Applied Math. 48, 1073 (1988).

[00101] In some embodiments, the polypeptide variant may have the same or a similar activity as the reference polypeptide. Alternatively, the variant may have an altered activity (e.g., increased or decreased) relative to a reference polypeptide. Generally, variants of a particular polynucleotide or polypeptide of the invention will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (Stephen F. Altschul, Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.) Other tools are described herein, specifically in the definition of "identity."

[00102] Default parameters in the BLAST algorithm include, for example, an expect threshold of 10, Word size of 28, Match/Mismatch Scores 1, -2, Gap costs Linear. Any filter can be applied as well as a selection for species specific repeats, e.g., Homo sapiens.

[00103] In one embodiment, the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may be used to treat a disease, disorder and/or condition in a subject.

[00104] In one embodiment, the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may be used to reduce, eliminate or prevent tumor growth in a subject.

[00105] In one embodiment, the polynucleotides, primary constructs and/or mmRNA may be used to reduce and/or ameliorate at least one symptom of cancer in a subject. A symptom of cancer may include, but is not limited to, weakness, aches and pains, fever, fatigue, weight loss, blood clots, increased blood calcium levels, low white blood cell count, short of breath, dizziness, headaches, hyperpigmentation, jaundice, erythema, pruritis, excessive hair growth, change in bowel habits, change in bladder function, long-lasting sores, white patches inside the mouth, white spots on the tongue, unusual bleeding

or discharge, thickening or lump on parts of the body, indigestion, trouble swallowing, changes in warts or moles, change in new skin and nagging cough or hoarseness.

Further, the polynucleotides, primary constructs, modified nucleic acid and/or mmRNA may reduce a side-effect associated with cancer such as, but not limited to, chemo brain, peripheral neuropathy, fatigue, depression, nausea, vomiting, pain, anemia, lymphedema, infections, sexual side effects, reduced fertility or infertility, ostomies, insomnia and hair loss.

Terminal Architecture Modifications: Untranslated Regions (UTRs)

[00106] Untranslated regions (UTRs) of a gene are transcribed but not translated. The 5'UTR starts at the transcription start site and continues to the start codon but does not include the start codon; whereas, the 3'UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into the nucleic acids or modified RNA of the present invention to enhance the stability of the molecule. The specific features can also be incorporated to ensure controlled down-regulation of the transcript in case they are misdirected to undesired organs sites. The untranslated regions may be incorporated into a vector system which can produce mRNA and/or be delivered to a cell, tissue and/or organism to produce a polypeptide of interest.

5' UTR and Translation Initiation

[00107] Natural 5'UTRs bear features which play roles in for translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5'UTR also have been known to form secondary structures which are involved in elongation factor binding.

[00108] 5'UTR secondary structures involved in elongation factor binding can interact with other RNA binding molecules in the 5'UTR or 3'UTR to regulate gene expression. For example, the elongation factor EIF4A2 binding to a secondarily structured element in

the 5'UTR is necessary for microRNA mediated repression (Meijer HA et al., Science, 2013, 340, 82-85, herein incorporated by reference in its entirety). The different secondary structures in the 5'UTR can be incorporated into the flanking region to either stabilize or selectively destabilized mRNAs in specific tissues or cells.

[00109] By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and protein production of the nucleic acids or mRNA of the invention. For example, introduction of 5' UTR of liver-expressed mRNA, such as albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII, could be used to enhance expression of a nucleic acid molecule, such as a mrnRNA, in hepatic cell lines or liver. Likewise, use of 5' UTR from other tissue-specific mRNA to improve expression in that tissue is possible - for muscle (MyoD, Myosin, Myoglobin, Myogenin, Herculin), for endothelial cells (Tie-1, CD36), for myeloid cells (C/EBP, AML1, G-CSF, GM-CSF, CD11b, MSR, Fr-1, i-NOS), for leukocytes (CD45, CD18), for adipose tissue (CD36, GLUT4, ACRP30, adiponectin) and for lung epithelial cells (SP-A/B/C/D).

[00110] Other non-UTR sequences may be incorporated into the 5' (or 3' UTR) UTRs. For example, introns or portions of introns sequences may be incorporated into the flanking regions of the nucleic acids or mRNA of the invention. Incorporation of intronic sequences may increase protein production as well as mRNA levels.

[00111] In one embodiment, at least one fragment of IRES sequences from a GTX gene may be included in the 5'UTR. As a non-limiting example, the fragment may be an 18 nucleotide sequence from the IRES of the GTX gene. As another non-limiting example, an 18 nucleotide sequence fragment from the IRES sequence of a GTX gene may be tandemly repeated in the 5'UTR of a polynucleotide described herein. The 18 nucleotide sequence may be repeated in the 5'UTR at least one, at least twice, at least three times, at least four times, at least five times, at least six times, at least seven times, at least eight times, at least nine times or more than ten times

[00112] In one embodiment, a 5'UTR may include at least five 18 nucleotide fragments of IRES sequences from a GTX gene may be included in the 5'UTR (see e.g., the 18 nucleotide fragment described in Table 62).

[00113] Nucleotides may be mutated, replaced and/or removed from the 5' (or 3') UTRs. For example, one or more nucleotides upstream of the start codon may be replaced with another nucleotide. The nucleotide or nucleotides to be replaced may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 nucleotides upstream of the start codon. As another example, one or more nucleotides upstream of the start codon may be removed from the UTR.

[00114] In one embodiment, at least one purine upstream of the start codon may be replaced with a pyrimidine. The purine to be replaced may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 nucleotides upstream of the start codon. As a non-limiting example, an adenine which is three nucleotides upstream of the start codon may be replaced with a thymine. As another non-limiting example, an adenine which is nine nucleotides upstream of the start codon may be replaced with a thymine.

[00115] In one embodiment, at least one nucleotide upstream of the start codon may be removed from the UTR. In one aspect, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 nucleotides upstream of the start codon may be removed from the UTR of the polynucleotides described herein. As a non-limiting example, the nine nucleotides upstream of the start codon may be removed from the UTR (See e.g., the G-CSF 9del5' construct described in Table 60).

5'UTR, 3'UTR and Translation Enhancer Elements (TEEs)

[00116] In one embodiment, the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least one translational enhancer polynucleotide, translation enhancer element, translational enhancer elements (collectively referred to as "TEE"s). As a non-limiting example, the TEE may be located between the transcription promoter and the start codon. The polynucleotides, primary constructs, modified nucleic acids and/or mmRNA with at least one TEE in the 5'UTR may include a cap at the 5'UTR. Further, at least one TEE may be located in the 5'UTR of polynucleotides, primary constructs, modified nucleic acids and/or mmRNA undergoing cap-dependent or cap-independent translation.

[00117] The term "translational enhancer element" or "translation enhancer element" (herein collectively referred to as "TEE") refers to sequences that increase the amount of polypeptide or protein produced from an mRNA.

[00118] In one aspect, TEEs are conserved elements in the UTR which can promote translational activity of a nucleic acid such as, but not limited to, cap-dependent or cap-independent translation. The conservation of these sequences has been previously shown by Panek et al (Nucleic Acids Research, 2013, 1-10; herein incorporated by reference in its entirety) across 14 species including humans.

[00119] In one embodiment, the TEE may be any of the TEEs listed in Table 32 in Example 45, including portion and/or fragments thereof. The TEE sequence may include at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more than 99% of the TEE sequences disclosed in Table 32 and/or the TEE sequence may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in Table 32.

[00120] In one non-limiting example, the TEEs known may be in the 5'-leader of the Gtx homeodomain protein (Chappell et al, Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004, herein incorporated by reference in their entirety).

[00121] In another non-limiting example, TEEs are disclosed as SEQ ID NOs: 1-35 in US Patent Publication No. US20090226470, SEQ ID NOs: 1-35 in US Patent Publication US20130177581, SEQ ID NOs: 1-35 in International Patent Publication No. WO2009075886, SEQ ID NOs: 1-5, and 7-645 in International Patent Publication No. WO2012009644, SEQ ID NO: 1 in International Patent Publication No. WO1999024595, SEQ ID NO: 1 in US Patent No. US63 10197, and SEQ ID NO: 1 in US Patent No. US6849405, each of which is herein incorporated by reference in its entirety.

[00122] In yet another non-limiting example, the TEE may be an internal ribosome entry site (IRES), HCV-IRES or an IRES element such as, but not limited to, those described in US Patent No. US7468275, US Patent Publication Nos. US20070048776 and US201 10124100 and International Patent Publication Nos. WO2007025008 and

WO200 1055369, each of which is herein incorporated by reference in its entirety. The IRES elements may include, but are not limited to, the Gtx sequences (e.g., Gtx9-nt, Gtx8-nt, Gtx7-nt) described by Chappell et al. (Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005) and in US Patent Publication Nos. US20070048776 and US201 10124100 and International Patent Publication No. WO2007025008, each of which is herein incorporated by reference in its entirety.

[00123] "Translational enhancer polynucleotides" or "translation enhancer polynucleotide sequences" are polynucleotides which include one or more of the specific TEE exemplified herein and/or disclosed in the art (see e.g., US63 10197, US6849405, US7456273, US7183395, US20090226470, US20070048776, US201 10124100, US20090093049, US20130177581, WO2009075886, WO2007025008, WO2012009644, WO2001055371 WO1999024595, and EP2610341A1 and EP2610340A1; each of which is herein incorporated by reference in its entirety) or their variants, homologs or functional derivatives. One or multiple copies of a specific TEE can be present in the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA. The TEEs in the translational enhancer polynucleotides can be organized in one or more sequence segments. A sequence segment can harbor one or more of the specific TEEs exemplified herein, with each TEE being present in one or more copies. When multiple sequence segments are present in a translational enhancer polynucleotide, they can be homogenous or heterogeneous. Thus, the multiple sequence segments in a translational enhancer polynucleotide can harbor identical or different types of the specific TEEs exemplified herein, identical or different number of copies of each of the specific TEEs, and/or identical or different organization of the TEEs within each sequence segment.

[00124] In one embodiment, the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least one TEE that is described in International Patent Publication No. WO1999024595, WO2012009644, WO2009075886, WO2007025008, WO1999024595, European Patent Publication No. EP2610341A1 and EP2610340A1, US Patent No. US6310197, US6849405, US7456273, US7183395, US Patent Publication No. US20090226470, US201 10124100, US20070048776, US20090093049, and US20130177581 each of which is herein incorporated by reference

in its entirety. The TEE may be located in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA.

[00125] In another embodiment, the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least one TEE that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identity with the TEEs described in US Patent Publication Nos. US20090226470, US20070048776, US20130177581 and US201 10124100, International Patent Publication No. WO1999024595, WO2012009644, WO2009075886 and WO2007025008, European Patent Publication No. EP2610341A1 and EP2610340A1, US Patent No. US6310197, US6849405, US7456273, US7183395, each of which is herein incorporated by reference in its entirety.

[00126] In one embodiment, the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55 or more than 60 TEE sequences. The TEE sequences in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may be the same or different TEE sequences. The TEE sequences may be in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

[00127] In one embodiment, the 5'UTR may include a spacer to separate two TEE sequences. As a non-limiting example, the spacer may be a 15 nucleotide spacer and/or other spacers known in the art. As another non-limiting example, the 5'UTR may include a TEE sequence-spacer module repeated at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times and at least 9 times or more than 9 times in the 5'UTR.

[00128] In another embodiment, the spacer separating two TEE sequences may include other sequences known in the art which may regulate the translation of the

polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention such as, but not limited to, miR sequences described herein (e.g., miR binding sites and miR seeds). As a non-limiting example, each spacer used to separate two TEE sequences may include a different miR sequence or component of a miR sequence (e.g., miR seed sequence).

[00129] In one embodiment, the TEE in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may include at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more than 99% of the TEE sequences disclosed in US Patent Publication Nos. US20090226470, US20070048776, US20130177581 and US201 10124100, International Patent Publication No. WO1999024595, WO2012009644, WO2009075886 and WO2007025008, European Patent Publication No. EP2610341A1 and EP2610340A1, US Patent No. US6310197, US6849405, US7456273, US7183395. In another embodiment, the TEE in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in US Patent Publication Nos. US20090226470, US20070048776, US20130177581 and US201 10124100, International Patent Publication No. WO1999024595, WO2012009644, WO2009075886 and WO2007025008, European Patent Publication No. EP2610341A1 and EP2610340A1, US Patent No. US6310197, US6849405, US7456273, US7183395; each of which are herein incorporated by reference in their entirety.

[00130] In one embodiment, the TEE in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may include at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more than 99% of the TEE sequences disclosed in Chappell et al. (Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005), in

Supplemental Table 1 and in Supplemental Table 2 disclosed by Wellensiek et al (Genome-wide profiling of human cap-independent translation-enhancing elements, Nature Methods, 2013; DOL10.1038/NMETH.2522); each of which is herein incorporated by reference in its entirety. In another embodiment, the TEE in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in Chappell et al. (Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005), in Supplemental Table 1 and in Supplemental Table 2 disclosed by Wellensiek et al (Genome-wide profiling of human cap-independent translation-enhancing elements, Nature Methods, 2013; DOL10.1038/NMETH.2522); each of which is herein incorporated by reference in its entirety.

[00131] In one embodiment, the TEE used in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention is an IRES sequence such as, but not limited to, those described in US Patent No. US7468275 and International Patent Publication No. WO200 1055369, each of which is herein incorporated by reference in its entirety.

[00132] In one embodiment, the TEEs used in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may be identified by the methods described in US Patent Publication No. US20070048776 and US201 10124100 and International Patent Publication Nos. WO2007025008 and WO20 12009644, each of which is herein incorporated by reference in its entirety.

[00133] In another embodiment, the TEEs used in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may be a transcription regulatory element described in US Patent No. US7456273 and US7183395, US Patent Publication No. US20090093049, and International Publication No. WO200 1055371, each of which is herein incorporated by reference in their entirety. The transcription regulatory elements may be identified by methods known in the art, such as, but not limited to, the methods described in US Patent No. US7456273 and

US7183395, US Patent Publication No. US20090093049, and International Publication No. WO200 1055371, each of which is herein incorporated by reference in their entirety.

[00134] In yet another embodiment, the TEE used in the 5'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention is an oligonucleotide or portion thereof as described in US Patent No. US7456273 and US7183395, US Patent Publication No. US20090093049, and International Publication No. WO200 1055371, each of which is herein incorporated by reference in their entirety.

[00135] The 5' UTR comprising at least one TEE described herein may be incorporated in a monocistronic sequence such as, but not limited to, a vector system or a nucleic acid vector. As a non-limiting example, the vector systems and nucleic acid vectors may include those described in US Patent Nos. 7456273 and US7183395, US Patent Publication No. US20070048776, US20090093049 and US201 10124100 and International Patent Publication Nos. WO2007025008 and WO200 1055371, each of which is herein incorporated by reference in its entirety.

[00136] In one embodiment, the TEEs described herein may be located in the 5'UTR and/or the 3'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA. The TEEs located in the 3'UTR may be the same and/or different than the TEEs located in and/or described for incorporation in the 5'UTR.

[00137] In one embodiment, the 3'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55 or more than 60 TEE sequences. The TEE sequences in the 3'UTR of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may be the same or different TEE sequences. The TEE sequences may be in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

[00138] In one embodiment, the 3'UTR may include a spacer to separate two TEE sequences. As a non-limiting example, the spacer may be a 15 nucleotide spacer and/or other spacers known in the art. As another non-limiting example, the 3'UTR may include a TEE sequence-spacer module repeated at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times and at least 9 times or more than 9 times in the 3'UTR.

[00139] In another embodiment, the spacer separating two TEE sequences may include other sequences known in the art which may regulate the translation of the polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention such as, but not limited to, miR sequences described herein (e.g., miR binding sites and miR seeds). As a non-limiting example, each spacer used to separate two TEE sequences may include a different miR sequence or component of a miR sequence (e.g., miR seed sequence).

[00140] In one embodiment, the incorporation of a miR sequence and/or a TEE sequence changes the shape of the stem loop region which may increase and/or decrease translation. (see e.g., Kedde et al. A Pumilio-induced RNA structure switch in p27-3'UTR controls miR-221 and miR-22 accessibility. Nature Cell Biology. 2010, herein incorporated by reference in its entirety).

Heterologous 5'UTRs

[00141] A 5' UTR may be provided as a flanking region to the modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids of the invention. 5'UTR may be homologous or heterologous to the coding region found in the modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids of the invention. Multiple 5' UTRs may be included in the flanking region and may be the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical modifications, before and/or after codon optimization.

[00142] Shown in Lengthy Table 21 in US Provisional Application No 61/775,509, filed March 9, 2013, entitled Heterologous Untranslated Regions for mRNA and in Lengthy Table 21 and in Table 22 in US Provisional Application No 61/829,372, filed May 31, 2013, entitled Heterologous Untranslated Regions for mRNA, the contents of

each of which is herein incorporated by reference in its entirety, is a listing of the start and stop site of the modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids of the invention. In Table 21 each 5'UTR (5'UTR-005 to 5'UTR 685 11) is identified by its start and stop site relative to its native or wild type (homologous) transcript (ENST; the identifier used in the ENSEMBL database).

[00143] Additional 5'UTR which may be used with the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention are shown in the present disclosure in Table 6, Table 38 and Table 41.

[00144] To alter one or more properties of the polynucleotides, primary constructs or mmRNA of the invention, 5'UTRs which are heterologous to the coding region of the modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids of the invention are engineered into compounds of the invention. The modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids are then administered to cells, tissue or organisms and outcomes such as protein level, localization and/or half life are measured to evaluate the beneficial effects the heterologous 5'UTR may have on the modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids of the invention. Variants of the 5' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G. 5'UTRs may also be codon-optimized or modified in any manner described herein.

Incorporating microRNA Binding Sites

[00145] In one embodiment modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids of the invention would not only encode a polypeptide but also a sensor sequence. Sensor sequences include, for example, microRNA binding sites, transcription factor binding sites, structured mRNA sequences and/or motifs, artificial binding sites engineered to act as pseudo-receptors for endogenous nucleic acid binding molecules. Non-limiting examples, of polynucleotides comprising at least one sensor sequence are described in co-pending and co-owned U.S. Provisional Patent Application No. US 61/753,661, filed January 17, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S. Provisional Patent Application No. US 61/754,159, filed January 18, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S.

Provisional Patent Application No. US61/781,097, filed March 14, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S. Provisional Patent Application No. US 61/829,334, filed May 31, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S. Provisional Patent Application No. US 61/839,893, filed June 27, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironments, U.S. Provisional Patent Application No. US 61/842,733, filed July 3, 2013, entitled Signal-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironment, and US Provisional Patent Application No. US 61/857,304, filed July 23, 2013, entitled Signla-Sensor Polynucleotide for the Alteration of Cellular Phenotypes and Microenvironment, the contents of each of which are herein incorporated by reference in its entirety.

[00146] In one embodiment, microRNA (miRNA) profiling of the target cells or tissues is conducted to determine the presence or absence of miRNA in the cells or tissues.

[00147] microRNAs (or miRNA) are 19-25 nucleotide long noncoding RNAs that bind to the 3'UTR of nucleic acid molecules and down-regulate gene expression either by reducing nucleic acid molecule stability or by inhibiting translation. The modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids of the invention may comprise one or more microRNA target sequences, microRNA sequences, or microRNA seeds. Such sequences may correspond to any known microRNA such as those taught in US Publication US2005/0261218 and US Publication US2005/0059005, the contents of which are incorporated herein by reference in their entirety. As a non-limiting embodiment, known microRNAs, their sequences and seed sequences in human genome are listed below in Table 11.

[00148] A microRNA sequence comprises a "seed" region, i.e., a sequence in the region of positions 2-8 of the mature microRNA, which sequence has perfect Watson-Crick complementarity to the miRNA target sequence. A microRNA seed may comprise positions 2-8 or 2-7 of the mature microRNA. In some embodiments, a microRNA seed may comprise 7 nucleotides (e.g., nucleotides 2-8 of the mature microRNA), wherein the seed-complementary site in the corresponding miRNA target is flanked by an adenine (A)

opposed to microRNA position 1. In some embodiments, a microRNA seed may comprise 6 nucleotides (e.g., nucleotides 2-7 of the mature microRNA), wherein the seed-complementary site in the corresponding miRNA target is flanked by an adenine (A) opposed to microRNA position 1. See for example, Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Barrell DP; *Mol Cell*. 2007 Jul 6;27(1):91-105. The bases of the microRNA seed have complete complementarity with the target sequence. By engineering microRNA target sequences into the 3'UTR of nucleic acids or mRNA of the invention one can target the molecule for degradation or reduced translation, provided the microRNA in question is available. This process will reduce the hazard of off target effects upon nucleic acid molecule delivery. Identification of microRNA, microRNA target regions, and their expression patterns and role in biology have been reported (Bonauer et al., *Curr Drug Targets* 2010 11:943-949; Anand and Cheresch *Curr Opin Hematol* 2011 18:171-176; Contreras and Rao *Leukemia* 2012 26:404-413 (2011 Dec 20. doi: 10.1038/leu.2011.356); Barrell *Cell* 2009 136:215-233; Landgraf et al, *Cell*, 2007 129:1401-1414; Gentner and Naldini, *Tissue Antigens*. 2012 80:393-403 and all references therein; each of which is herein incorporated by reference in its entirety).

[00149] For example, if the mRNA is not intended to be delivered to the liver but ends up there, then miR-122, a microRNA abundant in liver, can inhibit the expression of the gene of interest if one or multiple target sites of miR-122 are engineered into the 3'UTR of the modified nucleic acids, enhanced modified RNA or ribonucleic acids. Introduction of one or multiple binding sites for different microRNA can be engineered to further decrease the longevity, stability, and protein translation of a modified nucleic acids, enhanced modified RNA or ribonucleic acids. As used herein, the term "microRNA site" refers to a microRNA target site or a microRNA recognition site, or any nucleotide sequence to which a microRNA binds or associates. It should be understood that "binding" may follow traditional Watson-Crick hybridization rules or may reflect any stable association of the microRNA with the target sequence at or adjacent to the microRNA site.

[00150] Conversely, for the purposes of the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention, microRNA binding sites can be engineered out of (i.e. removed from) sequences in which they naturally occur in order to

increase protein expression in specific tissues. For example, miR-122 binding sites may be removed to improve protein expression in the liver.

[00151] In one embodiment, the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention may include at least one miRNA-binding site in the 3'UTR in order to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells (e.g., HEP3B or SNU449).

[00152] In another embodiment, the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention may include three miRNA-binding sites in the 3'UTR in order to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells (e.g., HEP3B or SNU449).

[00153] Regulation of expression in multiple tissues can be accomplished through introduction or removal of one or several microRNA binding sites. Shown below in Table 12, microRNAs which are differentially expressed in different tissues and cells, and often associated with different types of diseases (e.g. cancer cells). The decision of removal or insertion of microRNA binding sites, or any combination, is dependent on microRNA expression patterns and their profilings in diseases.

[00154] Examples of tissues where microRNA are known to regulate mRNA, and thereby protein expression, include, but are not limited to, liver (miR-122), muscle (miR-133, miR-206, miR-208), endothelial cells (miR-17-92, miR-126), myeloid cells (miR-142-3p, miR-142-5p, miR-16, miR-21, miR-223, miR-24, miR-27), adipose tissue (let-7, miR-30c), heart (miR-142, miR-149), kidney (miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126).

[00155] Specifically, microRNAs are known to be differentially expressed in immune cells (also called hematopoietic cells), such as antigen presenting cells (APCs) (e.g. dendritic cells and macrophages), macrophages, monocytes, B lymphocytes, T lymphocytes, granulocytes, natural killer cells, etc. Immune cell specific microRNAs are involved in immunogenicity, autoimmunity, the immune response to infection, inflammation, as well as unwanted immune response after gene therapy and tissue/organ transplantation. Immune cells specific microRNAs also regulate many aspects of development, proliferation, differentiation and apoptosis of hematopoietic cells (immune cells). For example, miR-142 and miR-146 are exclusively expressed in the immune

cells, particularly abundant in myeloid dendritic cells. It was demonstrated in the art that the immune response to exogenous nucleic acid molecules was shut-off by adding miR-142 binding sites to the 3'UTR of the delivered gene construct, enabling more stable gene transfer in tissues and cells. miR-142 efficiently degrades the exogenous mRNA in antigen presenting cells and suppresses cytotoxic elimination of transduced cells (Annoni A et al, blood, 2009, 114, 5152-5161; Brown BD, et al, Nat med. 2006, 12(5), 585-591; Brown BD, et al, blood, 2007, 110(13): 4144-4152, each of which is herein incorporated by reference in its entirety).

[00156] An antigen-mediated immune response can refer to an immune response triggered by foreign antigens, which, when entering an organism, are processed by the antigen presenting cells and displayed on the surface of the antigen presenting cells. T cells can recognize the presented antigen and induce a cytotoxic elimination of cells that express the antigen.

[00157] Introducing the miR-142 binding site into the 3'-UTR of a polypeptide of the present invention can selectively repress the gene expression in the antigen presenting cells through miR-142 mediated mRNA degradation, limiting antigen presentation in APCs (e.g. dendritic cells) and thereby preventing antigen-mediated immune response after the delivery of the polynucleotides. The polynucleotides are therefore stably expressed in target tissues or cells without triggering cytotoxic elimination.

[00158] In one embodiment, microRNAs binding sites that are known to be expressed in immune cells, in particular, the antigen presenting cells, can be engineered into the polynucleotide to suppress the expression of the sensor-signal polynucleotide in APCs through microRNA mediated RNA degradation, subduing the antigen-mediated immune response, while the expression of the polynucleotide is maintained in non-immune cells where the immune cell specific microRNAs are not expressed. For example, to prevent the immunogenic reaction caused by a liver specific protein expression, the miR-122 binding site can be removed and the miR-142 (and/or mirR-146) binding sites can be engineered into the 3-UTR of the polynucleotide.

[00159] To further drive the selective degradation and suppression of mRNA in APCs and macrophage, the polynucleotide may include another negative regulatory element in the 3-UTR, either alone or in combination with mir-142 and/or mir-146 binding sites. As

a non-limiting example, one regulatory element is the Constitutive Decay Elements (CDEs).

[00160] Immune cells specific microRNAs include, but are not limited to, hsa-let-7a-2-3p, hsa-let-7a-3p, hsa-7a-5p, hsa-let-7c, hsa-let-7e-3p, hsa-let-7e-5p, hsa-let-7g-3p, hsa-let-7g-5p, hsa-let-7i-3p, hsa-let-7i-5p, miR-10a-3p, miR-10a-5p, miR-184, hsa-let-7f-l-3p, hsa-let-7f-2--5p, hsa-let-7f-5p, miR-125b-l-3p, miR-125b-2-3p, miR-125b-5p, miR-1279, miR-130a-3p, miR-130a-5p, miR-132-3p, miR-132-5p, miR-142-3p, miR-142-5p, miR-143-3p, miR-143-5p, miR-146a-3p, miR-146a-5p, miR-146b-3p, miR-146b-5p, miR-147a, miR-147b, miR-148a-5p, miR-148a-3p, miR-150-3p, miR-150-5p, miR-151b, miR-155-3p, miR-155-5p, miR-15a-3p, miR-15a-5p, miR-15b-5p, miR-15b-3p, miR-16-l-3p, miR-16-2-3p, miR-16-5p, miR-17-5p, miR-181a-3p, miR-181a-5p, miR-181a-2-3p, miR-182-3p, miR-182-5p, miR-197-3p, miR-197-5p, miR-21-5p, miR-21-3p, miR-214-3p, miR-214-5p, miR-223-3p, miR-223-5p, miR-221-3p, miR-221-5p, miR-23b-3p, miR-23b-5p, miR-24-l-5p, miR-24-2-5p, miR-24-3p, miR-26a-l-3p, miR-26a-2-3p, miR-26a-5p, miR-26b-3p, miR-26b-5p, miR-27a-3p, miR-27a-5p, miR-27b-3p, miR-27b-5p, miR-28-3p, miR-28-5p, miR-2909, miR-29a-3p, miR-29a-5p, miR-29b-l-5p, miR-29b-2-5p, miR-29c-3p, miR-29c-5p,, miR-30e-3p, miR-30e-5p, miR-331-5p, miR-339-3p, miR-339-5p, miR-345-3p, miR-345-5p, miR-346, miR-34a-3p, miR-34a-5p, , miR-363-3p, miR-363-5p, miR-372, miR-377-3p, miR-377-5p, miR-493-3p, miR-493-5p, miR-542, miR-548b-5p, miR-548c-5p, miR-548i, miR-548j, miR-548n, miR-574-3p, miR-598, miR-718, miR-935, miR-99a-3p, miR-99a-5p, miR-99b-3p and miR-99b-5p. microRNAs that are enriched in specific types of immune cells are listed in Table 13. Furthermore, novel microRNAs are discovered in the immune cells in the art through micro-array hybridization and microtome analysis (Jima DD et al, Blood, 2010, 116:el 18-el27; Vaz C et al, BMC Genomics, 2010, 11,288, the content of each of which is incorporated herein by reference in its entirety.)

[00161] MicroRNAs that are known to be expressed in the liver include, but are not limited to, miR-107, miR-122-3p, miR-122-5p, miR-1228-3p, miR-1228-5p, miR-1249, miR-129-5p, miR-1303, miR-151a-3p, miR-151a-5p, miR-152, miR-194-3p, miR-194-5p, miR-199a-3p, miR-199a-5p, miR-199b-3p, miR-199b-5p, miR-296-5p, miR-557, miR-581, miR-939-3p, miR-939-5p. MicroRNA binding sites from any liver specific

microRNA can be introduced to or removed from the polynucleotides to regulate the expression of the polynucleotides in the liver. Liver specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent immune reaction against protein expression in the liver.

[00162] MicroRNAs that are known to be expressed in the lung include, but are not limited to, let-7a-2-3p, let-7a-3p, let-7a-5p, miR-126-3p, miR-126-5p, miR-127-3p, miR-127-5p, miR-130a-3p, miR-130a-5p, miR-130b-3p, miR-130b-5p, miR-133a, miR-133b, miR-134, miR-18a-3p, miR-18a-5p, miR-18b-3p, miR-18b-5p, miR-24-l-5p, miR-24-2-5p, miR-24-3p, miR-296-3p, miR-296-5p, miR-32-3p, miR-337-3p, miR-337-5p, miR-381-3p, miR-381-5p. MicroRNA binding sites from any lung specific microRNA can be introduced to or removed from the polynucleotide to regulate the expression of the polynucleotide in the lung. Lung specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent an immune reaction against protein expression in the lung.

[00163] MicroRNAs that are known to be expressed in the heart include, but are not limited to, miR-1, miR-133a, miR-133b, miR-149-3p, miR-149-5p, miR-186-3p, miR-186-5p, miR-208a, miR-208b, miR-210, miR-296-3p, miR-320, miR-451a, miR-451b, miR-499a-3p, miR-499a-5p, miR-499b-3p, miR-499b-5p, miR-744-3p, miR-744-5p, miR-92b-3p and miR-92b-5p. MicroRNA binding sites from any heart specific microRNA can be introduced to or removed from the polynucleotides to regulate the expression of the polynucleotides in the heart. Heart specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites to prevent an immune reaction against protein expression in the heart.

[00164] MicroRNAs that are known to be expressed in the nervous system include, but are not limited to, miR-124-5p, miR-125a-3p, miR-125a-5p, miR-125b-l-3p, miR-125b-2-3p, miR-125b-5p, miR-1271-3p, miR-1271-5p, miR-128, miR-132-5p, miR-135a-3p, miR-135a-5p, miR-135b-3p, miR-135b-5p, miR-137, miR-139-5p, miR-139-3p, miR-149-3p, miR-149-5p, miR-153, miR-181c-3p, miR-181c-5p, miR-183-3p, miR-183-5p, miR-190a, miR-190b, miR-212-3p, miR-212-5p, miR-219-l-3p, miR-219-2-3p, miR-

23a-3p, miR-23a-5p, miR-30a-5p, miR-30b-3p, miR-30b-5p, miR-30c-1-3p, miR-30c-2-3p, miR-30c-5p, miR-30d-3p, miR-30d-5p, miR-329, miR-342-3p, miR-3665, miR-3666, miR-380-3p, miR-380-5p, miR-383, miR-410, miR-425-3p, miR-425-5p, miR-454-3p, miR-454-5p, miR-483, miR-510, miR-516a-3p, miR-548b-5p, miR-548c-5p, miR-571, miR-7-1-3p, miR-7-2-3p, miR-7-5p, miR-802, miR-922, miR-9-3p and miR-9-5p.

MicroRNAs enriched in the nervous system further include those specifically expressed in neurons, including, but not limited to, miR-132-3p, miR-132-5p, miR-148b-3p, miR-148b-5p, miR-151a-3p, miR-151a-5p, miR-212-3p, miR-212-5p, miR-320b, miR-320e, miR-323a-3p, miR-323a-5p, miR-324-5p, miR-325, miR-326, miR-328, miR-922 and those specifically expressed in glial cells, including, but not limited to, miR-1250, miR-219-1-3p, miR-219-2-3p, miR-219-5p, miR-23a-3p, miR-23a-5p, miR-3065-3p, miR-3065-5p, miR-30e-3p, miR-30e-5p, miR-32-5p, miR-338-5p, miR-657. MicroRNA binding sites from any CNS specific microRNA can be introduced to or removed from the polynucleotides to regulate the expression of the polynucleotide in the nervous system. Nervous system specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent immune reaction against protein expression in the nervous system.

[00165] MicroRNAs that are known to be expressed in the pancreas include, but are not limited to, miR-105-3p, miR-105-5p, miR-184, miR-195-3p, miR-195-5p, miR-196a-3p, miR-196a-5p, miR-214-3p, miR-214-5p, miR-216a-3p, miR-216a-5p, miR-30a-3p, miR-33a-3p, miR-33a-5p, miR-375, miR-7-1-3p, miR-7-2-3p, miR-493-3p, miR-493-5p and miR-944. MicroRNA binding sites from any pancreas specific microRNA can be introduced to or removed from the polynucleotide to regulate the expression of the polynucleotide in the pancreas. Pancreas specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent an immune reaction against protein expression in the pancreas.

[00166] MicroRNAs that are known to be expressed in the kidney further include, but are not limited to, miR-122-3p, miR-145-5p, miR-17-5p, miR-192-3p, miR-192-5p, miR-194-3p, miR-194-5p, miR-20a-3p, miR-20a-5p, miR-204-3p, miR-204-5p, miR-210, miR-216a-3p, miR-216a-5p, miR-296-3p, miR-30a-3p, miR-30a-5p, miR-30b-3p, miR-

30b-5p, miR-30c-l-3p, miR-30c-2-3p, miR30c-5p, miR-324-3p, miR-335-3p, miR-335-5p, miR-363-3p, miR-363-5p and miR-562. MicroRNA binding sites from any kidney specific microRNA can be introduced to or removed from the polynucleotide to regulate the expression of the polynucleotide in the kidney. Kidney specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites to prevent an immune reaction against protein expression in the kidney.

[00167] MicroRNAs that are known to be expressed in the muscle further include, but are not limited to, let-7g-3p, let-7g-5p, miR-1, miR-1286, miR-133a, miR-133b, miR-140-3p, miR-143-3p, miR-143-5p, miR-145-3p, miR-145-5p, miR-188-3p, miR-188-5p, miR-206, miR-208a, miR-208b, miR-25-3p and miR-25-5p. MicroRNA binding sites from any muscle specific microRNA can be introduced to or removed from the polynucleotide to regulate the expression of the polynucleotide in the muscle. Muscle specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites to prevent an immune reaction against protein expression in the muscle.

[00168] MicroRNAs are differentially expressed in different types of cells, such as endothelial cells, epithelial cells and adipocytes. For example, microRNAs that are expressed in endothelial cells include, but are not limited to, let-7b-3p, let-7b-5p, miR-100-3p, miR-100-5p, miR-101-3p, miR-101-5p, miR-126-3p, miR-126-5p, miR-1236-3p, miR-1236-5p, miR-130a-3p, miR-130a-5p, miR-17-5p, miR-17-3p, miR-18a-3p, miR-18a-5p, , miR-19a-3p, miR-19a-5p, miR-19b-l-5p, miR-19b-2-5p, miR-19b-3p, miR-20a-3p, miR-20a-5p, miR-217, miR-210, miR-21-3p, miR-21-5p, miR-221-3p, miR-221-5p, miR-222-3p, miR-222-5p, miR-23a-3p, miR-23a-5p, miR-296-5p, miR-361-3p, miR-361-5p, miR-421, miR-424-3p, miR-424-5p, miR-513a-5p, miR-92a-l-5p, miR-92a-2-5p, miR-92a-3p, miR-92b-3p and miR-92b-5p. Many novel microRNAs are discovered in endothelial cells from deep-sequencing analysis (Voellenkle C et al, RNA, 2012, 18, 472-484, herein incorporated by reference in its entirety) microRNA binding sites from any endothelial cell specific microRNA can be introduced to or removed from the polynucleotide to modulate the expression of the polynucleotide in the endothelial cells in various conditions.

[00169] For further example, microRNAs that are expressed in epithelial cells include, but are not limited to, let-7b-3p, let-7b-5p, miR-1246, miR-200a-3p, miR-200a-5p, miR-200b-3p, miR-200b-5p, miR-200c-3p, miR-200c-5p, miR-338-3p, miR-429, miR-451a, miR-451b, miR-494, miR-802 and miR-34a, miR-34b-5p, miR-34c-5p, miR-449a, miR-449b-3p, miR-449b-5p specific in respiratory ciliated epithelial cells; let-7 family, miR-133a, miR-133b, miR-126 specific in lung epithelial cells; miR-382-3p, miR-382-5p specific in renal epithelial cells and miR-762 specific in corneal epithelial cells.

MicroRNA binding sites from any epithelial cell specific MicroRNA can be introduced to or removed from the polynucleotide to modulate the expression of the polynucleotide in the epithelial cells in various conditions.

[00170] In addition, a large group of microRNAs are enriched in embryonic stem cells, controlling stem cell self-renewal as well as the development and/or differentiation of various cell lineages, such as neural cells, cardiac, hematopoietic cells, skin cells, osteogenic cells and muscle cells (Kuppusamy KT et al, *Curr. Mol Med*, 2013, 13(5), 757-764; Vidigal JA and Ventura A, *Semin Cancer Biol.* 2012, 22(5-6), 428-436; Goff LA et al, *PLoS One*, 2009, 4:e7192; Morin RD et al, *Genome Res*, 2008, 18, 610-621; Yoo JK et al, *Stem Cells Dev.* 2012, 21(11), 2049-2057, each of which is herein incorporated by reference in its entirety). MicroRNAs abundant in embryonic stem cells include, but are not limited to, let-7a-2-3p, let-7a-3p, let-7a-5p, let-7d-3p, let-7d-5p, miR-103a-2-3p, miR-103a-5p, miR-106b-3p, miR-106b-5p, miR-1246, miR-1275, miR-138-1-3p, miR-138-2-3p, miR-138-5p, miR-154-3p, miR-154-5p, miR-200c-3p, miR-200c-5p, miR-290, miR-301a-3p, miR-301a-5p, miR-302a-3p, miR-302a-5p, miR-302b-3p, miR-302b-5p, miR-302c-3p, miR-302c-5p, miR-302d-3p, miR-302d-5p, miR-302e, miR-367-3p, miR-367-5p, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-373, miR-380-5p, miR-423-3p, miR-423-5p, miR-486-5p, miR-520c-3p, miR-548e, miR-548f, miR-548g-3p, miR-548g-5p, miR-548i, miR-548k, miR-548l, miR-548m, miR-548n, miR-548o-3p, miR-548o-5p, miR-548p, miR-664a-3p, miR-664a-5p, miR-664b-3p, miR-664b-5p, miR-766-3p, miR-766-5p, miR-885-3p, miR-885-5p, miR-93-3p, miR-93-5p, miR-941, miR-96-3p, miR-96-5p, miR-99b-3p and miR-99b-5p. Many predicted novel microRNAs are discovered by deep sequencing in human embryonic stem cells (Morin RD et al, *Genome Res*, 2008, 18, 610-621; Goff LA et al, *PLoS One*, 2009, 4:e7192; Bar

M et al, Stem cells, 2008, 26, 2496-2505, the content of each of which is incorporated herein by references in its entirety).

[00171] In one embodiment, the binding sites of embryonic stem cell specific microRNAs can be included in or removed from the 3-UTR of the polynucleotide to modulate the development and/or differentiation of embryonic stem cells, to inhibit the senescence of stem cells in a degenerative condition (e.g. degenerative diseases), or to stimulate the senescence and apoptosis of stem cells in a disease condition (e.g. cancer stem cells).

[00172] Many microRNA expression studies are conducted in the art to profile the differential expression of microRNAs in various cancer cells /tissues and other diseases. Some microRNAs are abnormally over-expressed in certain cancer cells and others are under-expressed. For example, microRNAs are differentially expressed in cancer cells (WO2008/154098, US2013/0059015, US2013/0042333, WO20 11/157294); cancer stem cells (US2012/0053224); pancreatic cancers and diseases (US2009/0131348, US201 1/0171646, US20 10/0286232, US8389210); asthma and inflammation (US84 15096); prostate cancer (US20 13/0053264); hepatocellular carcinoma (WO2012/151212, US2012/0329672, WO2008/054828, US8252538); lung cancer cells (WO201 1/076143, WO20 13/033640, WO2009/070653, US2010/0323357); cutaneous T cell lymphoma (WO20 13/01 1378); colorectal cancer cells (WO201 1/0281756, WO201 1/076142); cancer positive lympho nodes (WO2009/1 00430, US2009/0263803); nasopharyngeal carcinoma (EP21 12235); chronic obstructive pulmonary disease (US20 12/0264626, US2013/0053263); thyroid cancer (WO20 13/066678); ovarian cancer cells (US2012/0309645, WO201 1/095623); breast cancer cells (WO2008/154098, WO2007/081740, US20 12/02 14699), leukemia and lymphoma (WO2008/073915, US2009/0092974, US20 12/03 16081, US2012/0283310, WO2010/018563, the content of each of which is incorporated herein by reference in their entirety.)

[00173] As a non-limiting example, microRNA sites that are over-expressed in certain cancer and/or tumor cells can be removed from the 3-UTR of the polynucleotide encoding the polypeptide of interest, restoring the expression suppressed by the over-expressed microRNAs in cancer cells, thus ameliorating the corresponsive biological function, for instance, transcription stimulation and/or repression, cell cycle arrest,

apoptosis and cell death. Normal cells and tissues, wherein microRNAs expression is not up-regulated, will remain unaffected.

[00174] MicroRNA can also regulate complex biological processes such as angiogenesis (miR-132) (Anand and Cheresch Curr Opin Hematol 2011 18:171-176). In the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention, binding sites for microRNAs that are involved in such processes may be removed or introduced, in order to tailor the expression of the modified nucleic acids, enhanced modified RNA or ribonucleic acids expression to biologically relevant cell types or to the context of relevant biological processes. In this context, the mRNA are defined as auxotrophic mRNA.

[00175] MicroRNA gene regulation may be influenced by the sequence surrounding the microRNA such as, but not limited to, the species of the surrounding sequence, the type of sequence (e.g., heterologous, homologous and artificial), regulatory elements in the surrounding sequence and/or structural elements in the surrounding sequence. The microRNA may be influenced by the 5'UTR and/or the 3'UTR. As a non-limiting example, a non-human 3'UTR may increase the regulatory effect of the microRNA sequence on the expression of a polypeptide of interest compared to a human 3'UTR of the same sequence type.

[00176] In one embodiment, other regulatory elements and/or structural elements of the 5'-UTR can influence microRNA mediated gene regulation. One example of a regulatory element and/or structural element is a structured IRES (Internal Ribosome Entry Site) in the 5'UTR, which is necessary for the binding of translational elongation factors to initiate protein translation. EIF4A2 binding to this secondarily structured element in the 5'UTR is necessary for microRNA mediated gene expression (Meijer HA et al, Science, 2013, 340, 82-85, herein incorporated by reference in its entirety). The modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention can further be modified to include this structured 5'-UTR in order to enhance microRNA mediated gene regulation.

[00177] At least one microRNA site can be engineered into the 3' UTR of the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention. In this context, at least two, at least three, at least four, at least five, at least

six, at least seven, at least eight, at least nine, at least ten or more microRNA sites may be engineered into the 3' UTR of the ribonucleic acids of the present invention. In one embodiment, the microRNA sites incorporated into the modified nucleic acids, enhanced modified RNA or ribonucleic acids may be the same or may be different microRNA sites. In another embodiment, the microRNA sites incorporated into the modified nucleic acids, enhanced modified RNA or ribonucleic acids may target the same or different tissues in the body. As a non-limiting example, through the introduction of tissue-, cell-type-, or disease-specific microRNA binding sites in the 3' UTR of a modified nucleic acid mRNA, the degree of expression in specific cell types (e.g. hepatocytes, myeloid cells, endothelial cells, cancer cells, etc.) can be reduced.

[00178] In one embodiment, a microRNA site can be engineered near the 5' terminus of the 3'UTR, about halfway between the 5' terminus and 3'terminus of the 3'UTR and/or near the 3'terminus of the 3'UTR. As a non-limiting example, a microRNA site may be engineered near the 5' terminus of the 3'UTR and about halfway between the 5' terminus and 3'terminus of the 3'UTR. As another non-limiting example, a microRNA site may be engineered near the 3'terminus of the 3'UTR and about halfway between the 5' terminus and 3'terminus of the 3'UTR. As yet another non-limiting example, a microRNA site may be engineered near the 5' terminus of the 3'UTR and near the 3' terminus of the 3'UTR.

[00179] In another embodiment, a 3'UTR can comprise 4 microRNA sites. The microRNA sites may be complete microRNA binding sites, microRNA seed sequences and/or microRNA binding site sequences without the seed sequence.

[00180] In one embodiment, a nucleic acid of the invention may be engineered to include at least one microRNA in order to dampen the antigen presentation by antigen presenting cells. The microRNA may be the complete microRNA sequence, the microRNA seed sequence, the microRNA sequence without the seed or a combination thereof. As a non-limiting example, the microRNA incorporated into the nucleic acid may be specific to the hematopoietic system. As another non-limiting example, the microRNA incorporated into the nucleic acid of the invention to dampen antigen presentation is miR-142-3p.

[00181] In one embodiment, a nucleic acid may be engineered to include microRNA sites which are expressed in different tissues of a subject. As a non-limiting example, a modified nucleic acid, enhanced modified RNA or ribonucleic acid of the present invention may be engineered to include miR-192 and miR-122 to regulate expression of the modified nucleic acid, enhanced modified RNA or ribonucleic acid in the liver and kidneys of a subject. In another embodiment, a modified nucleic acid, enhanced modified RNA or ribonucleic acid may be engineered to include more than one microRNA sites for the same tissue. For example, a modified nucleic acid, enhanced modified RNA or ribonucleic acid of the present invention may be engineered to include miR-17-92 and miR-126 to regulate expression of the modified nucleic acid, enhanced modified RNA or ribonucleic acid in endothelial cells of a subject.

[00182] In one embodiment, the therapeutic window and or differential expression associated with the target polypeptide encoded by the modified nucleic acid, enhanced modified RNA or ribonucleic acid encoding a signal (also referred to herein as a polynucleotide) of the invention may be altered. For example, polynucleotides may be designed whereby a death signal is more highly expressed in cancer cells (or a survival signal in a normal cell) by virtue of the miRNA signature of those cells. Where a cancer cell expresses a lower level of a particular miRNA, the polynucleotide encoding the binding site for that miRNA (or miRNAs) would be more highly expressed. Hence, the target polypeptide encoded by the polynucleotide is selected as a protein which triggers or induces cell death. Neighboring noncancer cells, harboring a higher expression of the same miRNA would be less affected by the encoded death signal as the polynucleotide would be expressed at a lower level due to the effects of the miRNA binding to the binding site or "sensor" encoded in the 3'UTR. Conversely, cell survival or cytoprotective signals may be delivered to tissues containing cancer and non cancerous cells where a miRNA has a higher expression in the cancer cells—the result being a lower survival signal to the cancer cell and a larger survival signature to the normal cell. Multiple polynucleotides may be designed and administered having different signals according to the previous paradigm.

[00183] In one embodiment, the expression of a nucleic acid may be controlled by incorporating at least one sensor sequence in the nucleic acid and formulating the nucleic

acid. As a non-limiting example, a nucleic acid may be targeted to an orthotopic tumor by having a nucleic acid incorporating a miR-122 binding site and formulated in a lipid nanoparticle comprising the cationic lipid DLin-KC2-DMA (see e.g., the experiments described in Example 49A and 49B).

[00184] According to the present invention, the polynucleotides may be modified as to avoid the deficiencies of other polypeptide-encoding molecules of the art. Hence, in this embodiment the polynucleotides are referred to as modified polynucleotides.

[00185] Through an understanding of the expression patterns of microRNA in different cell types, modified nucleic acids, enhanced modified RNA or ribonucleic acids such as polynucleotides can be engineered for more targeted expression in specific cell types or only under specific biological conditions. Through introduction of tissue-specific microRNA binding sites, modified nucleic acids, enhanced modified RNA or ribonucleic acids, could be designed that would be optimal for protein expression in a tissue or in the context of a biological condition.

[00186] Transfection experiments can be conducted in relevant cell lines, using engineered modified nucleic acids, enhanced modified RNA or ribonucleic acids and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different microRNA binding site-engineering nucleic acids or mRNA and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hr, 12 hr, 24 hr, 48 hr, 72 hr and 7 days post-transfection. *In vivo* experiments can also be conducted using microRNA-binding site-engineered molecules to examine changes in tissue-specific expression of formulated modified nucleic acids, enhanced modified RNA or ribonucleic acids.

[00187] Non-limiting examples of cell lines which may be useful in these investigations include those from ATCC (Manassas, VA) including MRC-5, A549, T84, NCI-H2126 [H2126], NCI-H1688 [H1688], WI-38, WI-38 VA-13 subline 2RA, WI-26 VA4, C3A [HepG2/C3A, derivative of Hep G2 (ATCC HB-8065)], THLE-3, H69AR, NCI-H292 [H292], CFPAC-1, NTERA-2 cl.D1 [NT2/D1], DMS 79, DMS 53, DMS 153, DMS 114, MSTO-21 1H, SW 1573 [SW-1573, SW1573], SW 1271 [SW-1271, SW1271], SHP-77, SNU-398, SNU-449, SNU-182, SNU-475, SNU-387, SNU-423, NL20, NL20-TA [NL20T-A], THLE-2, HBE135-E6E7, HCC827, HCC4006, NCI-H23 [H23], NCI-

H1299, NCI-H187 [H187], NCI-H358 [H358, H358], NCI-H378 [H378], NCI-H522 [H522], NCI-H526 [H526], NCI-H727 [H727], NCI-H810 [H810], NCI-H889 [H889], NCI-H1155 [H1155], NCI-H1404 [H1404], NCI-N87 [N87], NCI-H196 [H196], NCI-H211 [H211], NCI-H220 [H220], NCI-H250 [H250], NCI-H524 [H524], NCI-H647 [H647], NCI-H650 [H650], NCI-H711 [H711], NCI-H719 [H719], NCI-H740 [H740], NCI-H748 [H748], NCI-H774 [H774], NCI-H838 [H838], NCI-H841 [H841], NCI-H847 [H847], NCI-H865 [H865], NCI-H920 [H920], NCI-H1048 [H1048], NCI-H1092 [H1092], NCI-H1105 [H1105], NCI-H1184 [H1184], NCI-H1238 [H1238], NCI-H1341 [H1341], NCI-H1385 [H1385], NCI-H1417 [H1417], NCI-H1435 [H1435], NCI-H1436 [H1436], NCI-H1437 [H1437], NCI-H1522 [H1522], NCI-H1563 [H1563], NCI-H1568 [H1568], NCI-H1573 [H1573], NCI-H1581 [H1581], NCI-H1618 [H1618], NCI-H1623 [H1623], NCI-H1650 [H1650, H1650], NCI-H1651 [H1651], NCI-H1666 [H1666], NCI-H1666 [H1666], NCI-H1672 [H1672], NCI-H1693 [H1693], NCI-H1694 [H1694], NCI-H1703 [H1703], NCI-H1734 [H1734, H1734], NCI-H1755 [H1755], NCI-H1755 [H1755], NCI-H1770 [H1770], NCI-H1793 [H1793], NCI-H1836 [H1836], NCI-H1838 [H1838], NCI-H1869 [H1869], NCI-H1876 [H1876], NCI-H1882 [H1882], NCI-H1915 [H1915], NCI-H1930 [H1930], NCI-H1944 [H1944], NCI-H1975 [H1975, H1975], NCI-H1993 [H1993], NCI-H2023 [H2023], NCI-H2029 [H2029], NCI-H2030 [H2030], NCI-H2066 [H2066], NCI-H2073 [H2073], NCI-H2081 [H2081], NCI-H2085 [H2085], NCI-H2087 [H2087], NCI-H2106 [H2106], NCI-H2110 [H2110], NCI-H2135 [H2135], NCI-H2141 [H2141], NCI-H2171 [H2171], NCI-H2172 [H2172], NCI-H2195 [H2195], NCI-H2196 [H2196], NCI-H2198 [H2198], NCI-H2227 [H2227], NCI-H2228 [H2228], NCI-H2286 [H2286], NCI-H2291 [H2291], NCI-H2330 [H2330], NCI-H2342 [H2342], NCI-H2347 [H2347], NCI-H2405 [H2405], NCI-H2444 [H2444], UMC-11, NCI-H64 [H64], NCI-H735 [H735], NCI-H735 [H735], NCI-H1963 [H1963], NCI-H2107 [H2107], NCI-H2108 [H2108], NCI-H2122 [H2122], Hs 573.T, Hs 573.Lu, PLC/PRF/5, BEAS-2B, Hep G2, Tera-1, Tera-2, NCI-H69 [H69], NCI-H128 [H128], ChaGo-K-1, NCI-H446 [H446], NCI-H209 [H209], NCI-H146 [H146], NCI-H441 [H441], NCI-H82 [H82], NCI-H460 [H460], NCI-H596 [H596], NCI-H676B [H676B], NCI-H345 [H345], NCI-H820 [H820], NCI-H520 [H520], NCI-H661 [H661], NCI-H510A [H510A, NCI-H510],

SK-HEP-1, A-427, Calu-1, Calu-3, Calu-6, SK-LU-1, SK-MES-1, SW 900 [SW-900, SW900], Malme-3M, and Capan-1.

[00188] In some embodiments, modified messenger RNA can be designed to incorporate microRNA binding region sites that either have 100% identity to known seed sequences or have less than 100% identity to seed sequences. The seed sequence can be partially mutated to decrease microRNA binding affinity and as such result in reduced downmodulation of that mRNA transcript. In essence, the degree of match or mis-match between the target mRNA and the microRNA seed can act as a rheostat to more finely tune the ability of the microRNA to modulate protein expression. In addition, mutation in the non-seed region of a microRNA binding site may also impact the ability of a microRNA to modulate protein expression.

[00189] In one embodiment, a miR sequence may be incorporated into the loop of a stem loop.

[00190] In another embodiment, a miR seed sequence may be incorporated in the loop of a stem loop and a miR binding site may be incorporated into the 5' or 3' stem of the stem loop.

[00191] In one embodiment, a TEE may be incorporated on the 5' end of the stem of a stem loop and a miR seed may be incorporated into the stem of the stem loop. In another embodiment, a TEE may be incorporated on the 5' end of the stem of a stem loop, a miR seed may be incorporated into the stem of the stem loop and a miR binding site may be incorporated into the 3' end of the stem or the sequence after the stem loop. The miR seed and the miR binding site may be for the same and/or different miR sequences.

[00192] In one embodiment, the incorporation of a miR sequence and/or a TEE sequence changes the shape of the stem loop region which may increase and/or decrease translation. (see e.g, Kedde et al. A Pumilio-induced RNA structure switch in p27-3'UTR controls miR-221 and miR-22 accessibility. Nature Cell Biology. 2010, herein incorporated by reference in its entirety).

[00193] In one embodiment, the incorporation of a miR sequence and/or a TEE sequence changes the shape of the stem loop region which may increase and/or decrease translation. (see e.g, Kedde et al. A Pumilio-induced RNA structure switch in p27-3'UTR

controls miR-221 and miR-22 accessibility. Nature Cell Biology. 2010, herein incorporated by reference in its entirety).

[00194] In one embodiment, the 5'UTR may comprise at least one microRNA sequence. The microRNA sequence may be, but is not limited to, a 19 or 22 nucleotide sequence and/or a microRNA sequence without the seed.

[00195] In one embodiment the microRNA sequence in the 5'UTR may be used to stabilize the nucleic acid and/or mRNA described herein.

[00196] In another embodiment, a microRNA sequence in the 5'UTR may be used to decrease the accessibility of the site of translation initiation such as, but not limited to a start codon. Matsuda et al (PLoS One. 2010 11(5):e 15057; herein incorporated by reference in its entirety) used antisense locked nucleic acid (LNA) oligonucleotides and exon-junction complexes (EJCs) around a start codon (-4 to +37 where the A of the AUG codon is +1) in order to decrease the accessibility to the first start codon (AUG). Matsuda showed that altering the sequence around the start codon with an LNA or EJC the efficiency, length and structural stability of the nucleic acid or mRNA is affected. The nucleic acids or mRNA of the present invention may comprise a microRNA sequence, instead of the LNA or EJC sequence described by Matsuda et al, near the site of translation initiation in order to decrease the accessibility to the site of translation initiation. The site of translation initiation may be prior to, after or within the microRNA sequence. As a non-limiting example, the site of translation initiation may be located within a microRNA sequence such as a seed sequence or binding site. As another non-limiting example, the site of translation initiation may be located within a miR-122 sequence such as the seed sequence or the mir-122 binding site.

[00197] In one embodiment, the nucleic acids or mRNA of the present invention may include at least one microRNA in order to dampen the antigen presentation by antigen presenting cells. The microRNA may be the complete microRNA sequence, the microRNA seed sequence, the microRNA sequence without the seed or a combination thereof. As a non-limiting example, the microRNA incorporated into the nucleic acids or mRNA of the present invention may be specific to the hematopoietic system. As another non-limiting example, the microRNA incorporated into the nucleic acids or mRNA of the present invention to dampen antigen presentation is miR-142-3p.

[00198] In one embodiment, the nucleic acids or mRNA of the present invention may include at least one microRNA in order to dampen expression of the encoded polypeptide in a cell of interest. As a non-limiting example, the nucleic acids or mRNA of the present invention may include at least one miR-122 binding site in order to dampen expression of an encoded polypeptide of interest in the liver. As another non-limiting example, the nucleic acids or mRNA of the present invention may include at least one miR-142-3p binding site, miR-142-3p seed sequence, miR-142-3p binding site without the seed, miR-142-5p binding site, miR-142-5p seed sequence, miR-142-5p binding site without the seed, miR-146 binding site, miR-146 seed sequence and/or miR-146 binding site without the seed sequence (see e.g., the experiment outlined in Example 24, 25, 26, 26, 36 and 48).

[00199] In one embodiment, the nucleic acids or mRNA of the present invention may comprise at least one microRNA binding site in the 3'UTR in order to selectively degrade mRNA therapeutics in the immune cells to subdue unwanted immunogenic reactions caused by therapeutic delivery. As a non-limiting example, the microRNA binding site may be the modified nucleic acids more unstable in antigen presenting cells. Non-limiting examples of these microRNA include mir-142-5p, mir-142-3p, mir-146a-5p and mir-146-3p.

[00200] In one embodiment, the nucleic acids or mRNA of the present invention comprises at least one microRNA sequence in a region of the nucleic acid or mRNA which may interact with a RNA binding protein.

RNA Motifs for RNA Binding Proteins (RBPs)

[00201] RNA binding proteins (RBPs) can regulate numerous aspects of co- and post-transcription gene expression such as, but not limited to, RNA splicing, localization, translation, turnover, polyadenylation, capping, modification, export and localization. RNA-binding domains (RBDs), such as, but not limited to, RNA recognition motif (RR) and hnRNP K-homology (KH) domains, typically regulate the sequence association between RBPs and their RNA targets (Ray et al. Nature 2013. 499:172-177; herein incorporated by reference in its entirety). In one embodiment, the canonical RBDs can bind short RNA sequences. In another embodiment, the canonical RBDs can recognize structure RNAs.

[00202] Non limiting examples of RNA binding proteins and related nucleic acid and protein sequences are shown in Table 26 in Example 23.

[00203] In one embodiment, to increase the stability of the mRNA of interest, an mRNA encoding HuR can be co-transfected or co-injected along with the mRNA of interest into the cells or into the tissue. These proteins can also be tethered to the mRNA of interest in vitro and then administered to the cells together. Poly A tail binding protein, PABP interacts with eukaryotic translation initiation factor eIF4G to stimulate translational initiation. Co-administration of mRNAs encoding these RBPs along with the mRNA drug and/or tethering these proteins to the mRNA drug in vitro and administering the protein-bound mRNA into the cells can increase the translational efficiency of the mRNA. The same concept can be extended to co-administration of mRNA along with mRNAs encoding various translation factors and facilitators as well as with the proteins themselves to influence RNA stability and/or translational efficiency.

[00204] In one embodiment, the nucleic acids and/or mRNA may comprise at least one RNA-binding motif such as, but not limited to a RNA-binding domain (RBD).

[00205] In one embodiment, the RBD may be any of the RBDs, fragments or variants thereof described by Ray et al. (Nature 2013. 499:172-177; herein incorporated by reference in its entirety).

[00206] In one embodiment, the nucleic acids or mRNA of the present invention may comprise a sequence for at least one RNA-binding domain (RBDs). When the nucleic acids or mRNA of the present invention comprise more than one RBD, the RBDs do not need to be from the same species or even the same structural class.

[00207] In one embodiment, at least one flanking region (e.g., the 5'UTR and/or the 3'UTR) may comprise at least one RBD. In another embodiment, the first flanking region and the second flanking region may both comprise at least one RBD. The RBD may be the same or each of the RBDs may have at least 60% sequence identity to the other RBD. As a non-limiting example, at least one RBD may be located before, after and/or within the 3'UTR of the nucleic acid or mRNA of the present invention. As another non-limiting example, at least one RBD may be located before or within the first 300 nucleosides of the 3'UTR.

[00208] In another embodiment, the nucleic acids and/or mRNA of the present invention may comprise at least one RBD in the first region of linked nucleosides. The RBD may be located before, after or within a coding region (e.g., the ORF).

[00209] In yet another embodiment, the first region of linked nucleosides and/or at least one flanking region may comprise at least one RBD. As a non-limiting example, the first region of linked nucleosides may comprise a RBD related to splicing factors and at least one flanking region may comprise a RBD for stability and/or translation factors.

[00210] In one embodiment, the nucleic acids and/or mRNA of the present invention may comprise at least one RBD located in a coding and/or non-coding region of the nucleic acids and/or mRNA.

[00211] In one embodiment, at least one RBD may be incorporated into at least one flanking region to increase the stability of the nucleic acid and/or mRNA of the present invention.

[00212] In one embodiment, a microRNA sequence in a RNA binding protein motif may be used to decrease the accessibility of the site of translation initiation such as, but not limited to a start codon. The nucleic acids or mRNA of the present invention may comprise a microRNA sequence, instead of the LNA or EJC sequence described by Matsuda et al, near the site of translation initiation in order to decrease the accessibility to the site of translation initiation. The site of translation initiation may be prior to, after or within the microRNA sequence. As a non-limiting example, the site of translation initiation may be located within a microRNA sequence such as a seed sequence or binding site. As another non-limiting example, the site of translation initiation may be located within a miR-122 sequence such as the seed sequence or the mir-122 binding site.

[00213] In another embodiment, an antisense locked nucleic acid (LNA) oligonucleotides and exon-junctino complexes (EJCs) may be used in the RNA binding protein motif. The LNA and EJCs may be used around a start codon (-4 to +37 where the A of the AUG codons is +1) in order to decrease the accessibility to the first start codon (AUG).

Other regulatory elements in 3'UTR

[00214] In addition to microRNA binding sites, other regulatory sequences in the 3'-UTR of natural mRNA, which regulate mRNA stability and translation in different

tissues and cells, can be removed or introduced into modified messenger RNA. Such cis-regulatory elements may include, but are not limited to, Cis- RNP (Ribonucleoprotein)/RBP (RNA binding protein) regulatory elements, AU-rich element (AUE), structured stem-loop, constitutive decay elements (CDEs), GC-richness and other structured mRNA motifs (Parker BJ et al, Genome Research, 2011, 21, 1929-1943, which is herein incorporated by reference in its entirety). For example, CDEs are a class of regulatory motifs that mediate mRNA degradation through their interaction with Roquin proteins. In particular, CDEs are found in many mRNAs that encode regulators of development and inflammation to limit cytokine production in macrophage (Leppek K et al, 2013, Cell, 153, 869-881, which is herein incorporated by reference in its entirety).

[00215] In one embodiment, a particular CDE can be introduced to the nucleic acids or mRNA when the degradation of polypeptides in a cell or tissue is desired. A particular CDE can also be removed from the nucleic acids or mRNA to maintain a more stable mRNA in a cell or tissue for sustaining protein expression.

Auxotrophic mRNA

[00216] In one embodiment, the nucleic acids or mRNA of the present invention may be auxotrophic. As used herein, the term "auxotrophic" refers to mRNA that comprises at least one feature that triggers, facilitates or induces the degradation or inactivation of the mRNA in response to spatial or temporal cues such that protein expression is substantially prevented or reduced. Such spatial or temporal cues include the location of the mRNA to be translated such as a particular tissue or organ or cellular environment. Also contemplated are cues involving temperature, pH, ionic strength, moisture content and the like.

[00217] In one embodiment, the feature is located in a terminal region of the nucleic acids or mRNA of the present invention. As a non-limiting example, the auxotrophic mRNA may contain a miR binding site in the terminal region which binds to a miR expressed in a selected tissue so that the expression of the auxotrophic mRNA is substantially prevented or reduced in the selected tissue. To this end and for example, an auxotrophic mRNA containing a miR- 122 binding site will not produce protein if localized to the liver since miR- 122 is expressed in the liver and binding of the miR would effectuate destruction of the auxotrophic mRNA. As a non-limiting example,

HEK293 cells do not express miR-122 so there would be little to no downregulation of a nucleic acid or mRNA of the present invention having a miR-122 sequence in HEK293 but for hepatocytes which do expression miR-122 there would be a downregulation of a nucleic acid or mRNA of the present invention having a miR-122 sequence in hepatocytes (see e.g., the study outlined Example 14). As another non-limiting example, the miR-122 level can be measured in HeLa cells, primary human hepatocytes and primary rat hepatocytes prior to administration with a nucleic acid or mRNA of the present invention encoding at least one miR-122 binding site, miR-122 binding site without the seed sequence or a miR-122 binding site. After administration the expression of the modified nucleic acid with a microRNA sequence can be measured to determine the dampening effect of the miR-122 in the modified nucleic acid (see e.g., the studies outlined in Examples 28, 29, 30, 35, 45, 46 and 47). As yet another non-limiting example, the effectiveness of the miR-122 binding site, miR-122 seed or the miR-122 binding site without the seed in different 3'UTRs may be evaluated in order to determine the proper UTR for the desired outcome such as, but not limited to, the highest dampening effect (see e.g., the study outlined in Example 35 and 46).

[00218] In one embodiment, the degradation or inactivation of auxotrophic mRNA may comprise a feature responsive to a change in pH. As a non-limiting example, the auxotrophic mRNA may be triggered in an environment having a pH of between pH 4.5 to 8.0 such as at a pH of 5.0 to 6.0 or a pH of 6.0 to 6.5. The change in pH may be a change of 0.1 unit, 0.2 units, 0.3 units, 0.4 units, 0.5 units, 0.6 units, 0.7 units, 0.8 units, 0.9 units, 1.0 units, 1.1 units, 1.2 units, 1.3 units, 1.4 units, 1.5 units, 1.6 units, 1.7 units, 1.8 units, 1.9 units, 2.0 units, 2.1 units, 2.2 units, 2.3 units, 2.4 units, 2.5 units, 2.6 units, 2.7 units, 2.8 units, 2.9 units, 3.0 units, 3.1 units, 3.2 units, 3.3 units, 3.4 units, 3.5 units, 3.6 units, 3.7 units, 3.8 units, 3.9 units, 4.0 units or more.

[00219] In another embodiment, the degradation or inactivation of auxotrophic mRNA may be triggered or induced by changes in temperature. As a non-limiting example, a change of temperature from room temperature to body temperature. The change of temperature may be less than 1°C, less than 5°C, less than 10°C, less than 15°C, less than 20°C, less than 25°C or more than 25°C.

[00220] In yet another embodiment, the degradation or inactivation of auxotrophic mRNA may be triggered or induced by a change in the levels of ions in the subject. The ions may be cations or anions such as, but not limited to, sodium ions, potassium ions, chloride ions, calcium ions, magnesium ions and/or phosphate ions.

3' UTR and the AU Rich Elements

[00221] 3'UTRs are known to have stretches of Adenosines and Uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF- α . Class III AREs are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

[00222] Introduction, removal or modification of 3' UTR AU rich elements (AREs) can be used to modulate the stability of nucleic acids or mRNA of the invention. When engineering specific nucleic acids or mRNA, one or more copies of an ARE can be introduced to make nucleic acids or mRNA of the invention less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein. Transfection experiments can be conducted in relevant cell lines, using nucleic acids or mRNA of the invention and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different ARE-engineering molecules and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hr, 12 hr, 24 hr, 48 hr, and 7 days post-transfection.

3' UTR and Triple Helices

[00223] In one embodiment, nucleic acids of the present invention may include a triple helix on the 3' end of the modified nucleic acid, enhanced modified RNA or ribonucleic acid. The 3' end of the nucleic acids of the present invention may include a triple helix alone or in combination with a Poly-A tail.

[00224] In one embodiment, the nucleic acid of the present invention may comprise at least a first and a second U-rich region, a conserved stem loop region between the first and second region and an A-rich region. The first and second U-rich region and the A-rich region may associate to form a triple helix on the 3' end of the nucleic acid. This triple helix may stabilize the nucleic acid, enhance the translational efficiency of the nucleic acid and/or protect the 3' end from degradation. Exemplary triple helices include, but are not limited to, the triple helix sequence of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), MEN- β and polyadenylated nuclear (PAN) RNA (See Wilusz et al, Genes & Development 2012 26:2392-2407; herein incorporated by reference in its entirety). In one embodiment, the 3' end of the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention comprises a first U-rich region comprising TTTTCTTTT (SEQ ID NO: 1), a second U-rich region comprising TTTTGCTTTT (SEQ ID NO: 2) or TTTTGCTTTT (SEQ ID NO: 3), an A-rich region comprising AAAAAGCAAAA (SEQ ID NO: 4). In another embodiment, the 3' end of the nucleic acids of the present invention comprises a triple helix formation structure comprising a first U-rich region, a conserved region, a second U-rich region and an A-rich region.

[00225] In one embodiment, the triple helix may be formed from the cleavage of a MALAT1 sequence prior to the cloverleaf structure. While not meaning to be bound by theory, MALAT1 is a long non-coding RNA which, when cleaved, forms a triple helix and a tRNA-like cloverleaf structure. The MALAT1 transcript then localizes to nuclear speckles and the tRNA-like cloverleaf localizes to the cytoplasm (Wilusz et al. Cell 2008 135(5): 919-932; herein incorporated by reference in its entirety).

[00226] As a non-limiting example, the terminal end of the nucleic acid of the present invention comprising the MALAT1 sequence can then form a triple helix structure, after RNaseP cleavage from the cloverleaf structure, which stabilizes the nucleic acid (Peart et

al. *Non-mRNA 3' endformation: how the other half lives*; WIREs RNA 2013; herein incorporated by reference in its entirety).

[00227] In one embodiment, the nucleic acids or mRNA described herein comprise a MALAT1 sequence. In another embodiment, the nucleic acids or mRNA may be polyadenylated. In yet another embodiment, the nucleic acids or mRNA is not polyadenylated but has an increased resistance to degradation compared to unmodified nucleic acids or mRNA.

[00228] In one embodiment, the nucleic acids of the present invention may comprise a MALAT1 sequence in the second flanking region (e.g., the 3'UTR). As a non-limiting example, the MALAT1 sequence may be human or mouse (see e.g., the polynucleotides described in Table 37 in Example 38).

[00229] In another embodiment, the cloverleaf structure of the MALAT1 sequence may also undergo processing by RNaseZ and CCA adding enzyme to form a tRNA-like structure called mascRNA (MALAT1-associated small cytoplasmic RNA). As a non-limiting example, the mascRNA may encode a protein or a fragment thereof and/or may comprise a microRNA sequence. The mascRNA may comprise at least one chemical modification described herein.

Stem Loop

[00230] In one embodiment, the nucleic acids of the present invention may include a stem loop such as, but not limited to, a histone stem loop. The stem loop may be a nucleotide sequence that is about 25 or about 26 nucleotides in length such as, but not limited to, SEQ ID NOs: 7-17 as described in International Patent Publication No. WO2013 103659, herein incorporated by reference in its entirety. The histone stem loop may be located 3' relative to the coding region (e.g., at the 3' terminus of the coding region). As a non-limiting example, the stem loop may be located at the 3' end of a nucleic acid described herein.

[00231] In one embodiment, the stem loop may be located in the second terminal region. As a non-limiting example, the stem loop may be located within an untranslated region (e.g., 3'UTR) in the second terminal region.

[00232] In one embodiment, the nucleic acid such as, but not limited to mRNA, which comprises the histone stem loop may be stabilized by the addition of at least one chain

terminating nucleoside. Not wishing to be bound by theory, the addition of at least one chain terminating nucleoside may slow the degradation of a nucleic acid and thus can increase the half-life of the nucleic acid.

[00233] In one embodiment, the chain terminating nucleoside may be, but is not limited to, those described in International Patent Publication No. WO2013 103659, herein incorporated by reference in its entirety. In another embodiment, the chain terminating nucleosides which may be used with the present invention includes, but is not limited to, 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, such as 2',3'-dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'-dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, or a -O- methyl nucleoside.

[00234] In another embodiment, the nucleic acid such as, but not limited to mRNA, which comprises the histone stem loop may be stabilized by a modification to the 3' region of the nucleic acid that can prevent and/or inhibit the addition of oligo(U) (see e.g., International Patent Publication No. WO2013 103659, herein incorporated by reference in its entirety).

[00235] In yet another embodiment, the nucleic acid such as, but not limited to mRNA, which comprises the histone stem loop may be stabilized by the addition of an oligonucleotide that terminates in a 3'-deoxynucleoside, 2',3'-dideoxynucleoside 3'-O-methyl nucleosides, 3'-O-ethyl nucleosides, 3'-arabinosides, and other modified nucleosides known in the art and/or described herein.

[00236] In one embodiment, the nucleic acids of the present invention may include a histone stem loop, a polyA tail sequence and/or a 5' cap structure. The histone stem loop may be before and/or after the polyA tail sequence. The nucleic acids comprising the histone stem loop and a polyA tail sequence may include a chain terminating nucleoside described herein.

[00237] In another embodiment, the nucleic acids of the present invention may include a histone stem loop and a 5' cap structure. The 5' cap structure may include, but is not limited to, those described herein and/or known in the art.

[00238] In one embodiment, the conserved stem loop region may comprise a miR sequence described herein. As a non-limiting example, the stem loop region may

comprise the seed sequence of a miR sequence described herein. In another non-limiting example, the stem loop region may comprise a miR- 122 seed sequence.

[00239] In another embodiment, the conserved stem loop region may comprise a miR sequence described herein and may also include a TEE sequence.

[00240] In one embodiment, the incorporation of a miR sequence and/or a TEE sequence changes the shape of the stem loop region which may increase and/or decrease translation. (see e.g, Kedde et al. A Pumilio-induced RNA structure switch in p27-3'UTR controls miR-221 and miR-22 accessibility. Nature Cell Biology. 2010, herein incorporated by reference in its entirety).

[00241] In one embodiment, the modified nucleic acids described herein may comprise at least one histone stem-loop and a polyA sequence or polyadenylation signal. Non-limiting examples of nucleic acid sequences encoding for at least one histone stem-loop and a polyA sequence or a polyadenylation signal are described in International Patent Publication No. WO2013 120497, WO2013 120629, WO2013 120500, WO2013 120627, WO2013 120498, WO2013 120626, WO2013 120499 and WO2013 120628, the contents of each of which is herein incorporated by reference in their entirety. In one embodiment, the nucleic acid encoding for a histone stem loop and a polyA sequence or a polyadenylation signal may code for a pathogen antigen or fragment thereof such as the nucleic acid sequences described in International Patent Publication No WO2013 120499 and WO2013 120628, the contents of which is herein incorporated by reference in its entirety. In another embodiment, the nucleic acid encoding for a histone stem loop and a polyA sequence or a polyadenylation signal may code for a therapeutic protein such as the nucleic acid sequences described in International Patent Publication No WO20 13 120497 and WO20 13 120629, the contents of which is herein incorporated by reference in its entirety. In one embodiment, the nucleic acid encoding for a histone stem loop and a polyA sequence or a polyadenylation signal may code for a tumor antigen or fragment thereof such as the nucleic acid sequences described in International Patent Publication No WO2013 120500 and WO20 13 120627, the contents of which is herein incorporated by reference in its entirety. In another embodiment, the nucleic acid encoding for a histone stem loop and a polyA sequence or a polyadenylation signal may code for a allergenic antigen or an autoimmune

self-antigen such as the nucleic acid sequences described in International Patent Publication No WO2013 120498 and WO2013 120626, the contents of which is herein incorporated by reference in its entirety.

5' Capping

[00242] The 5' cap structure of an mRNA is involved in nuclear export, increasing mRNA stability and binds the mRNA Cap Binding Protein (CBP), which is responsible for mRNA stability in the cell and translation competency through the association of CBP with poly(A) binding protein to form the mature cyclic mRNA species. The cap further assists the removal of 5' proximal introns removal during mRNA splicing.

[00243] Endogenous mRNA molecules may be 5'-end capped generating a 5'-ppp-5'-triphosphate linkage between a terminal guanosine cap residue and the 5'-terminal transcribed sense nucleotide of the mRNA. This 5'-guanylate cap may then be methylated to generate an N7-methyl-guanylate residue. The ribose sugars of the terminal and/or antiterminal transcribed nucleotides of the 5' end of the mRNA may optionally also be 2'-O-methylated. 5'-decapping through hydrolysis and cleavage of the guanylate cap structure may target a nucleic acid molecule, such as an mRNA molecule, for degradation.

[00244] Modifications to the nucleic acids of the present invention may generate a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5'-ppp-5' phosphodiester linkages, modified nucleotides may be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, MA) may be used with a-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5'-ppp-5' cap. Additional modified guanosine nucleotides may be used such as a-methyl-phosphonate and seleno-phosphate nucleotides.

[00245] Additional modifications include, but are not limited to, 2'-O-methylation of the ribose sugars of 5'-terminal and/or 5'-antiterminal nucleotides of the mRNA (as mentioned above) on the 2'-hydroxyl group of the sugar ring. Multiple distinct 5'-cap structures can be used to generate the 5'-cap of a nucleic acid molecule, such as an mRNA molecule.

[00246] Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (i.e. endogenous, wild-type or physiological) 5'-caps in their chemical structure, while retaining cap function. Cap analogs may be chemically (i.e. non-enzymatically) or enzymatically synthesized and/linked to a nucleic acid molecule.

[00247] For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5'-5'-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3'-O-methyl group (i.e., N7,3'-O-dimethyl-guanosine-5'-triphosphate-5'-guanosine (m^7G -3'mppp-G; which may equivalently be designated 3' O-Me- $m^7G(5')ppp(5')G$). The 3'-O atom of the other, unmodified, guanine becomes linked to the 5'-terminal nucleotide of the capped nucleic acid molecule (e.g. an mRNA or mmRNA). The N7- and 3'-O-methylated guanine provides the terminal moiety of the capped nucleic acid molecule (e.g. mRNA or mmRNA).

[00248] Another exemplary cap is mCAP, which is similar to ARCA but has a 2'-O-methyl group on guanosine (i.e., N7,2'-O-dimethyl-guanosine-5'-triphosphate-5'-guanosine, m^7Gm -ppp-G).

[00249] In one embodiment, the cap is a dinucleotide cap analog. As a non-limiting example, the dinucleotide cap analog may be modified at different phosphate positions with a boranophosphate group or a phosphoroselenoate group such as the dinucleotide cap analogs described in US Patent No. US 8,519,110, the contents of which are herein incorporated by reference in its entirety.

[00250] In another embodiment, the cap is a cap analog is a N7-(4-chlorophenoxyethyl) substituted dinucleotide form of a cap analog known in the art and/or described herein. Non-limiting examples of a N7-(4-chlorophenoxyethyl) substituted dinucleotide form of a cap analog include a N7-(4-chlorophenoxyethyl)- $G(5')ppp(5')G$ and a N7-(4-chlorophenoxyethyl)- $m^{3'-O}G(5')ppp(5')G$ cap analog (See e.g., the various cap analogs and the methods of synthesizing cap analogs described in Kore et al. *Bioorganic & Medicinal Chemistry* 2013 21:4570-4574; the contents of which are herein incorporated by reference in its entirety). In another embodiment, a cap analog of the present invention is a 4-chloro/bromophenoxyethyl analog.

[00251] While cap analogs allow for the concomitant capping of a nucleic acid molecule in an in vitro transcription reaction, up to 20% of transcripts remain uncapped. This, as well as the structural differences of a cap analog from an endogenous 5'-cap structures of nucleic acids produced by the endogenous, cellular transcription machinery, may lead to reduced translational competency and reduced cellular stability.

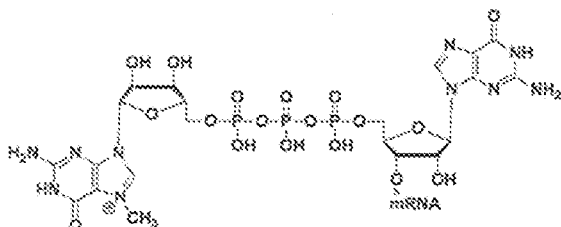
[00252] Modified nucleic acids of the invention may also be capped post-transcriptionally, using enzymes, in order to generate more authentic 5'-cap structures. As used herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an endogenous, wild-type, natural or physiological cellular function and/or structure as compared to synthetic features or analogs, etc., of the prior art, or which outperforms the corresponding endogenous, wild-type, natural or physiological feature in one or more respects. Non-limiting examples of more authentic 5'cap structures of the present invention are those which, among other things, have enhanced binding of cap binding proteins, increased half life, reduced susceptibility to 5' endonuc leases and/or reduced 5'decapping, as compared to synthetic 5'cap structures known in the art (or to a wild-type, natural or physiological 5'cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2'-O-methyltransferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-terminal nucleotide of an mRNA and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5'-terminal nucleotide of the mRNA contains a 2'-O -methyl. Such a structure is termed the Cap1 structure. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, e.g., to other 5'cap analog structures known in the art. Cap structures include 7mG(5')ppp(5')N,pN2p (cap 0), 7mG(5')ppp(5')NlmpNp (cap 1), 7mG(5')-ppp(5')NlmpN2mp (cap 2) and m(7)Gpppm(3)(6,6,2')Apm(2·)Apm(2·)Cpm(2)(3,2·)Up (cap 4).

[00253] Because the modified nucleic acids may be capped post-transcriptionally, and because this process is more efficient, nearly 100% of the modified nucleic acids may be capped. This is in contrast to ~80% when a cap analog is linked to an mRNA in the course of an in vitro transcription reaction.

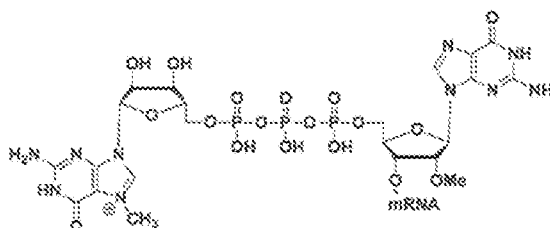
[00254] According to the present invention, 5' terminal caps may include endogenous caps or cap analogs. According to the present invention, a 5' terminal cap may comprise a guanine analog. Useful guanine analogs include inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[00255] In one embodiment, the nucleic acids described herein may contain a modified 5'cap. A modification on the 5'cap may increase the stability of mRNA, increase the half-life of the mRNA, and could increase the mRNA translational efficiency. The modified 5'cap may include, but is not limited to, one or more of the following modifications: modification at the 2' and/or 3' position of a capped guanosine triphosphate (GTP), a replacement of the sugar ring oxygen (that produced the carbocyclic ring) with a methylene moiety (C^{3/4}), a modification at the triphosphate bridge moiety of the cap structure, or a modification at the nucleobase (G) moiety.

[00256] The 5'cap structure that may be modified includes, but is not limited to, the caps described herein such as CapO having the substrate structure for cap dependent translation of :

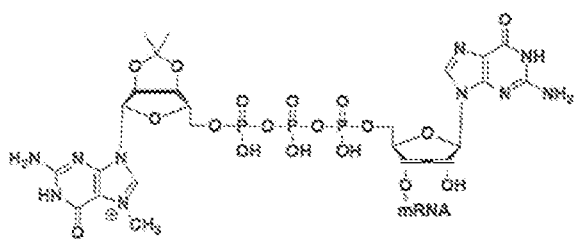


(CAP-001), or CapI having the substrate

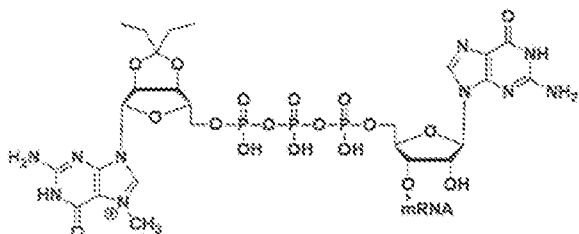


structure for cap dependent translation of:
(CAP-002).

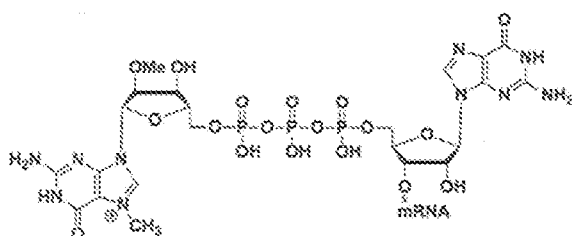
[00257] As a non-limiting example, the modified 5'cap may have the substrate structure for cap dependent translation of:



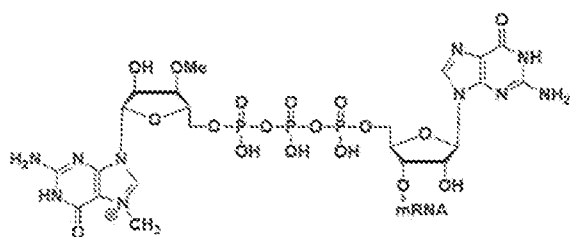
(CAP-003),



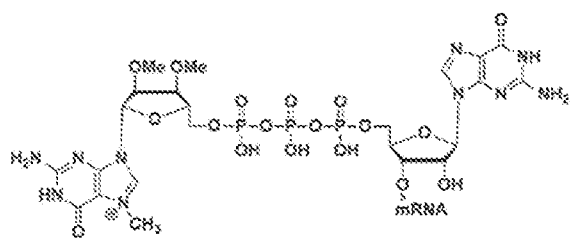
(CAP-004),



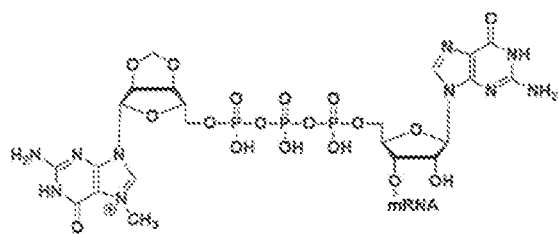
(CAP-005),



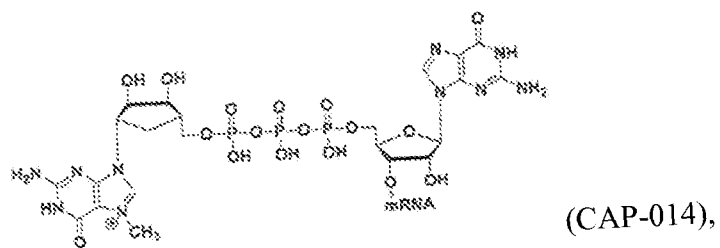
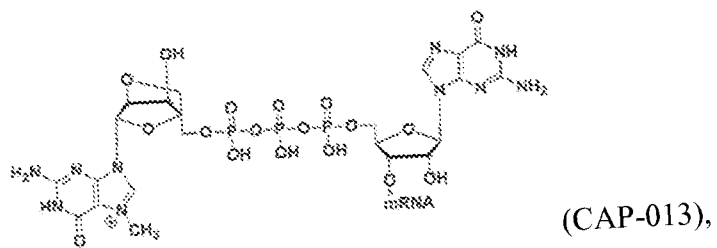
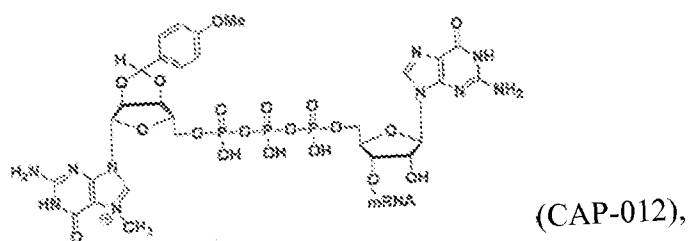
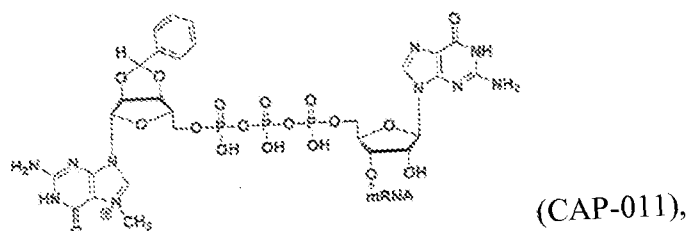
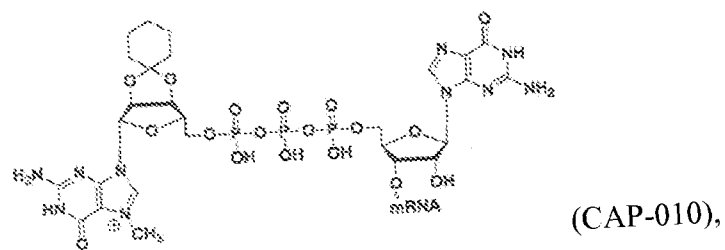
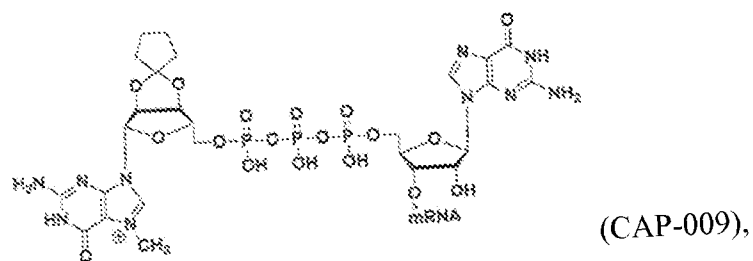
(CAP-006),

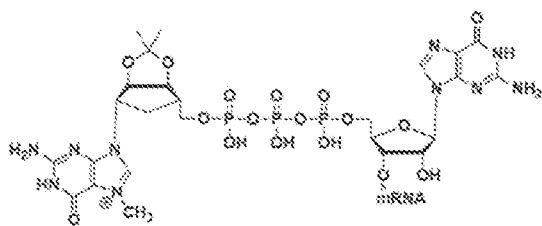


(CAP-007),

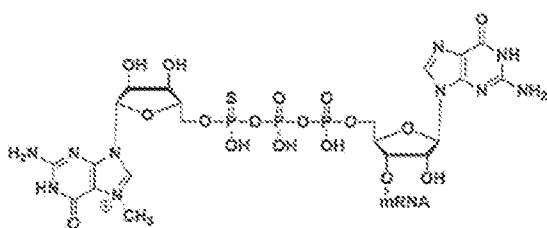


(CAP-008),

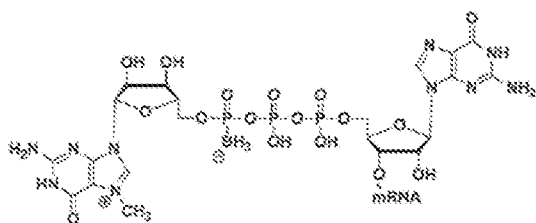




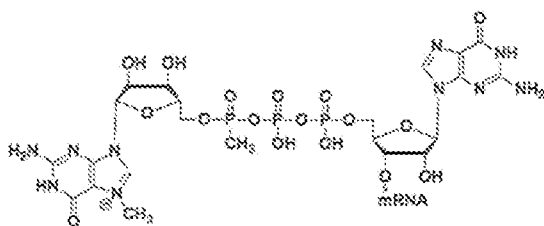
(CAP-015),



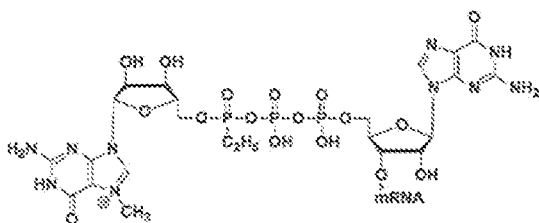
(CAP-016),



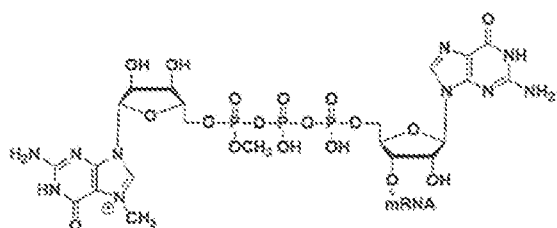
(CAP-017),



(CAP-018),



(CAP-019),



(CAP-020),

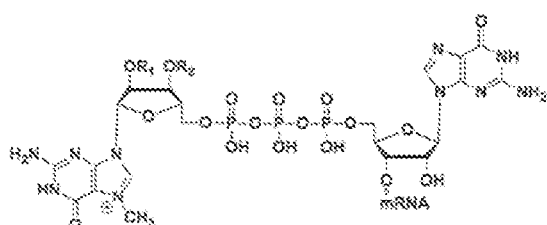
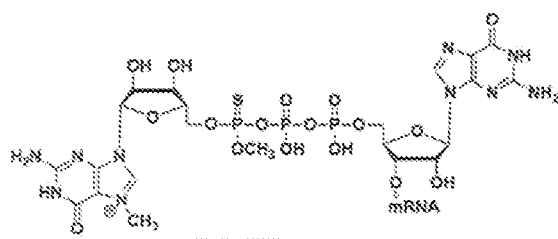
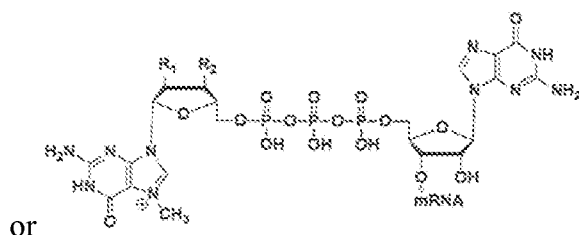


Table 1

Cap Structure Number	R ₁	R ₂
CAP-022	C ₂ H ₅ (Ethyl)	H
CAP-023	H	C ₂ H ₅ (Ethyl)
CAP-024	C ₂ H ₅ (Ethyl)	C ₂ H ₅ (Ethyl)
CAP-025	C ₃ H ₇ (Propyl)	H
CAP-026	H	C ₃ H ₇ (Propyl)
CAP-027	C ₃ H ₇ (Propyl)	C ₃ H ₇ (Propyl)
CAP-028	C ₄ H ₉ (Butyl)	H
CAP-029	H	C ₄ H ₉ (Butyl)
CAP-030	C ₄ H ₉ (Butyl)	C ₄ H ₉ (Butyl)
CAP-031	C ₅ H ₁₁ (Pentyl)	H
CAP-032	H	C ₅ H ₁₁ (Pentyl)
CAP-033	C ₅ H ₁₁ (Pentyl)	C ₅ H ₁₁ (Pentyl)
CAP-034	H ₂ C-C≡CH (Propargyl)	H
CAP-035	H	H ₂ C-C≡CH (Propargyl)
CAP-036	H ₂ C-C≡CH (Propargyl)	H ₂ C-C≡CH (Propargyl)
CAP-037	CH ₂ CH=CH ₂ (Allyl)	H
CAP-038	H	CH ₂ CH=CH ₂ (Allyl)
CAP-039	CH ₂ CH=CH ₂ (Allyl)	CH ₂ CH=CH ₂ (Allyl)
CAP-040	CH ₂ OCH ₃ (MOM)	H
CAP-041	H	CH ₂ OCH ₃ (MOM)
CAP-042	CH ₂ OCH ₃ (MOM)	CH ₂ OCH ₃ (MOM)
CAP-043	CH ₂ OCH ₂ CH ₂ OCH ₃ (MEM)	H
CAP-044	H	CH ₂ OCH ₂ CH ₂ OCH ₃ (MEM)
CAP-045	CH ₂ OCH ₂ CH ₂ OCH ₃ (MEM)	CH ₂ OCH ₂ CH ₂ OCH ₃ (MEM)
CAP-046	CH ₂ SCH ₃ (MTM)	H
CAP-047	H	CH ₂ SCH ₃ (MTM)
CAP-048	CH ₂ SCH ₃ (MTM)	CH ₂ SCH ₃ (MTM)
CAP-049	CH ₂ C ₆ H ₅ (Benzyl)	H
CAP-050	H	CH ₂ C ₆ H ₅ (Benzyl)
CAP-051	CH ₂ C ₆ H ₅ (Benzyl)	CH ₂ C ₆ H ₅ (Benzyl)

CAP-052	CH ₂ OCH ₂ C ₆ H ₅ (BOM)	H
CAP-053	H	CH ₂ OCH ₂ C ₆ H ₅ (BOM)
CAP-054	CH ₂ OCH ₂ C ₆ H ₅ (BOM)	CH ₂ OCH ₂ C ₆ H ₅ (BOM)
CAP-055	CH ₂ C ₆ H ₄ -OMe (<i>p</i> -Methoxybenzyl)	H
CAP-056	H	CH ₂ C ₆ H ₄ -OMe (<i>p</i> -Methoxybenzyl)
CAP-057	CH ₂ C ₆ H ₄ -OMe (<i>p</i> -Methoxybenzyl)	CH ₂ C ₆ H ₄ -OMe (<i>p</i> -Methoxybenzyl)
CAP-058	CH ₂ C ₆ H ₄ -NO ₂ (<i>p</i> -Nitrobenzyl)	H
CAP-059	H	CH ₂ C ₆ H ₄ -NO ₂ (<i>p</i> -Nitrobenzyl)
CAP-060	CH ₂ C ₆ H ₄ -NO ₂ (<i>p</i> -Nitrobenzyl)	CH ₂ C ₆ H ₄ -NO ₂ (<i>p</i> -Nitrobenzyl)
CAP-061	CH ₂ C ₆ H ₄ -X (<i>p</i> -Halobenzyl) where X=F, Cl, Br or I	H
CAP-062	H	CH ₂ C ₆ H ₄ -X (<i>p</i> -Halobenzyl) where X=F, Cl, Br or I
CAP-063	CH ₂ C ₆ H ₄ -X (<i>p</i> -Halobenzyl) where X=F, Cl, Br or I	CH ₂ C ₆ H ₄ -X (<i>p</i> -Halobenzyl) where X=F, Cl, Br or I
CAP-064	CH ₂ C ₆ H ₄ -N ₃ (<i>p</i> -Azidobenzyl)	H
CAP-065	H	CH ₂ C ₆ H ₄ -N ₃ (<i>p</i> -Azidobenzyl)
CAP-066	CH ₂ C ₆ H ₄ -N ₃ (<i>p</i> -Azidobenzyl)	CH ₂ C ₆ H ₄ -N ₃ (<i>p</i> -Azidobenzyl)
CAP-067	CH ₂ C ₆ H ₄ -CF ₃ (<i>p</i> -Trifluoromethylbenzyl)	H
CAP-068	H	CH ₂ C ₆ H ₄ -CF ₃ (<i>p</i> -Trifluoromethylbenzyl)
CAP-069	CH ₂ C ₆ H ₄ -CF ₃ (<i>p</i> -Trifluoromethylbenzyl)	CH ₂ C ₆ H ₄ -CF ₃ (<i>p</i> -Trifluoromethylbenzyl)
CAP-070	CH ₂ C ₆ H ₄ -OCF ₃ (<i>p</i> -Trifluoromethoxylbenzyl)	H
CAP-071	H	CH ₂ C ₆ H ₄ -OCF ₃ (<i>p</i> -Trifluoromethoxylbenzyl)
CAP-072	CH ₂ C ₆ H ₄ -OCF ₃ (<i>p</i> -Trifluoromethoxylbenzyl)	CH ₂ C ₆ H ₄ -OCF ₃ (<i>p</i> -Trifluoromethoxylbenzyl)
CAP-073	CH ₂ C ₆ H ₃ -(CF ₃) ₂ [2,4-bis(Trifluoromethyl)benzyl]	H
CAP-074	H	CH ₂ C ₆ H ₃ -(CF ₃) ₂ [2,4-bis(Trifluoromethyl)benzyl]
CAP-075	CH ₂ C ₆ H ₃ -(CF ₃) ₂ [2,4-bis(Trifluoromethyl)benzyl]	CH ₂ C ₆ H ₃ -(CF ₃) ₂ [2,4-bis(Trifluoromethyl)benzyl]
CAP-076	Si(C ₆ H ₅) ₂ C ₄ H ₉ (t-Butyldiphenylsilyl)	H
CAP-077	H	Si(C ₆ H ₅) ₂ C ₄ H ₉ (t-Butyldiphenylsilyl)
CAP-078	Si(C ₆ H ₅) ₂ C ₄ H ₉ (t-Butyldiphenylsilyl)	Si(C ₆ H ₅) ₂ C ₄ H ₉ (t-Butyldiphenylsilyl)
CAP-079	CH ₂ CH ₂ CH=CH ₂ (Homoallyl)	H
CAP-080	H	CH ₂ CH ₂ CH=CH ₂ (Homoallyl)
CAP-081	CH ₂ CH ₂ CH=CH ₂ (Homoallyl)	CH ₂ CH ₂ CH=CH ₂ (Homoallyl)
CAP-082	P(O)(OH) ₂ (MP)	H
CAP-083	H	P(O)(OH) ₂ (MP)

CAP-084	P(0)(OH) ₂ (MP)	P(0)(OH) ₂ (MP)
CAP-085	P(S)(OH) ₂ (Thio-MP)	H
CAP-086	H	P(S)(OH) ₂ (Thio-MP)
CAP-087	P(S)(OH) ₂ (Thio-MP)	P(S)(OH) ₂ (Thio-MP)
CAP-088	P(0)(CH ₃)(OH) (Methylphosphonate)	H
CAP-089	H	P(0)(CH ₃)(OH) (Methylphosphonate)
CAP-090	P(0)(CH ₃)(OH) (Methylphosphonate)	P(0)(CH ₃)(OH) (Methylphosphonate)
CAP-091	PNCPr) ₂ (OCH ₂ CH ₂ CN) (Phosphoramidite)	H
CAP-092	H	PNCPr) ₂ (OCH ₂ CH ₂ CN) (Phosphoramidite)
CAP-093	PNCPr) ₂ (OCH ₂ CH ₂ CN) (Phosphoramidite)	PNCPr) ₂ (OCH ₂ CH ₂ CN) (Phosphoramidite)
CAP-094	SO ₂ CH ₃ (Methanesulfomc_acid)	H
CAP-095	H	SO ₂ CH ₃ (Methanesulfomc_acid)
CAP-096	SO ₂ CH ₃ (Methanesulfomc_acid)	SO ₂ CH ₃ (Methanesulfomc_acid)



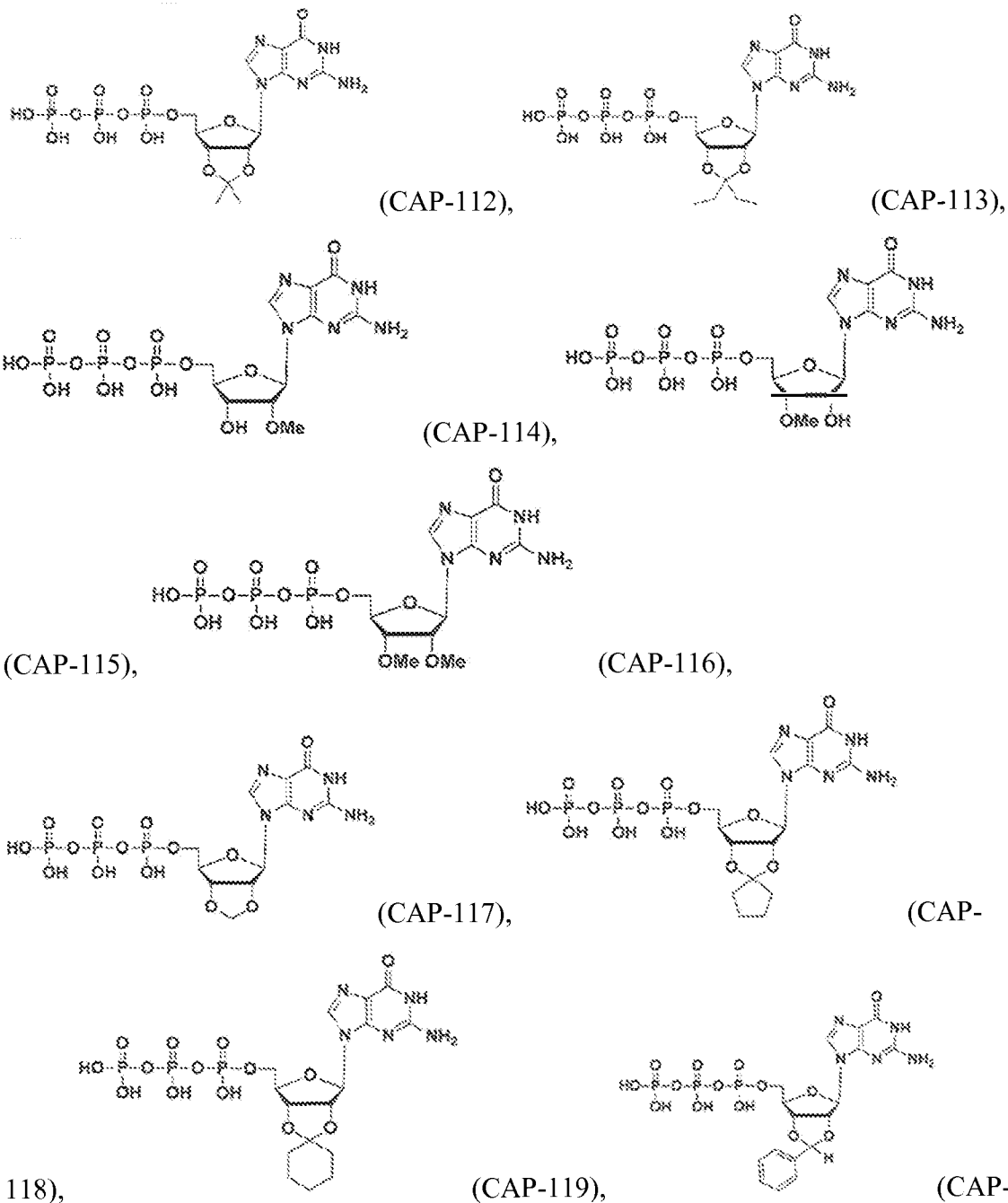
where R₁ and R₂ are defined in Table 2:

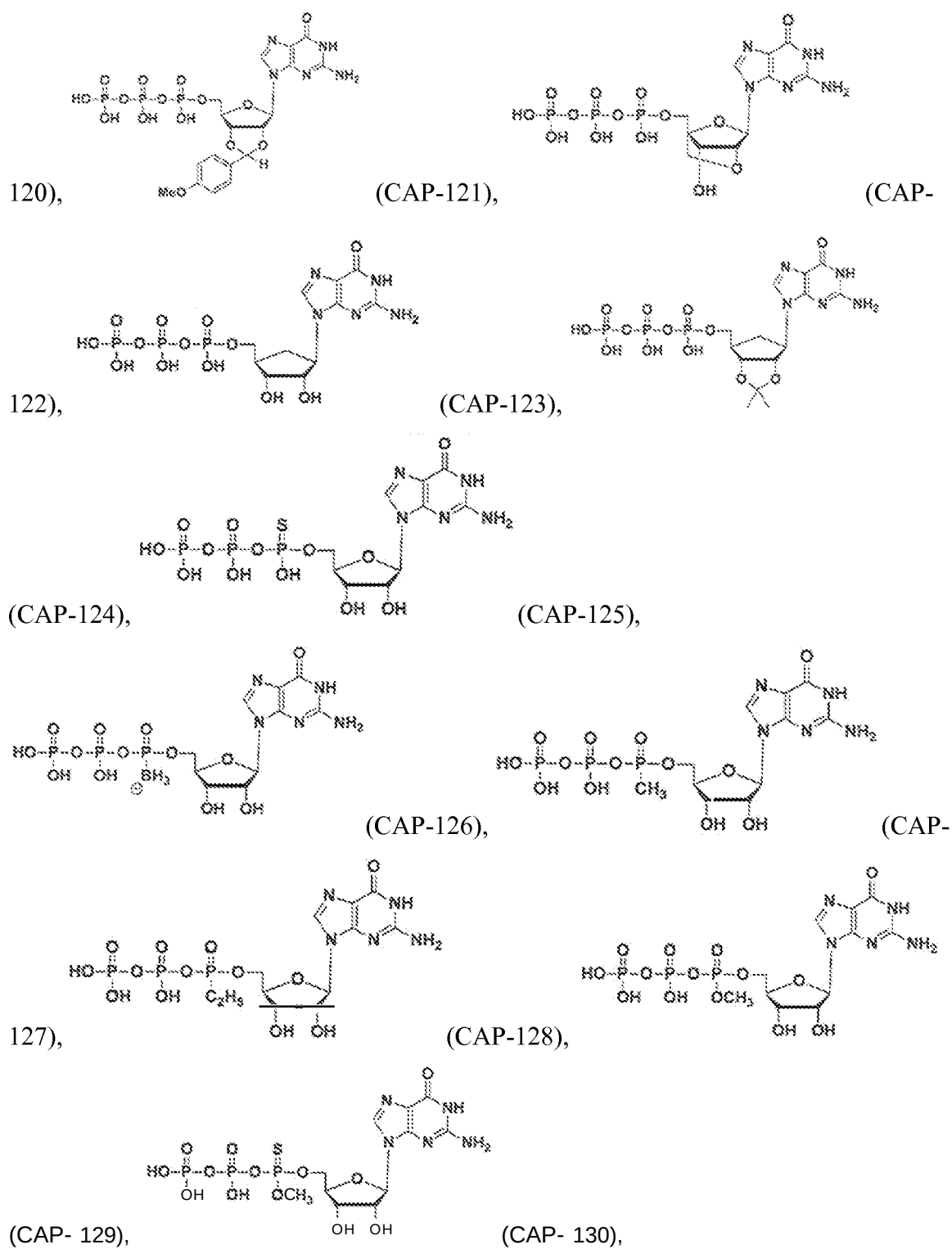
Table 2

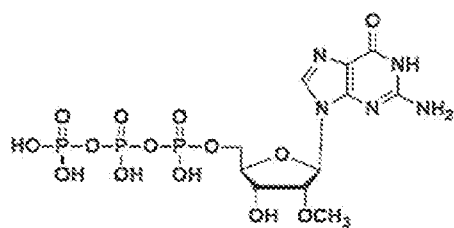
Cap Structure Number	R1	R2
CAP-097	NH ₂ (amino)	H
CAP-098	H	NH ₂ (amino)
CAP-099	NH ₂ (amino)	NH ₂ (amino)
CAP-100	N ₃ (Azido)	H
CAP-101	H	N ₃ (Azido)
CAP-102	N ₃ (Azido)	N ₃ (Azido)
CAP-103	X (Halo: F, Cl, Br, I)	H
CAP-104	H	X (Halo: F, Cl, Br, I)
CAP-105	X (Halo: F, Cl, Br, I)	X (Halo: F, Cl, Br, I)
CAP-106	SH (Thiol)	H
CAP-107	H	SH (Thiol)
CAP-108	SH (Thiol)	SH (Thiol)
CAP-109	SCH ₃ (Thiomethyl)	H
CAP-110	H	SCH ₃ (Thiomethyl)
CAP-111	SCH ₃ (Thiomethyl)	SCH ₃ (Thiomethyl)

[00258] In Table 1, "MOM" stands for methoxymethyl, "MEM" stands for methoxyethoxymethyl, "MTM" stands for methylthiomethyl, "BOM" stands for benzyloxymethyl and "MP" stands for monophosphonate. In Table 1 and 2, "F" stands for fluorine, "Cl" stands for chlorine, "Br" stands for bromine and "I" stands for iodine.

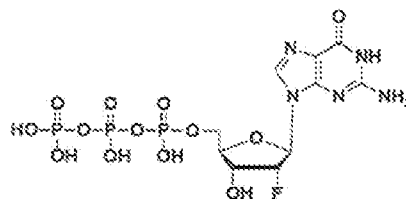
[00259] In a non-limiting example, the modified 5'cap may have the substrate structure for vaccinia mRNA capping enzyme of:





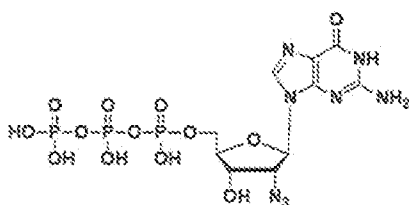


(CAP-131),

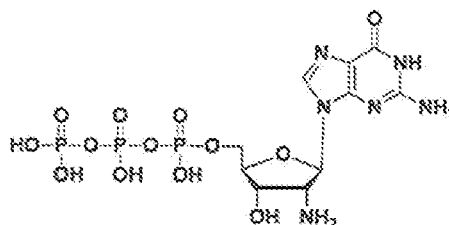


(CAP-

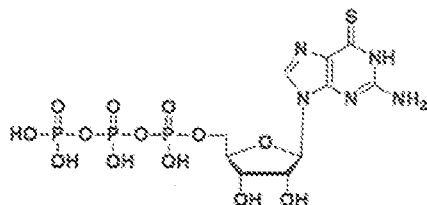
132),



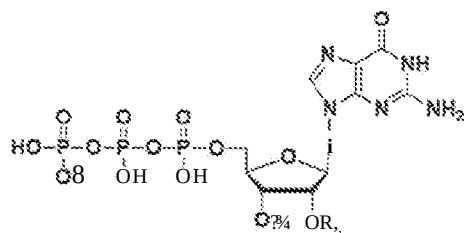
(CAP-133),



(CAP-134),



(CAP-135),

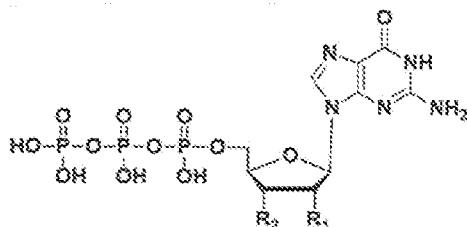
where **R₁** and **R₂** are defined in Table 3:**Table 3**

Cap Structure Number	R ₁	R ₂
CAP-136	C ₂ H ₅ (Ethyl)	H
CAP-137	H	C ₂ H ₅ (Ethyl)
CAP-138	C ₂ H ₅ (Ethyl)	C ₂ H ₅ (Ethyl)
CAP-139	C ₃ H ₇ (Propyl)	H
CAP-140	H	C ₃ H ₇ (Propyl)
CAP-141	C ₃ H ₇ (Propyl)	C ₃ H ₇ (Propyl)
CAP-142	C ₄ H ₉ (Butyl)	H
CAP-143	H	C ₄ H ₉ (Butyl)
CAP-144	C ₄ H ₉ (Butyl)	C ₄ H ₉ (Butyl)
CAP-145	C ₅ H ₁₁ (Pentyl)	H
CAP-146	H	C ₅ H ₁₁ (Pentyl)
CAP-147	C ₅ H ₁₁ (Pentyl)	C ₅ H ₁₁ (Pentyl)
CAP-148	H ₂ C-C≡CH (Propargyl)	H
CAP-149	H	H ₂ C-C≡CH (Propargyl)
CAP-150	H ₂ C-C≡CH (Propargyl)	H ₂ C-C≡CH (Propargyl)

CAP-151	CH ₂ CH=CH ₂ (Allyl)	H
CAP-152	H	CH ₂ CH=CH ₂ (Allyl)
CAP-153	CH ₂ CH=CH ₂ (Allyl)	CH ₂ CH=CH ₂ (Allyl)
CAP-154	CH ₂ OCH ₃ (MOM)	H
CAP-155	H	CH ₂ OCH ₃ (MOM)
CAP-156	CH ₂ OCH ₃ (MOM)	CH ₂ OCH ₃ (MOM)
CAP-157	CH ₂ OCH ₂ CH ₂ OCH ₃ (MEM)	H
CAP-158	H	CH ₂ OCH ₂ CH ₂ OCH ₃ (MEM)
CAP-159	CH ₂ OCH ₂ CH ₂ OCH ₃ (MEM)	CH ₂ OCH ₂ CH ₂ OCH ₃ (MEM)
CAP-160	CH ₂ SCH ₃ (MTM)	H
CAP-161	H	CH ₂ SCH ₃ (MTM)
CAP-162	CH ₂ SCH ₃ (MTM)	CH ₂ SCH ₃ (MTM)
CAP-163	CH ₂ C ₆ H ₅ (Benzyl)	H
CAP-164	H	CH ₂ C ₆ H ₅ (Benzyl)
CAP-165	CH ₂ C ₆ H ₅ (Benzyl)	CH ₂ C ₆ H ₅ (Benzyl)
CAP-166	CH ₂ OCH ₂ C ₆ H ₅ (BOM)	H
CAP-167	H	CH ₂ OCH ₂ C ₆ H ₅ (BOM)
CAP-168	CH ₂ OCH ₂ C ₆ H ₅ (BOM)	CH ₂ OCH ₂ C ₆ H ₅ (BOM)
CAP-169	CH ₂ C ₆ H ₄ -OMe (<i>p</i> -Methoxybenzyl)	H
CAP-170	H	CH ₂ C ₆ H ₄ -OMe (<i>p</i> -Methoxybenzyl)
CAP-171	CH ₂ C ₆ H ₄ -OMe (<i>p</i> -Methoxybenzyl)	CH ₂ C ₆ H ₄ -OMe (<i>p</i> -Methoxybenzyl)
CAP-172	CH ₂ C ₆ H ₄ -NO ₂ (<i>p</i> -Nitrobenzyl)	H
CAP-173	H	CH ₂ C ₆ H ₄ -NO ₂ (<i>p</i> -Nitrobenzyl)
CAP-174	CH ₂ C ₆ H ₄ -NO ₂ (<i>p</i> -Nitrobenzyl)	CH ₂ C ₆ H ₄ -NO ₂ (<i>p</i> -Nitrobenzyl)
CAP-175	CH ₂ C ₆ H ₄ -X (<i>p</i> -Halobenzyl) where X=F, Cl, Br or I	H
CAP-176	H	CH ₂ C ₆ H ₄ -X (<i>p</i> -Halobenzyl) where X=F, Cl, Br or I
CAP-177	CH ₂ C ₆ H ₄ -X (<i>p</i> -Halobenzyl) where X=F, Cl, Br or I	CH ₂ C ₆ H ₄ -X (<i>p</i> -Halobenzyl) where X=F, Cl, Br or I
CAP-178	CH ₂ C ₆ H ₄ -N ₃ (<i>p</i> -Azidobenzyl)	H
CAP-179	H	CH ₂ C ₆ H ₄ -N ₃ (<i>p</i> -Azidobenzyl)
CAP-180	CH ₂ C ₆ H ₄ -N ₃ (<i>p</i> -Azidobenzyl)	CH ₂ C ₆ H ₄ -N ₃ (<i>p</i> -Azidobenzyl)
CAP-181	CH ₂ C ₆ H ₄ -CF ₃ (<i>p</i> -Trifluoromethylbenzyl)	H
CAP-182	H	CH ₂ C ₆ H ₄ -CF ₃ (<i>p</i> -Trifluoromethylbenzyl)
CAP-183	CH ₂ C ₆ H ₄ -CF ₃ (<i>p</i> -Trifluoromethylbenzyl)	CH ₂ C ₆ H ₄ -CF ₃ (<i>p</i> -Trifluoromethylbenzyl)
CAP-184	CH ₂ C ₆ H ₄ -OCF ₃ (<i>p</i> -Trifluoromethoxybenzyl)	H
CAP-185	H	CH ₂ C ₆ H ₄ -OCF ₃ (<i>p</i> -Trifluoromethoxybenzyl)
CAP-186	CH ₂ C ₆ H ₄ -OCF ₃ (<i>p</i> -Trifluoromethoxybenzyl)	CH ₂ C ₆ H ₄ -OCF ₃ (<i>p</i> -Trifluoromethoxybenzyl)

CAP-187	CH ₂ C ₆ H ₃ -(CF ₃) ₂ [2,4-bis(Trifluoromethyl)benzyl]	H
CAP-188	H	CH ₂ C ₆ H ₃ -(CF ₃) ₂ [2,4-bis(Trifluoromethyl)benzyl]
CAP-189	CH ₂ C ₆ H ₃ -(CF ₃) ₂ [2,4-bis(Trifluoromethyl)benzyl]	CH ₂ C ₆ H ₃ -(CF ₃) ₂ [2,4-bis(Trifluoromethyl)benzyl]
CAP-190	Si(C ₆ H ₅) ₂ C ₄ H ₉ (t-Butyldiphenylsilyl)	H
CAP-191	H	Si(C ₆ H ₅) ₂ C ₄ H ₉ (t-Butyldiphenylsilyl)
CAP-192	Si(C ₆ H ₅) ₂ C ₄ H ₉ (t-Butyldiphenylsilyl)	Si(C ₆ H ₅) ₂ C ₄ H ₉ (t-Butyldiphenylsilyl)
CAP-193	CH ₂ CH ₂ CH=CH ₂ (Homoallyl)	H
CAP-194	H	CH ₂ CH ₂ CH=CH ₂ (Homoallyl)
CAP-195	CH ₂ CH ₂ CH=CH ₂ (Homoallyl)	CH ₂ CH ₂ CH=CH ₂ (Homoallyl)
CAP-196	P(O)(OH) ₂ (MP)	H
CAP-197	H	P(O)(OH) ₂ (MP)
CAP-198	P(O)(OH) ₂ (MP)	P(O)(OH) ₂ (MP)
CAP-199	P(S)(OH) ₂ (Thio-MP)	H
CAP-200	H	P(S)(OH) ₂ (Thio-MP)
CAP-201	P(S)(OH) ₂ (Thio-MP)	P(S)(OH) ₂ (Thio-MP)
CAP-202	P(O)(CH ₃)(OH) (Methylphosphonate)	H
CAP-203	H	P(O)(CH ₃)(OH) (Methylphosphonate)
CAP-204	P(O)(CH ₃)(OH) (Methylphosphonate)	P(O)(CH ₃)(OH) (Methylphosphonate)
CAP-205	PN(ⁱ Pr) ₂ (OCH ₂ CH ₂ CN) (Phosphoramidite)	H
CAP-206	H	PN(ⁱ Pr) ₂ (OCH ₂ CH ₂ CN) (Phosphoramidite)
CAP-207	PN(ⁱ Pr) ₂ (OCH ₂ CH ₂ CN) (Phosphoramidite)	PN(ⁱ Pr) ₂ (OCH ₂ CH ₂ CN) (Phosphoramidite)
CAP-208	SO ₂ CH ₃ (Methanesulfonic acid)	H
CAP-209	H	SO ₂ CH ₃ (Methanesulfonic acid)
CAP-210	SO ₂ CH ₃ (Methanesulfonic acid)	SO ₂ CH ₃ (Methanesulfonic acid)

or

where R₁ and R₂ are defined in Table 4:**Table 4**

Cap Structure	R ₁	R ₂

Number		
CAP-211	NH ₂ (amino)	H
CAP-212	H	NH ₂ (amino)
CAP-213	NH ₂ (amino)	NH ₂ (amino)
CAP-214	N ₃ (Azido)	H
CAP-215	H	N ₃ (Azido)
CAP-216	N ₃ (Azido)	N ₃ (Azido)
CAP-217	X (Halo: F, Cl, Br, I)	H
CAP-218	H	X (Halo: F, Cl, Br, I)
CAP-219	X (Halo: F, Cl, Br, I)	X (Halo: F, Cl, Br, I)
CAP-220	SH (Thiol)	H
CAP-221	H	SH (Thiol)
CAP-222	SH (Thiol)	SH (Thiol)
CAP-223	SCH ₃ (Thiomethyl)	H
CAP-224	H	SCH ₃ (Thiomethyl)
CAP-225	SCH ₃ (Thiomethyl)	SCH ₃ (Thiomethyl)

[00260] In Table 3, "MOM" stands for methoxymethyl, "MEM" stands for methoxyethoxymethyl, "MTM" stands for methylthiomethyl, "BOM" stands for benzyloxymethyl and "MP" stands for monophosphonate. In Table 3 and 4, "F" stands for fluorine, "Cl" stands for chlorine, "Br" stands for bromine and "I" stands for iodine.

[00261] In another non-limiting example, of the modified capping structure substrates CAP-112 - CAP-225 could be added in the presence of vaccinia capping enzyme with a component to create enzymatic activity such as, but not limited to, S-adenosylmethionine (AdoMet), to form a modified cap for mRNA.

[00262] In one embodiment, the replacement of the sugar ring oxygen (that produced the carbocyclic ring) with a methylene moiety (CH₂) could create greater stability to the C-N bond against phosphorylases as the C-N bond is resistant to acid or enzymatic hydrolysis. The methylene moiety may also increase the stability of the triphosphate bridge moiety and thus increasing the stability of the mRNA. As a non-limiting example, the cap substrate structure for cap dependent translation may have the structure such as, but not limited to, CAP-014 and CAP-015 and/or the cap substrate structure for vaccinia mRNA capping enzyme such as, but not limited to, CAP-123 and CAP-124. In another example, CAP-112 - CAP-122 and/or CAP-125 - CAP-225, can be modified by replacing the sugar ring oxygen (that produced the carbocyclic ring) with a methylene moiety (CH₂).

[00263] In another embodiment, the triphosphate bridge may be modified by the replacement of at least one oxygen with sulfur (thio), a borane (BH_3) moiety, a methyl group, an ethyl group, a methoxy group and/or combinations thereof. This modification could increase the stability of the mRNA towards decapping enzymes. As a non-limiting example, the cap substrate structure for cap dependent translation may have the structure such as, but not limited to, CAP-016 - CAP-021 and/or the cap substrate structure for vaccinia mRNA capping enzyme such as, but not limited to, CAP-125 - CAP-130. In another example, CAP-003 - CAP-015, CAP-022 - CAP-124 and/or CAP-131 - CAP-225, can be modified on the triphosphate bridge by replacing at least one of the triphosphate bridge oxygens with sulfur (thio), a borane (BH_3) moiety, a methyl group, an ethyl group, a methoxy group and/or combinations thereof.

[00264] In one embodiment, CAP-001 - 134 and/or CAP-136 - CAP-225 may be modified to be a thioguanosine analog similar to CAP-135. The thioguanosine analog may comprise additional modifications such as, but not limited to, a modification at the triphosphate moiety (e.g., thio, BH_3 , CH_3 , C_2H_5 , OCH_3 , S and S with OCH_3), a modification at the 2' and/or 3' positions of 6-thio guanosine as described herein and/or a replacement of the sugar ring oxygen (that produced the carbocyclic ring) as described herein.

[00265] In one embodiment, CAP-001 - 121 and/or CAP-123 - CAP-225 may be modified to be a modified 5'cap similar to CAP-122. The modified 5'cap may comprise additional modifications such as, but not limited to, a modification at the triphosphate moiety (e.g., thio, BH_3 , CH_3 , C_2H_5 , OCH_3 , S and S with OCH_3), a modification at the 2' and/or 3' positions of 6-thio guanosine as described herein and/or a replacement of the sugar ring oxygen (that produced the carbocyclic ring) as described herein.

[00266] In one embodiment, the 5'cap modification may be the attachment of biotin or conjugation at the 2' or 3' position of a GTP.

[00267] In another embodiment, the 5' cap modification may include a CF_2 modified triphosphate moiety.

3' UTR and Viral Sequences

[00268] Additional viral sequences such as, but not limited to, the translation enhancer sequence of the barley yellow dwarf virus (BYDV-PAV) can be engineered and inserted

in the 3' UTR of the nucleic acids or mRNA of the invention and can stimulate the translation of the construct *in vitro* and *in vivo*. Transfection experiments can be conducted in relevant cell lines and protein production can be assayed by ELISA at 12hr, 24hr, 48hr, 72 hr and day 7 post-transfection.

IRES Sequences

[00269] Further, provided are nucleic acids containing an internal ribosome entry site (IRES). First identified as a feature Picorna virus RNA, IRES plays an important role in initiating protein synthesis in absence of the 5' cap structure. An IRES may act as the sole ribosome binding site, or may serve as one of multiple ribosome binding sites of an mRNA. Nucleic acids or mRNA containing more than one functional ribosome binding site may encode several peptides or polypeptides that are translated independently by the ribosomes ("multicistronic nucleic acid molecules"). When nucleic acids or mRNA are provided with an IRES, further optionally provided is a second translatable region. Examples of IRES sequences that can be used according to the invention include without limitation, those from picornaviruses (e.g. FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV).

Terminal Architecture Modifications: Poly-A tails

[00270] During RNA processing, a long chain of adenine nucleotides (poly-A tail) is normally added to a messenger RNA (mRNA) molecules to increase the stability of the molecule. Immediately after transcription, the 3' end of the transcript is cleaved to free a 3' hydroxyl. Then poly-A polymerase adds a chain of adenine nucleotides to the RNA. The process, called polyadenylation, adds a poly-A tail that is between 100 and 250 residues long.

[00271] It has been discovered that unique poly-A tail lengths provide certain advantages to the modified RNAs of the present invention.

[00272] Generally, the length of a poly-A tail of the present invention is greater than 30 nucleotides in length. In another embodiment, the poly-A tail is greater than 35 nucleotides in length. In another embodiment, the length is at least 40 nucleotides. In another embodiment, the length is at least 45 nucleotides. In another embodiment, the

length is at least 55 nucleotides. In another embodiment, the length is at least 60 nucleotides. In another embodiment, the length is at least 60 nucleotides. In another embodiment, the length is at least 80 nucleotides. In another embodiment, the length is at least 90 nucleotides. In another embodiment, the length is at least 100 nucleotides. In another embodiment, the length is at least 120 nucleotides. In another embodiment, the length is at least 140 nucleotides. In another embodiment, the length is at least 160 nucleotides. In another embodiment, the length is at least 180 nucleotides. In another embodiment, the length is at least 200 nucleotides. In another embodiment, the length is at least 250 nucleotides. In another embodiment, the length is at least 300 nucleotides. In another embodiment, the length is at least 350 nucleotides. In another embodiment, the length is at least 400 nucleotides. In another embodiment, the length is at least 450 nucleotides. In another embodiment, the length is at least 500 nucleotides. In another embodiment, the length is at least 600 nucleotides. In another embodiment, the length is at least 700 nucleotides. In another embodiment, the length is at least 800 nucleotides. In another embodiment, the length is at least 900 nucleotides. In another embodiment, the length is at least 1000 nucleotides. In another embodiment, the length is at least 1100 nucleotides. In another embodiment, the length is at least 1200 nucleotides. In another embodiment, the length is at least 1300 nucleotides. In another embodiment, the length is at least 1400 nucleotides. In another embodiment, the length is at least 1500 nucleotides. In another embodiment, the length is at least 1600 nucleotides. In another embodiment, the length is at least 1700 nucleotides. In another embodiment, the length is at least 1800 nucleotides. In another embodiment, the length is at least 1900 nucleotides. In another embodiment, the length is at least 2000 nucleotides. In another embodiment, the length is at least 2500 nucleotides. In another embodiment, the length is at least 3000 nucleotides.

[00273] In some embodiments, the nucleic acid or mRNA includes from about 30 to about 3,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 750, from 30 to 1,000, from 30 to 1,500, from 30 to 2,000, from 30 to 2,500, from 50 to 100, from 50 to 250, from 50 to 500, from 50 to 750, from 50 to 1,000, from 50 to 1,500, from 50 to 2,000, from 50 to 2,500, from 50 to 3,000, from 100 to 500, from 100 to 750, from 100 to 1,000, from 100 to 1,500, from 100 to 2,000, from 100 to 2,500, from 100 to 3,000, from 500 to 750, from 500 to 1,000, from 500 to 1,500, from

500 to 2,000, from 500 to 2,500, from 500 to 3,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 2,500, from 1,000 to 3,000, from 1,500 to 2,000, from 1,500 to 2,500, from 1,500 to 3,000, from 2,000 to 3,000, from 2,000 to 2,500, and from 2,500 to 3,000).

[00274] In one embodiment, the poly-A tail may be 80 nucleotides, 120 nucleotides, 160 nucleotides in length on a modified RNA molecule described herein such as, but not limited to, the polyA tail length on the modified RNA described in Example 13.

[00275] In another embodiment, the poly-A tail may be 20, 40, 80, 100, 120, 140 or 160 nucleotides in length on a modified RNA molecule described herein such as, but not limited to, the polyA tail length on the modified RNA described in Example 44.

[00276] In one embodiment, the poly-A tail is designed relative to the length of the overall modified RNA molecule. This design may be based on the length of the coding region of the modified RNA, the length of a particular feature or region of the modified RNA (such as the mRNA), or based on the length of the ultimate product expressed from the modified RNA. When relative to any additional feature of the modified RNA (e.g., other than the mRNA portion which includes the poly-A tail) the poly-A tail may be 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% greater in length than the additional feature. The poly-A tail may also be designed as a fraction of the modified RNA to which it belongs. In this context, the poly-A tail may be 10, 20, 30, 40, 50, 60, 70, 80, or 90% or more of the total length of the construct or the total length of the construct minus the poly-A tail.

[00277] In one embodiment, engineered binding sites and/or the conjugation of nucleic acids or mRNA for Poly-A binding protein may be used to enhance expression. The engineered binding sites may be sensor sequences which can operate as binding sites for ligands of the local microenvironment of the nucleic acids and/or mRNA. As a non-limiting example, the nucleic acids and/or mRNA may comprise at least one engineered binding site to alter the binding affinity of Poly-A binding protein (PABP) and analogs thereof. The incorporation of at least one engineered binding site may increase the binding affinity of the PABP and analogs thereof.

[00278] Additionally, multiple distinct nucleic acids or mRNA may be linked together to the PABP (Poly-A binding protein) through the 3'-end using modified nucleotides at the 3'-terminus of the poly-A tail. Transfection experiments can be conducted in relevant

cell lines at and protein production can be assayed by ELISA at 12hr, 24hr, 48hr, 72 hr and day 7 post-transfection. As a non-limiting example, the transfection experiments may be used to evaluate the effect on PABP or analogs thereof binding affinity as a result of the addition of at least one engineered binding site.

[00279] In one embodiment, a polyA tail may be used to modulate translation initiation. While not wishing to be bound by theory, the polyA tail recruits PABP which in turn can interact with translation initiation complex and thus may be essential for protein synthesis.

[00280] In another embodiment, a polyA tail may also be used in the present invention to protect against 3'-5' exonuclease digestion.

[00281] In one embodiment, the nucleic acids or mRNA of the present invention are designed to include a polyA-G Quartet. The G-quartet is a cyclic hydrogen bonded array of four guanine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In this embodiment, the G-quartet is incorporated at the end of the poly-A tail. The resultant nucleic acid or mRNA may be assayed for stability, protein production and other parameters including half-life at various time points. It has been discovered that the polyA-G quartet results in protein production equivalent to at least 75% of that seen using a poly-A tail of 120 nucleotides alone.

[00282] In one embodiment, the nucleic acids or mRNA of the present invention may comprise a polyA tail and may be stabilized by the addition of a chain terminating nucleoside. The nucleic acids and/or mRNA with a polyA tail may further comprise a 5'cap structure.

[00283] In another embodiment, the nucleic acids or mRNA of the present invention may comprise a polyA-G Quartet. The nucleic acids and/or mRNA with a polyA-G Quartet may further comprise a 5'cap structure.

[00284] In one embodiment, the chain terminating nucleoside which may be used to stabilize the nucleic acid or mRNA comprising a polyA tail or polyA-G Quartet may be, but is not limited to, those described in International Patent Publication No. WO2013/103659, herein incorporated by reference in its entirety. In another embodiment, the chain terminating nucleosides which may be used with the present invention includes, but is not limited to, 3'-deoxyadenosine (cordycepin), 3'-

deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, such as 2',3'- dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'- dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, or a -O- methylnucleoside.

[00285] In another embodiment, the nucleic acid such as, but not limited to mRNA, which comprise a polyA tail or a polyA-G Quartet may be stabilized by a modification to the 3' region of the nucleic acid that can prevent and/or inhibit the addition of oligo(U) (see e.g., International Patent Publication No. WO2013 103659, herein incorporated by reference in its entirety).

[00286] In yet another embodiment, the nucleic acid such as, but not limited to mRNA, which comprise a polyA tail or a polyA-G Quartet may be stabilized by the addition of an oligonucleotide that terminates in a 3'-deoxynucleoside, 2', 3'-dideoxynucleoside 3'-O- methylnucleosides, S'-O-ethylnucleosides, S'-arabinosides, and other modified nucleosides known in the art and/or described herein.

Quantification

[00287] In one embodiment, the polynucleotides, primary constructs, modified nucleic acids or mmRNA of the present invention may be quantified in exosomes derived from one or more bodily fluid. As used herein "bodily fluids" include peripheral blood, serum, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, bronchoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyst cavity fluid, and umbilical cord blood. Alternatively, exosomes may be retrieved from an organ selected from the group consisting of lung, heart, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colon, breast, prostate, brain, esophagus, liver, and placenta.

[00288] In the quantification method, a sample of not more than 2mL is obtained from the subject and the exosomes isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration,

immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof. In the analysis, the level or concentration of the polynucleotides, primary construct, modified nucleic acid or mmRNA may be an expression level, presence, absence, truncation or alteration of the administered construct. It is advantageous to correlate the level with one or more clinical phenotypes or with an assay for a human disease biomarker. The assay may be performed using construct specific probes, cytometry, qRT-PCR, real-time PCR, PCR, flow cytometry, electrophoresis, mass spectrometry, or combinations thereof while the exosomes may be isolated using immunohistochemical methods such as enzyme linked immunosorbent assay (ELISA) methods. Exosomes may also be isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof.

[00289] These methods afford the investigator the ability to monitor, in real time, the level of the polynucleotides, primary constructs, modified nucleic acid or mmRNA remaining or delivered. This is possible because the polynucleotides, primary constructs, modified nucleic acid or mmRNA of the present invention differ from the endogenous forms due to the structural and/or chemical modifications.

II. Design and Synthesis of Polynucleotides

[00290] Polynucleotides, primary constructs modified nucleic acids or mmRNA for use in accordance with the invention may be prepared according to any available technique including, but not limited to chemical synthesis, enzymatic synthesis, which is generally termed *in vitro* transcription (IVT) or enzymatic or chemical cleavage of a longer precursor, etc. Methods of synthesizing RNAs are known in the art (see, *e.g.*, Gait, M.J. (ed.) *Oligonucleotide synthesis: a practical approach*, Oxford [Oxfordshire], Washington, DC: IRL Press, 1984; and Herdewijn, P. (ed.) *Oligonucleotide synthesis: methods and applications*, Methods in Molecular Biology, v. 288 (Clifton, N.J.) Totowa, N.J.: Humana Press, 2005; both of which are incorporated herein by reference).

[00291] The process of design and synthesis of the primary constructs of the invention generally includes the steps of gene construction, mRNA production (either with or without modifications) and purification. In the enzymatic synthesis method, a target

polynucleotide sequence encoding the polypeptide of interest is first selected for incorporation into a vector which will be amplified to produce a cDNA template. Optionally, the target polynucleotide sequence and/or any flanking sequences may be codon optimized. The cDNA template is then used to produce mRNA through *in vitro* transcription (IVT). After production, the mRNA may undergo purification and clean-up processes. The steps of which are provided in more detail below.

Gene Construction

[00292] The step of gene construction may include, but is not limited to gene synthesis, vector amplification, plasmid purification, plasmid linearization and clean-up, and cDNA template synthesis and clean-up.

Gene Synthesis

[00293] Once a polypeptide of interest, or target, is selected for production, a primary construct is designed. Within the primary construct, a first region of linked nucleosides encoding the polypeptide of interest may be constructed using an open reading frame (ORF) of a selected nucleic acid (DNA or RNA) transcript. The ORF may comprise the wild type ORF, an isoform, variant or a fragment thereof. As used herein, an "open reading frame" or "ORF" is meant to refer to a nucleic acid sequence (DNA or RNA) which is capable of encoding a polypeptide of interest. ORFs often begin with the start codon, ATG and end with a nonsense or termination codon or signal.

[00294] Further, the nucleotide sequence of the first region may be codon optimized. Codon optimization methods are known in the art and may be useful in efforts to achieve one or more of several goals. These goals include to match codon frequencies in target and host organisms to ensure proper folding, bias GC content to increase mRNA stability or reduce secondary structures, minimize tandem repeat codons or base runs that may impair gene construction or expression, customize transcriptional and translational control regions, insert or remove protein trafficking sequences, remove/add post translation modification sites in encoded protein (e.g. glycosylation sites), add, remove or shuffle protein domains, insert or delete restriction sites, modify ribosome binding sites and mRNA degradation sites, to adjust translational rates to allow the various domains of the protein to fold properly, or to reduce or eliminate problem secondary structures within the mRNA. Codon optimization tools, algorithms and services are known in the art, non-

limiting examples include services from GeneArt (Life Technologies) and/or DNA2.0 (Menlo Park CA). In one embodiment, the ORF sequence is optimized using optimization algorithms. Codon options for each amino acid are given in Table 5.

Table 5. Codon Options

Amino Acid	Single Letter Code	Codon Options
Isoleucine	I	ATT, ATC, ATA
Leucine	L	CTT, CTC, CTA, CTG, TTA, TTG
Valine	V	GTT, GTC, GTA, GTG
Phenylalanine	F	TTT, TTC
Methionine	M	ATG
Cysteine	C	TGT, TGC
Alanine	A	GCT, GCC, GCA, GCG
Glycine	G	GGT, GGC, GGA, GGG
Proline	P	CCT, CCC, CCA, CCG
Threonine	T	ACT, ACC, ACA, ACG
Serine	S	TCT, TCC, TCA, TCG, AGT, AGC
Tyrosine	Y	TAT, TAC
Tryptophan	W	TGG
Glutamine	Q	CAA, CAG
Asparagine	N	AAT, AAC
Histidine	H	CAT, CAC
Glutamic acid	E	GAA, GAG
Aspartic acid	D	GAT, GAC
Lysine	K	AAA, AAG
Arginine	R	CGT, CGC, CGA, CGG, AGA, AGG
Selenocysteine	Sec	UGA in mRNA in presence of Selenocystein insertion element (SECIS)
Stop codons	Stop	TAA, TAG, TGA

[00295] In one embodiment, after a nucleotide sequence has been codon optimized it may be further evaluated for regions containing restriction sites. At least one nucleotide within the restriction site regions may be replaced with another nucleotide in order to remove the restriction site from the sequence but the replacement of nucleotides does alter the amino acid sequence which is encoded by the codon optimized nucleotide sequence.

[00296] Features, which may be considered beneficial in some embodiments of the present invention, may be encoded by the primary construct and may flank the ORF as a first or second flanking region. The flanking regions may be incorporated into the primary construct before and/or after optimization of the ORF. It is not required that a primary construct contain both a 5' and 3' flanking region. Examples of such features

include, but are not limited to, untranslated regions (UTRs), Kozak sequences, an oligo(dT) sequence, and detectable tags and may include multiple cloning sites which may have XbaI recognition.

[00297] In some embodiments, a 5' UTR and/or a 3' UTR may be provided as flanking regions. Multiple 5' or 3' UTRs may be included in the flanking regions and may be the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical modifications, before and/or after codon optimization. Combinations of features may be included in the first and second flanking regions and may be contained within other features. For example, the ORF may be flanked by a 5' UTR which may contain a strong Kozak translational initiation signal and/or a 3' UTR which may include an oligo(dT) sequence for templated addition of a poly-A tail.

[00298] Tables 2 and 3 provide a listing of exemplary UTRs which may be utilized in the primary construct of the present invention as flanking regions. Shown in Table 6 is a representative listing of a 5'-untranslated region of the invention. Variants of 5' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G.

Table 6. 5'-Untranslated Regions

5' UTR Identifier	Name/Description	Sequence	SEQ ID NO.
Native	Wild type UTR	See wild type sequence	-
5UTR-001	Synthetic UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAG AAGAAATATAAGAGCCACC	5
5UTR-002	Upstream UTR	GGGAGATCAGAGAGAAAAGAAGAGTAAGA AGAAATATAAGAGCCACC	6
5UTR-003	Upstream UTR	GGAATAAAAGTCTCAACACAACATATACA AAACAAACGAATCTCAAGCAATCAAGCAT TCTACTTCTATTGCAGCAATTTAAATCATTT CTTTTAAAGCAAAAGCAATTTTCTGAAAAT TTTCACCATTACGAACGATAGCAAC	7
5UTR-004	Upstream UTR	GGGAGACAAGCUUGGCAUUCGGGUACUGU UGGUAAAGCCACC	8

[00299] In another embodiment, the 5' UTR may comprise a first polynucleotide fragment and a second polynucleotide fragment where the first and second fragments

may be from the same or different gene. (See e.g., US20 100293625 and US201 10247090, each of which is herein incorporated by reference in its entirety). As a non-limiting example, the first polynucleotide may be a fragment of the canine, human or mouse SERCA2 gene and/or the second polynucleotide fragment is a fragment of the bovine, mouse, rat or sheep beta-casein gene.

[00300] In one embodiment, the first polynucleotide fragment may be located on the 5' end of the second polynucleotide fragment. (See e.g., US20100293625 and US201 10247090, each of which is herein incorporated by reference in its entirety).

[00301] In another embodiment, the first polynucleotide fragment may comprise the second intron of a sarcoplasmic/endoplasmic reticulum calcium ATPase gene and/or the second polynucleotide fragment comprises at least a portion of the 5' UTR of a eukaryotic casein gene. (See e.g., US20100293625 and US201 10247090, each of which is herein incorporated by reference in its entirety). The first polynucleotide fragment may also comprise at least a portion of exon 2 and/or exon 3 of the sarcoplasmic/endoplasmic reticulum calcium ATPase gene. (See e.g., US20 100293625 and US201 10247090, each of which is herein incorporated by reference in its entirety).

[00302] Shown in Table 7 is a representative listing of 3'-untranslated regions of the invention. Variants of 3' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G.

Table 7. 3'-Untranslated Regions

3' UTR Identifier	Name/Description	Sequence	SEQ ID NO.
3UTR-001	Creatine Kinase	GCGCCTGCCCACCTGCCACCGACTGCTGGAAC CCAGCCAGTGGGAGGGCCTGGCCCACCAGAGT CCTGCTCCCTCACTCCTCGCCCCGCCCCCTGTC CCAGAGTCCCACCTGGGGGCTCTCTCCACCCTT CTCAGAGTTCCAGTTTCAACCAGAGTTCCAACC AATGGGCTCCATCCTCTGGATTCTGGCCAATGA AATATCTCCCTGGCAGGGTCCTTCTTTTCCC AGAGCTCCACCCCAACCAGGAGCTCTAGTTAA TGGAGAGCTCCCAGCACACTCGGAGCTTGTGC TTTGTCTCCACGCAAAGCGATAAATAAAAGCA TTGGTGGCCTTTGGTCTTTGAATAAAGCCTGAG TAGGAAGTCTAGA	9
3UTR-002	Myoglobin	GCCCCTGCCGCTCCCACCCCAACCATCTGGGC CCCGGGTTCAAGAGAGAGCGGGGTCTGATCTC GTGTAGCCATATAGAGTTTGCTTCTGAGTGTCT	10

		GCTTTGTTTAGTAGAGGTGGGCAGGAGGAGCT GAGGGGCTGGGGCTGGGGTGTGGAAGTTGGCT TTGCATGCCCAGCGATGCGCCTCCCTGTGGGAT GTCATCACCTTGGGAACCGGGAGTGGCCCTTG GCTCACTGTGTTCTGCATGGTTTGGATCTGAAT TAATTGTCCTTTCTTCTAAATCCCAACCGAACT TCTTCCAACCTCCAAACTGGCTGTAACCCCAAA TCCAAGCCATTAACTACACCTGACAGTAGCAA TTGTCTGATTAATCACTGGCCCCTTGAAGACAG CAGAATGTCCCTTTGCAATGAGGAGGAGATCT GGGCTGGGCGGGCCAGCTGGGGAAGCATTTGA CTATCTGGAACCTTGTGTGTGCCTCCTCAGGTAT GGCAGTGACTCACCTGGTTTTAATAAAACAAC CTGCAACATCTCATGGTCTTTGAATAAAGCCTG AGTAGGAAGTCTAGA	
3UTR-003	α -actin	ACACACTCCACCTCCAGCACGCGACTTCTCAG GACGACGAATCTTCTCAATGGGGGGGCGGCTG AGCTCCAGCCACCCCGCAGTCACTTTCTTTGTA ACAACCTCCGTTGCTGCCATCGTAAACTGACAC AGTGTTTATAACGTGTACATACATTAACCTTATT ACCTCATTTTGTATTATTTTCGAAACAAAGCCCT GTGGAAGAAAATGGAAAACCTGAAGAAGCATT AAAGTCATTCTGTTAAGCTGCGTAAATGGTCTT TGAATAAAGCCTGAGTAGGAAGTCTAGA	11
3UTR-004	Albumin	CATCACATTTAAAAGCATCTCAGCCTACCATG AGAATAAGAGAAAGAAAATGAAGATCAAAAG CTTATTCATCTGTTTTTCTTTTTCGTTGGTGTA AGCCAACACCCTGTCTAAAAAACATAAATTC TTAATCATTTTGCCTCTTTTCTCTGTGCTTCAA TTAATAAAAAATGGAAAGAATCTAATAGAGTG GTACAGCACTGTTATTTTCAAAGATGTGTTGC TATCCTGAAAATTCTGTAGGTTCTGTGGAAGTT CCAGTGTTCTCTCTTATTCCACTTCGGTAGAGG ATTTCTAGTTTCTTGTGGGCTAATTAAATAAAT CATTAATACTCTTCTAATGGTCTTTGAATAAAG CCTGAGTAGGAAGTCTAGA	12
3UTR-005	α -globin	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATG CCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGG TCTTTGAATAAAGCCTGAGTAGGAAGGCGGCC GCTCGAGCATGCATCTAGA	13
3UTR-006	G-CSF	GCCAAGCCCTCCCCATCCCATGTATTTATCTCT ATTTAATATTTATGTCTATTTAAGCCTCATATTT AAAGACAGGGAAGAGCAGAACGGAGCCCCAG GCCTCTGTGTCCTTCCCTGCATTTCTGAGTTT ATTCTCCTGCCTGTAGCAGTGAGAAAAAGCTC CTGTCCTCCCATCCCCTGGACTGGGAGGTAGAT AGGTAAATACCAAGTATTTATTACTATGACTGC TCCCCAGCCCTGGCTCTGCAATGGGCACTGGG ATGAGCCGCTGTGAGCCCCTGGTCCTGAGGGT CCCCACCTGGGACCCTTGAGAGTATCAGGTCT	14

		CCCACGTGGGAGACAAGAAATCCCTGTTTAAT ATTTAAACAGCAGTGTTCCTCATCTGGGTCCTT GCACCCCTCACTCTGGCCTCAGCCGACTGCAC AGCGGCCCCCTGCATCCCCCTTGGCTGTGAGGCC CCTGGACAAGCAGAGGTGGCCAGAGCTGGGA GGCATGGCCCTGGGGTCCCACGAATTTGCTGG GGAATCTCGTTTTTCTTCTTAAGACTTTTGGGA CATGGTTTGACTCCCGAACATCACCGACGCGT CTCCTGTTTTTCTGGGTGGCCTCGGGACACCTG CCCTGCCCCCACGAGGGTCAGGACTGTGACTC TTTTTAGGGCCAGGCAGGTGCCTGGACATTTGC CTTGCTGGACGGGGACTGGGGATGTGGGAGGG AGCAGACAGGAGGAATCATGTCAGGCCTGTGT GTGAAAGGAAGCTCCACTGTCACCCTCCACCT CTTCACCCCCCACTCACCACTGTCCCCTCCACT GTCACATTGTAAGTGAAGTTCAGGATAATAAA GTGTTTGCCTCCATGGTCTTTGAATAAAGCCTG AGTAGGAAGGCGGCCGCTCGAGCATGCATCTA GA	
3UTR-007	Colla2; collagen, type I, alpha 2	ACTCAATCTAAATTAAAAAAGAAAGAAATTTG AAAAAACTTTCTCTTTGCCATTTCTTCTTCTCT TTTTAACTGAAAGCTGAATCCTTCCATTTCTT CTGCACATCTACTTGCTTAAATTGTGGGCAAAA GAGAAAAAGAAGGATTGATCAGAGCATTGTGC AATACAGTTTCATTAAGTCTTCCCCCGCTCCC CCAAAAATTTGAATTTTTTTTTCAACACTCTTA CACCTGTTATGGAAAATGTCAACCTTTGTAAG AAAACCAAATAAAAAATTGAAAAATAAAAAAC CATAAACATTTGCACCACTTGTGGCTTTTGAAT ATCTTCCACAGAGGGAAGTTTAAAACCCAAAC TTCCAAAGGTTTAACTACCTCAAAACACTTTC CCATGAGTGTGATCCACATTGTTAGGTGCTGAC CTAGACAGAGATGAAGTGAAGTCTTGTTTTGT TTTGTTTATAATACAAAGGTGCTAATTAATAGT ATTTAGATACTTGAAGAATGTTGATGGTGCTA GAAGAATTTGAGAAGAAATACTCCTGTATTGA GTTGTATCGTGTGGTGTATTTTTTAAAAAATTT GATTTAGCATTATATTTTCCATCTTATTTCCA ATTAAAAGTATGCAGATTATTTGCCCAAATCTT CTTCAGATTACAGCATTGTTCTTTGCCAGTCTC ATTTTCATCTTCTTCCATGGTTCCACAGAAGCT TTGTTTCTTGGGCAAGCAGAAAAATTAAATTGT ACCTATTTTGTATATGTGAGATGTTTAAATAAA TTGTGAAAAAATGAAATAAAGCATGTTTGGT TTTCCAAAAGAACATAT	15
3UTR-008	Col6a2; collagen, type VI, alpha 2	CGCCGCCGCCCGGGCCCCGAGTCGAGGGTCG TGAGCCCACCCCGTCCATGGTGCTAAGCGGGC CCGGGTCCCACACGGCCAGCACCGCTGCTCAC TCGGACGACGCCCTGGGCCTGCACCTCTCCAG CTCCTCCCACGGGGTCCCCGTAGCCCCGGCCC CCGCCAGCCCCAGGTCTCCCCAGGCCCTCCG	16

		CAGGCTGCCCCGGCCTCCCTCCCCCTGCAGCCAT CCCAAGGCTCCTGACCTACCTGGCCCCCTGAGCT CTGGAGCAAGCCCTGACCCAATAAAGGCTTTG AACCCAT	
3UTR-009	RPN1; ribophorin I	GGGGCTAGAGCCCTCTCCGCACAGCGTGGAGA CGGGGCAAGGAGGGGGTATTAGGATTGGTG GTTTTGTTTTGCTTTGTTTAAAGCCGTGGGAAA ATGGCACAACCTTACCTCTGTGGGAGATGCAA CACTGAGAGCCAAGGGGTGGGAGTTGGGATAA TTTTTATATAAAAGAAGTTTTTCCACTTTGAAT TGCTAAAAGTGGCATTTTTCCCTATGTGCAGTCA CTCCTCTCATTTCTAAAATAGGGACGTGGCCAG GCACGGTGGCTCATGCCTGTAATCCCAGCACTT TGGGAGGCCGAGGCAGGCGGCTCACGAGGTCA GGAGATCGAGACTATCCTGGCTAACACGGTAA AACCCTGTCTCTACTAAAAGTACAAAAAATTA GCTGGGCGTGGTGGTGGGCACCTGTAGTCCCA GCTACTCGGGAGGCTGAGGCAGGAGAAAGGC ATGAATCCAAGAGGCAGAGCTTGCAGTGAGCT GAGATCACGCCATTGCACTCCAGCCTGGGCAA CAGTGTTAAGACTCTGTCTCAAATATAAATAA ATAAATAAATAAATAAATAAATAAATAAATAA AAAGCGAGATGTTGCCCTCAA	17
3UTR-010	LRP1; low density lipoprotein receptor- related protein 1	GGCCCTGCCCCGTCGGACTGCCCCCAGAAAGC CTCCTGCCCCCTGCCAGTGAAGTCCTTCAGTGA GCCCCCTCCCAGCCAGCCCTTCCCTGGCCCCGC CGGATGTATAAATGTAAAAATGAAGGAATTAC ATTTTATATGTGAGCGAGCAAGCCGGCAAGCG AGCACAGTATTATTTCTCCATCCCCTCCCTGCC TGCTCCTTGGCACCCCCATGCTGCCTTCAGGGA GACAGGCAGGGAGGGCTTGGGGCTGCACCTCC TACCCTCCCACCAGAACGCACCCCCTGAGGAG AGCTGGTGGTGCAGCCTTCCCCTCCCTGTATAA GACACTTTGCCAAGGCTCTCCCCTCTCGCCCCA TCCCTGCTTGCCCGCTCCCACAGCTTCCTGAGG GCTAATTCTGGGAAGGGAGAGTTCTTTGCTGC CCCTGTCTGGAAGACGTGGCTCTGGGTGAGGT AGGCGGGAAGGATGGAGTGTTTTAGTTCTTG GGGGAGGCCACCCCAAACCCAGCCCCAACTC CAGGGGCACCTATGAGATGGCCATGCTCAACC CCCCTCCCAGACAGGCCCTCCCTGTCTCCAGG GCCCCACCGAGGTTCCAGGGCTGGAGACTT CCTCTGGTAAACATTCTCCAGCCTCCCCTCCC CTGGGGACGCCAAGGAGGTGGGCCACACCCAG GAAGGGAAAGCGGGCAGCCCCGTTTTGGGGAC GTGAACGTTTTAATAATTTTGTGAATTCTT TACAATAAATAACACAGATATTGTTATAAAT AAAATTGT	18
3UTR-011	Nnt1; cardiotrophin-like	ATATTAAGGATCAAGCTGTTAGCTAATAATGC CACCTCTGCAGTTTTGGGAACAGGCAAATAAA GTATCAGTATACATGGTGATGTACATCTGTAGC	19

	cytokine factor 1	AAAGCTCTTGGAGAAAATGAAGACTGAAGAA AGCAAAGCAAAAACGTATAGAGAGATTTTTC AAAAGCAGTAATCCCTCAATTTTAAAAAAGGA TTGAAAATTCTAAATGTCTTTCTGTGCATATTT TTTGTGTTAGGAATCAAAAGTATTTTATAAAAG GAGAAAGAACAGCCTCATTTTAGATGTAGTCC TGTTGGATTTTTTATGCCTCCTCAGTAACCAGA AATGTTTTAAAAAACTAAGTGTTTAGGATTTCA AGACAACATTATACATGGCTCTGAAATATCTG ACACAATGTAAACATTGCAGGCACCTGCATTT TATGTTTTTTTTTCAACAAATGTGACTAATTT GAAACTTTTATGAACTTCTGAGCTGTCCCCTTG CAATTC AACCGCAGTTTGAATTAATCATATCAA ATCAGTTTTAATTTTTTAAATTGTACTTCAGAG TCTATATTTCAAGGGCACATTTTCTCACTACTA TTTTAATACATTAAAGGACTAAATAATCTTTCA GAGATGCTGGAAACAAATCATTTGCTTTATAT GTTTCATTAGAATACCAATGAAACATACAAC TGAAAATTAGTAATAGTATTTTTGAAGATCCCA TTTCTAATTGGAGATCTCTTAATTTTCGATCAA CTTATAATGTGTAGTACTATATTAAGTGCACCT GAGTGG AATTCAACATTTGACTAATAAAATGA GTTTCATCATGTTGGCAAGTGATGTGGCAATTAT CTCTGGTGACAAAAGAGTAAAATCAAATATTT CTGCCTGTTACAAATATCAAGGAAGACCTGCT ACTATGAAATAGATGACATTAATCTGTCTTCAC TGTTTATAATACGGATGGATTTTTTTTCAAATC AGTGTGTGTTTTGAGGTCTTATGTAATTGATGA CATTTGAGAGAAATGGTGGCTTTTTTTAGCTAC CTCTTTGTTTCATTTAAGCACCAGTAAAGATCAT GTCTTTTTATAGAAGTGTAGATTTTCTTTGTGA CTTTGCTATCGTGCCTAAAGCTCTAAATATAGG TGAATGTGTGATGAATACTCAGATTATTTGTCT CTCTATATAATTAGTTTGGTACTAAGTTTCTCA AAAAATTATTAACACATGAAAGACAATCTCTA AACCAGAAAAAGAAGTAGTACAAATTTTGTTA CTGTAATGCTCGCGTTTAGTGAGTTTAAACAC ACAGTATCTTTTGGTTTTATAATCAGTTTCTATT TTGCTGTGCCTGAGATTAAGATCTGTGTATGTG TGTGTGTGTGTGTGTGCGTTTGTGTGTTAAAGC AGAAAAGACTTTTTTAAAAGTTTTAAGTGATA AATGCAATTTGTTAATTGATCTTAGATCACTAG TAAACTCAGGGCTGAATTATACCATGTATATTC TATTAGAAGAAAGTAAACACCATCTTTATTCCT GCCCTTTTTCTTCTCTCAAAGTAGTTGTAGTTA TATCTAGAAAGAAGCAATTTTGATTTCTTGAAA AGGTAGTTCCTGCACTCAGTTTAAACTAAAAA TAATCATACTTGATTTTATTTATTTTGTGATA GTAAAAATTTTAAATTTATATATATTTTATTTA GTATTATCTTATTCTTTGCTATTTGCCAATCCTT TGTCATCAATTGTGTTAAATGAATTGAAAATTC	

		ATGCCCTGTTTCATTTTATTTTACTTTATTGGTTA GGATATTTAAAGGATTTTGTATATATAATTTC TAAATTAATATTCCAAAAGGTTAGTGGACTTA GATTATAAATTATGGCAAAAATCTAAAAACAA CAAAAATGATTTTTATACATTCTATTTTCATTAT TCCTCTTTTTCCAATAAGTCATACAATTGGTAG ATATGACTTATTTTATTTTGTATTATTCACTAT ATCTTTATGATATTTAAGTATAAATAATTAATA AAATTTATTGTACCTTATAGTCTGTCACCAAAA AAAAAAATTATCTGTAGGTAGTGAAATGCTA ATGTTGATTTGTCTTTAAGGGCTTGTTAACTAT CCTTTATTTTCTCATTTGTCTTAAATTAGGAGTT TGTGTTTAAATTACTCATCTAAGCAAAAAATGT ATATAAATCCCATTACTGGGTATATACCCAAA GGATTATAAATCATGCTGCTATAAAGACACAT GCACACGTATGTTTATTGCAGCACTATTCACAA TAGCAAAGACTTGGAACCAACCCAAATGTCCA TCAATGATAGACTTGATTAAGAAAATGTGCAC ATATACACCATGGAATACTATGCAGCCATAAA AAAGGATGAGTTCATGTCCTTTGTAGGGACAT GGATAAAGCTGGAAACCATCATTCTGAGCAAA CTATTGCAAGGACAGAAAACCAAACACTGCAT GTTCTCACTCATAGGTGGGAATTGAACAATGA GAACACTTGGACACAAGGTGGGGAACACCACA CACCAGGGCCTGTCATGGGGTGGGGGGAGTGG GGAGGGATAGCATTAGGAGATATACCTAATGT AAATGATGAGTTAATGGGTGCAGCACACCAAC ATGGCACATGTATACATATGTAGCAAACCTGC ACGTTGTGCACATGTACCCTAGAACTTAAAGT ATAATTAATAAAAAAAAAAGAAAACAGAAGCTA TTTATAAAGAAGTTATTTGCTGAAATAAATGTG ATCTTTCCCATTAATAAAAAATAAAGAAATTTG GGGTAAAAAAACACAATATATTGTATTCTTGA AAAATTCTAAGAGAGTGGATGTGAAGTGTTCT CACCACAAAAGTGATAACTAATTGAGGTAATG CACATATTAATTAGAAAGATTTTGTCAATCCAC AATGTATATATACTTAAAAATATGTTATACACA ATAAATACATACATTAAAAAATAAGTAAATGT A	
3UTR-012	Col6a1; collagen, type VI, alpha 1	CCCACCCTGCACGCCGGCACCAAACCCTGTCC TCCCACCCCTCCCCACTCATCACTAAACAGAGT AAAATGTGATGCGAATTTTCCCGACCAACCTG ATTCGCTAGATTTTTTTTAAAGGAAAAGCTTGGA AAGCCAGGACACAACGCTGCTGCCTGCTTTGT GCAGGGTCCTCCGGGGCTCAGCCCTGAGTTGG CATCACCTGCGCAGGGCCCTCTGGGGCTCAGC CCTGAGCTAGTGTACCTGCACAGGGCCCTCT GAGGCTCAGCCCTGAGCTGGCGTCACCTGTGC AGGGCCCTCTGGGGCTCAGCCCTGAGCTGGCC TCACCTGGGTTCCCCACCCCGGGCTCTCCTGCC CTGCCCTCCTGCCCGCCCTCCCTCCTGCCTGCG	20

		CAGCTCCTTCCCTAGGCACCTCTGTGCTGCATC CCACCAGCCTGAGCAAGACGCCCTCTCGGGGC CTGTGCCGCACTAGCCTCCCTCTCCTCTGTCCC CATAGCTGGTTTTTCCACCAATCCTCACCTAA CAGTTACTTTACAATTAAACTCAAAGCAAGCT CTTCTCCTCAGCTTGGGGCAGCCATTGGCCTCT GTCTCGTTTTTGGGAAACCAAGGTCAGGAGGCC GTTGCAGACATAAATCTCGGCGACTCGGCCCC GTCTCCTGAGGGTCCTGCTGGTGACCGGCCTG GACCTTGGCCCTACAGCCCTGGAGGCCGCTGC TGACCAGCACTGACCCCGACCTCAGAGAGTAC TCGCAGGGGCGCTGGCTGCACTCAAGACCCTC GAGATTAACGGTGCTAACCCCGTCTGCTCCTCC CTCCCGCAGAGACTGGGGCCTGGACTGGACAT GAGAGCCCCCTTGGTGCCACAGAGGGCTGTGTC TACTAGAAACAACGCAAACCTCTCCTTCCTCA GAATAGTGATGTGTTTCGACGTTTTATCAAAGG CCCCCTTTCTATGTTTCATGTTAGTTTTGCTCCTT CTGTGTTTTTTTCTGAACCATATCCATGTTGCT GACTTTTCCAAATAAAGGTTTTCACTCCTCTC	
3UTR-013	Calr; calreticulin	AGAGGCCTGCCTCCAGGGCTGGACTGAGGCCT GAGCGCTCCTGCCGCAGAGCTGGCCGCGCCAA ATAATGTCTCTGTGAGACTCGAGAACTTTCATT TTTTTCCAGGCTGGTTCGGATTTGGGGTGGATT TTGGTTTTGTCCCCTCCTCCACTCTCCCCACC CCCTCCCCGCCCTTTTTTTTTTTTTTTTAAAC TGGTATTTTATCTTTGATTCTCCTTCAGCCCTCA CCCCTGGTTCTCATCTTTCTTGATCAACATCTTT TCTTGCCTCTGTCCCCTTCTCTCATCTCTTAGCT CCCCTCCAACCTGGGGGGCAGTGGTGTGGAGA AGCCACAGGCCTGAGATTTTCATCTGCTCTCCTT CCTGGAGCCCAGAGGAGGGCAGCAGAAGGGG GTGGTGTCTCCAACCCCCCAGCACTGAGGAAG AACGGGGCTCTTCTCATTTACCCCTCCCTTTC TCCCCTGCCCCCAGGACTGGGCCACTTCTGGGT GGGGCAGTGGGTCCCAGATTGGCTCACACTGA GAATGTAAGAACTACAAACAAAATTTCTATTA AATTAAATTTTGTGTCTCC	21
3UTR-014	Colla1; collagen, type I, alpha 1	CTCCCTCCATCCCAACCTGGCTCCCTCCCACCC AACCAACTTTCCCCCAACCCGAAACAGACA AGCAACCCAACTGAACCCCTCAAAAGCCAA AAAATGGGAGACAATTTACATGGACTTTGGA AAATATTTTTTCTTTGCATTCATCTCTCAAAC TTAGTTTTTATCTTTGACCAACCGAACATGACC AAAAACCAAAAGTGCAATTCAACCTTACCAAAA AAAAAAAAAAAAAAAAAGAATAAATAAATAACT TTTTAAAAAAGGAAGCTTGGTCCACTTGCTTGA AGACCCATGCGGGGGTAAGTCCCTTTCTGCCC GTTGGGCTTATGAAACCCCAATGCTGCCCTTTC TGCTCCTTTCTCCACACCCCCCTTGGGGCCTCC CCTCCACTCCTTCCCAATCTGTCTCCCCAGAA	22

		GACACAGGAAACAATGTATTGTCTGCCCAGCA ATCAAAGGCAATGCTCAAACACCCAAGTGGCC CCCACCCTCAGCCCGCTCCTGCCCAGCA CCCCAGGCCCTGGGGGACCTGGGGTTCTCAG ACTGCCAAAGAAGCCTTGCCATCTGGCGCTCC CATGGCTCTTGCAACATCTCCCCTTCGTTTTTG AGGGGGTCATGCCGGGGGAGCCACCAGCCCCT CACTGGGTTCGGAGGAGAGTCAGGAAGGGCCA CGACAAAGCAGAAACATCGGATTTGGGGAACG CGTGTCATCCCTTGTGCCGCAGGGCTGGGCG GGAGAGACTGTTCTGTTCCCTTGTGTAAGTGTGT TGCTGAAAGACTACCTCGTTCCTTGTCTTGATGT GTCACCGGGGCAACTGCCTGGGGGCGGGGATG GGGGCAGGGTGGAAGCGGCTCCCCATTTTATA CCAAAGGTGCTACATCTATGTGATGGGTGGGG TGGGGAGGGAATCACTGGTGCTATAGAAATTG AGATGCCCCCCCAGGCCAGCAAATGTTCCCTTTT TGTTCAAAGTCTATTTTTTATTCCTTGATATTTTT CTTTTTTTTTTTTTTTTTTTGTGGATGGGGACTT GTGAATTTTTCTAAAGGTGCTATTTAACATGGG AGGAGAGCGTGTGCGGCTCCAGCCCAGCCCCG TGCTCACTTTCCACCCTCTCTCCACCTGCCTCT GGCTTCTCAGGCCTCTGCTCTCCGACCTCTCTC CTCTGAAACCCTCCTCCACAGCTGCAGCCCATC CTCCCGGCTCCCTCCTAGTCTGTCTGCGTCCT CTGTCCCCGGGTTTCAGAGACAACTTCCCAA GCACAAAGCAGTTTTTCCCCCTAGGGGTGGGA GGAAGCAAAGACTCTGTACCTATTTTGTATGT GTATAATAATTTGAGATGTTTTTAATTATTTTG ATTGCTGGAATAAAGCATGTGGAAATGACCCA AACATAATCCGCAGTGGCCTCCTAATTTCCCTTC TTTGAGTTGGGGGAGGGGTAGACATGGGGAA GGGGCTTTGGGGTGATGGGCTTGCCCTCCATTC CTGCCCTTTCCCTCCCCACTATTCTCTTCTAGAT CCCTCCATAACCCCACTCCCCTTTCTCTCACCC TTCTTATACCGCAAACCTTTCTACTTCCTCTTTC ATTTTCTATTCTTGCAATTTCCCTGACCTTTTC CAAATCCTCTTCTCCCCTGCAATACCATAACAGG CAATCCACGTGCACAACACACACACACTCT TCACATCTGGGGTTGTCCAAACCTCATAACCCAC TCCCCTTCAAGCCCATCCACTCTCCACCCCCTG GATGCCCTGCACTTGGTGGCGGTGGGATGCTC ATGGATACTGGGAGGGTGAGGGGAGTGGAAC CCGTGAGGAGGACCTGGGGGCCTCTCCTTGAA CTGACATGAAGGGTCATCTGGCCTCTGCTCCCT TCTCACCCACGCTGACCTCCTGCCGAAGGAGC AACGCAACAGGAGAGGGGTCTGCTGAGCCTGG CGAGGGTCTGGGAGGGACCAGGAGGAAGGCG TGCTCCCTGCTCGCTGTCTGGCCCTGGGGGAG TGAGGGAGACAGACACCTGGGAGAGCTGTGG GGAAGGCACTCGCACCGTGCTCTTGGGAAGGA	

		AGGAGACCTGGCCCTGCTCACCACGGACTGGG TGCCTCGACCTCCTGAATCCCCAGAACACAAC CCCCCTGGGCTGGGGTGGTCTGGGGAACCATC GTGCCCCCGCCTCCCGCCTACTCCTTTTAAGC TT	
3UTR-015	Plodl; procollagen -lysine, 2- oxoglutarat e 5- dioxygenas e 1	TTGGCCAGGCCTGACCCTCTTGGACCTTTCTTC TTTGCCGACAACCACTGCCCAGCAGCCTCTGG GACCTCGGGGTCCCAGGGAACCCAGTCCAGCC TCCTGGCTGTTGACTTCCCATTGCTCTTGGAGC CACCAATCAAAGAGATTCAAAGAGATTCTGC AGGCCAGAGGCGGAACACACCTTTATGGCTGG GGCTCTCCGTGGTGTCTGGACCCAGCCCCTGG AGACACCATTCACTTTTACTGCTTTGTAGTGAC TCGTGCTCTCCAACCTGTCTTCCTGAAAAACCA AGGCCCCCTTCCCCACCTCTTCCATGGGGTGA GACTTGAGCAGAACAGGGGCTTCCCCAAGTTG CCCAGAAAGACTGTCTGGGTGAGAAGCCATGG CCAGAGCTTCTCCCAGGCACAGGTGTTGCACC AGGGACTTCTGCTTCAAGTTTTGGGGTAAAGA CACCTGGATCAGACTCCAAGGGCTGCCCTGAG TCTGGGACTTCTGCCTCCATGGCTGGTCATGAG AGCAAACCGTAGTCCCCTGGAGACAGCGACTC CAGAGAACCTCTTGGGAGACAGAAGAGGCATC TGTGCACAGCTCGATCTTCTACTTGCCTGTGGG GAGGGGAGTGACAGGTCCACACACCACACTGG GTCACCCTGTCCTGGATGCCTCTGAAGAGAGG GACAGACCGTCAGAACTGGAGAGTTTCTATT AAAGGTCATTTAAACCA	23
3UTR-016	Nucbl; nucleobindi n 1	TCCTCCGGGACCCCAGCCCTCAGGATTCCTGAT GCTCCAAGGCGACTGATGGGCGCTGGATGAAG TGGCACAGTCAGCTTCCCTGGGGGCTGGTGTG ATGTTGGGCTCCTGGGGCGGGGGCACGGCCTG GCATTCACGCATTGCTGCCACCCCAGGTCCAC CTGTCTCCACTTTCACAGCCTCCAAGTCTGTGG CTCTTCCCTTCTGTCCTCCGAGGGGCTTGCTT CTCTCGTGTCCAGTGAGGTGCTCAGTGATCGGC TTAACTTAGAGAAGCCCGCCCCCTCCCCTTCTC CGTCTGTCCCAAGAGGGTCTGCTCTGAGCCTGC GTTCCTAGGTGGCTCGGCCTCAGCTGCCTGGGT TGTGGCCGCCCTAGCATCCTGTATGCCACAGC TACTGGAATCCCCGCTGCTGCTCCGGGCCAAG CTTCTGGTTGATTAATGAGGGCATGGGGTGGT CCCTCAAGACCTTCCCCTACCTTTTGTGGAACC AGTGATGCCTCAAAGACAGTGTCCCCTCCACA GCTGGGTGCCAGGGGCAGGGGATCCTCAGTAT AGCCGGTGAACCCTGATACCAGGAGCCTGGGC CTCCCTGAACCCCTGGCTTCCAGCCATCTCATC GCCAGCCTCCTCCTGGACCTCTTGGCCCCCAGC CCCTTCCCCACACAGCCCCAGAAGGGTCCCAG AGCTGACCCCACTCCAGGACCTAGGCCCAGCC CCTCAGCCTCATCTGGAGCCCCTGAAGACCAG	24

		TCCCACCCACCTTTCTGGCCTCATCTGACACTG CTCCGCATCCTGCTGTGTGTCCTGTTCCATGTT CCGGTTCCATCCAAATACACTTTCTGGAACAA A	
3UTR-017	α -globin	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCT TGGGCCTCCCCCAGCCCCCTCCTCCCCTTCCTG CACCCGTACCCCGTGGTCTTTGAATAAAGTCT GAGTGGGCGGC	25

[00303] It should be understood that those listed in the previous tables are examples and that any UTR from any gene may be incorporated into the respective first or second flanking region of the primary construct. Furthermore, multiple wild-type UTRs of any known gene may be utilized. It is also within the scope of the present invention to provide artificial UTRs which are not variants of wild type genes. These UTRs or portions thereof may be placed in the same orientation as in the transcript from which they were selected or may be altered in orientation or location. Hence a 5' or 3' UTR may be inverted, shortened, lengthened, made chimeric with one or more other 5' UTRs or 3' UTRs. As used herein, the term "altered" as it relates to a UTR sequence, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3' or 5' UTR may be altered relative to a wild type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides. Any of these changes producing an "altered" UTR (whether 3' or 5') comprise a variant UTR.

[00304] In one embodiment, a double, triple or quadruple UTR such as a 5' or 3' UTR may be used. As used herein, a "double" UTR is one in which two copies of the same UTR are encoded either in series or substantially in series. For example, a double beta-globin 3' UTR may be used as described in US Patent publication 20100129877, the contents of which are incorporated herein by reference in its entirety.

[00305] It is also within the scope of the present invention to have patterned UTRs. As used herein "patterned UTRs" are those UTRs which reflect a repeating or alternating pattern, such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than 3 times. In these patterns, each letter, A, B, or C represent a different UTR at the nucleotide level.

[00306] In one embodiment, flanking regions are selected from a family of transcripts whose proteins share a common function, structure, feature of property. For example, polypeptides of interest may belong to a family of proteins which are expressed in a particular cell, tissue or at some time during development. The UTRs from any of these genes may be swapped for any other UTR of the same or different family of proteins to create a new chimeric primary transcript. As used herein, a "family of proteins" is used in the broadest sense to refer to a group of two or more polypeptides of interest which share at least one function, structure, feature, localization, origin, or expression pattern.

[00307] After optimization (if desired), the primary construct components are reconstituted and transformed into a vector such as, but not limited to, plasmids, viruses, cosmids, and artificial chromosomes. For example, the optimized construct may be reconstituted and transformed into chemically competent *E. coli*, yeast, neurospora, maize, drosophila, etc. where high copy plasmid-like or chromosome structures occur by methods described herein. *Stop Codons*

[00308] In one embodiment, the primary constructs of the present invention may include at least two stop codons before the 3' untranslated region (UTR). The stop codon may be selected from TGA, TAA and TAG. In one embodiment, the primary constructs of the present invention include the stop codon TGA and one additional stop codon. In a further embodiment the addition stop codon may be TAA.

Vector Amplification

[00309] The vector containing the primary construct is then amplified and the plasmid isolated and purified using methods known in the art such as, but not limited to, a maxi prep using the Invitrogen PURELINK™ HiPure Maxiprep Kit (Carlsbad, CA).

Plasmid Linearization

[00310] The plasmid may then be linearized using methods known in the art such as, but not limited to, the use of restriction enzymes and buffers. The linearization reaction may be purified using methods including, for example Invitrogen's PURELINK™ PCR Micro Kit (Carlsbad, CA), and HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC) and Invitrogen's standard PURELINK™ PCR Kit (Carlsbad, CA). The purification method may be modified

depending on the size of the linearization reaction which was conducted. The linearized plasmid is then used to generate cDNA for *in vitro* transcription (IVT) reactions.

cDNA Template Synthesis

[00311] A cDNA template may be synthesized by having a linearized plasmid undergo polymerase chain reaction (PCR). Table 8 is a listing of primers and probes that may be useful in the PCR reactions of the present invention. It should be understood that the listing is not exhaustive and that primer-probe design for any amplification is within the skill of those in the art. Probes may also contain chemically modified bases to increase base-pairing fidelity to the target molecule and base-pairing strength. Such modifications may include 5-methyl-Cytidine, 2, 6-di-amino-purine, 2'-fluoro, phosphoro-thioate, or locked nucleic acids.

Table 8. Primers and Probes

Primer/ Probe Identifier	Sequence (5'-3')	Hybridization target	SEQ ID NO.
UFP	TTGGACCCTCGTACAGAAGCTAA TACG	cDNA Template	26
URP	T _{x160} CTTCCTACTCAGGCTTTATTC AAAGACCA	cDNA Template	27
GBA1	CCTTGACCTTCTGGAACTTC	Acid glucocerebrosidase	28
GBA2	CCAAGCACTGAAACGGATAT	Acid glucocerebrosidase	29
LUC1	GATGAAAAGTGCTCCAAGGA	Luciferase	30
LUC2	AACCGTGATGAAAAGGTACC	Luciferase	31
LUC3	TCATGCAGATTGGAAAGGTC	Luciferase	32
GCSF1	CTTCTTGGA CTGTCCAGAGG	G-CSF	33
GCSF2	GCAGTCCCTGATACAAGAAC	G-CSF	34
GCSF3	GATTGAAGGTGGCTCGCTAC	G-CSF	35

*UFP is universal forward primer; URP is universal reverse primer.

[00312] In one embodiment, the cDNA may be submitted for sequencing analysis before undergoing transcription.

Polynucleotide Production

[00313] The process of polynucleotide production may include, but is not limited to, *in vitro* transcription, cDNA template removal and RNA clean-up, and capping and/or tailing reactions.

In Vitro Transcription

[00314] The cDNA produced in the previous step may be transcribed using an *in vitro* transcription (IVT) system. The system typically comprises a transcription buffer, nucleotide triphosphates (NTPs), an RNase inhibitor and a polymerase. The NTPs may be manufactured in house, may be selected from a supplier, or may be synthesized as described herein. The NTPs may be selected from, but are not limited to, those described herein including natural and unnatural (modified) NTPs. The polymerase may be selected from, but is not limited to, T7 RNA polymerase, T3 RNA polymerase and mutant polymerases such as, but not limited to, polymerases able to be incorporated into modified nucleic acids.

RNA Polymerases

[00315] Any number of RNA polymerases or variants may be used in the design of the primary constructs of the present invention.

[00316] RNA polymerases may be modified by inserting or deleting amino acids of the RNA polymerase sequence. As a non-limiting example, the RNA polymerase may be modified to exhibit an increased ability to incorporate a 2'-modified nucleotide triphosphate compared to an unmodified RNA polymerase (see International Publication WO2008078180 and U.S. Patent 8,101,385; herein incorporated by reference in their entireties).

[00317] Variants may be obtained by evolving an RNA polymerase, optimizing the RNA polymerase amino acid and/or nucleic acid sequence and/or by using other methods known in the art. As a non-limiting example, T7 RNA polymerase variants may be evolved using the continuous directed evolution system set out by Esvelt *et al.* (Nature (2011) 472(7344):499-503; herein incorporated by reference in its entirety) where clones of T7 RNA polymerase may encode at least one mutation such as, but not limited to, lysine at position 93 substituted for threonine (K93T), I4M, A7T, E63V, V64D, A65E, D66Y, T76N, C125R, S128R, A136T, N165S, G175R, H176L, Y178H, F182L, L196F, G198V, D208Y, E222K, S228A, Q239R, T243N, G259D, M267I, G280C, H300R, D351A, A354S, E356D, L360P, A383V, Y385C, D388Y, S397R, M401T, N410S, K450R, P451T, G452V, E484A, H523L, H524N, G542V, E565K, K577E, K577M, N601S, S684Y, L699I, K713E, N748D, Q754R, E775K, A827V, D851N or L864F. As another non-limiting example, T7 RNA polymerase variants may encode at least

mutation as described in U.S. Pub. Nos. 20100120024 and 200701 171 12; herein incorporated by reference in their entireties. Variants of RNA polymerase may also include, but are not limited to, substitutional variants, conservative amino acid substitution, insertional variants, deletional variants and/or covalent derivatives.

[00318] In one embodiment, the primary construct may be designed to be recognized by the wild type or variant RNA polymerases. In doing so, primary construct may be modified to contain sites or regions of sequence changes from the wild type or parent primary construct.

[00319] In one embodiment, the primary construct may be designed to include at least one substitution and/or insertion upstream of an RNA polymerase binding or recognition site, downstream of the RNA polymerase binding or recognition site, upstream of the TATA box sequence, downstream of the TATA box sequence of the primary construct but upstream of the coding region of the primary construct, within the 5'UTR, before the 5'UTR and/or after the 5'UTR.

[00320] In one embodiment, the 5'UTR of the primary construct may be replaced by the insertion of at least one region and/or string of nucleotides of the same base. The region and/or string of nucleotides may include, but is not limited to, at least 3, at least 4, at least 5, at least 6, at least 7 or at least 8 nucleotides and the nucleotides may be natural and/or unnatural. As a non-limiting example, the group of nucleotides may include 5-8 adenine, cytosine, thymine, a string of any of the other nucleotides disclosed herein and/or combinations thereof.

[00321] In one embodiment, the 5'UTR of the primary construct may be replaced by the insertion of at least two regions and/or strings of nucleotides of two different bases such as, but not limited to, adenine, cytosine, thymine, any of the other nucleotides disclosed herein and/or combinations thereof. For example, the 5'UTR may be replaced by inserting 5-8 adenine bases followed by the insertion of 5-8 cytosine bases. In another example, the 5'UTR may be replaced by inserting 5-8 cytosine bases followed by the insertion of 5-8 adenine bases.

[00322] In one embodiment, the primary construct may include at least one substitution and/or insertion downstream of the transcription start site which may be recognized by an RNA polymerase. As a non-limiting example, at least one substitution

and/or insertion may occur downstream the transcription start site by substituting at least one nucleic acid in the region just downstream of the transcription start site (such as, but not limited to, +1 to +6). Changes to region of nucleotides just downstream of the transcription start site may affect initiation rates, increase apparent nucleotide triphosphate (NTP) reaction constant values, and increase the dissociation of short transcripts from the transcription complex curing initial transcription (Briebe et al, Biochemistry (2002) 41: 5144-5149; herein incorporated by reference in its entirety). The modification, substitution and/or insertion of at least one nucleic acid may cause a silent mutation of the nucleic acid sequence or may cause a mutation in the amino acid sequence.

[00323] In one embodiment, the primary construct may include the substitution of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12 or at least 13 guanine bases downstream of the transcription start site.

[00324] In one embodiment, the primary construct may include the substitution of at least 1, at least 2, at least 3, at least 4, at least 5 or at least 6 guanine bases in the region just downstream of the transcription start site. As a non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be substituted by at least 1, at least 2, at least 3 or at least 4 adenine nucleotides. In another non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be substituted by at least 1, at least 2, at least 3 or at least 4 cytosine bases. In another non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be substituted by at least 1, at least 2, at least 3 or at least 4 thymine, and/or any of the nucleotides described herein.

[00325] In one embodiment, the primary construct may include at least one substitution and/or insertion upstream of the start codon. For the purpose of clarity, one of skill in the art would appreciate that the start codon is the first codon of the protein coding region whereas the transcription start site is the site where transcription begins. The primary construct may include, but is not limited to, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 or at least 8 substitutions and/or insertions of nucleotide bases. The nucleotide bases may be inserted or substituted at 1, at least 1, at

least 2, at least 3, at least 4 or at least 5 locations upstream of the start codon. The nucleotides inserted and/or substituted may be the same base (e.g., all A or all C or all T or all G), two different bases (e.g., A and C, A and T, or C and T), three different bases (e.g., A, C and T or A, C and T) or at least four different bases. As a non-limiting example, the guanine base upstream of the coding region in the primary construct may be substituted with adenine, cytosine, thymine, or any of the nucleotides described herein. In another non-limiting example the substitution of guanine bases in the primary construct may be designed so as to leave one guanine base in the region downstream of the transcription start site and before the start codon (see Esvelt *et al.* Nature (2011) 472(7344):499-503; herein incorporated by reference in its entirety). As a non-limiting example, at least 5 nucleotides may be inserted at 1 location downstream of the transcription start site but upstream of the start codon and the at least 5 nucleotides may be the same base type.

cDNA Template Removal and Clean-Up

[00326] The cDNA template may be removed using methods known in the art such as, but not limited to, treatment with Deoxyribonuclease I (DNase I). RNA clean-up may also include a purification method such as, but not limited to, AGENCOURT® CLEANSEQ® system from Beckman Coulter (Danvers, MA), HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC) .

Capping and/or Tailing Reactions

[00327] The primary construct or mmRNA may also undergo capping and/or tailing reactions. A capping reaction may be performed by methods known in the art to add a 5' cap to the 5' end of the primary construct. Methods for capping include, but are not limited to, using a Vaccinia Capping enzyme (New England Biolabs, Ipswich, MA).

[00328] A poly-A tailing reaction may be performed by methods known in the art, such as, but not limited to, 2' O-methyltransferase and by methods as described herein. If the primary construct generated from cDNA does not include a poly-T, it may be beneficial to perform the poly-A-tailing reaction before the primary construct is cleaned.

Purification

[00329] The primary construct or mmRNA purification may include, but is not limited to, mRNA or mmRNA clean-up, quality assurance and quality control. mRNA or mmRNA clean-up may be performed by methods known in the arts such as, but not limited to, AGENCOURT® beads (Beckman Coulter Genomics, Danvers, MA), poly-T beads, LNA™ oligo-T capture probes (EXIQON® Inc, Vedbaek, Denmark) or HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC). The term "purified" when used in relation to a polynucleotide such as a "purified mRNA or mmRNA" refers to one that is separated from at least one contaminant. As used herein, a "contaminant" is any substance which makes another unfit, impure or inferior. Thus, a purified polynucleotide (e.g., DNA and RNA) is present in a form or setting different from that in which it is found in nature, or a form or setting different from that which existed prior to subjecting it to a treatment or purification method.

[00330] A quality assurance and/or quality control check may be conducted using methods such as, but not limited to, gel electrophoresis, UV absorbance, or analytical HPLC.

[00331] In another embodiment, the mRNA or mmRNA may be sequenced by methods including, but not limited to reverse-transcriptase-PCR.

[00332] In one embodiment, the mRNA or mmRNA may be quantified using methods such as, but not limited to, ultraviolet visible spectroscopy (UV/Vis). A non-limiting example of a UV/Vis spectrometer is a NANODROP® spectrometer (ThermoFisher, Waltham, MA). The quantified mRNA or mmRNA may be analyzed in order to determine if the mRNA or mmRNA may be of proper size, check that no degradation of the mRNA or mmRNA has occurred. Degradation of the mRNA and/or mmRNA may be checked by methods such as, but not limited to, agarose gel electrophoresis, HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC), liquid chromatography-mass spectrometry (LCMS), capillary electrophoresis (CE) and capillary gel electrophoresis (CGE).

Signal Peptides or Proteins

[00333] The primary constructs or mmRNA may also encode additional features which facilitate trafficking of the polypeptides to therapeutically relevant sites. One such feature which aids in protein trafficking is the signal peptide sequence. As used herein, a "signal sequence" or "signal peptide" is a polynucleotide or polypeptide, respectively, which is from about 9 to 200 nucleotides (3-60 amino acids) in length which is incorporated at the 5' (or N-terminus) of the coding region or polypeptide encoded, respectively. Addition of these sequences result in trafficking of the encoded polypeptide to the endoplasmic reticulum through one or more secretory pathways. Some signal peptides are cleaved from the protein by signal peptidase after the proteins are transported.

[00334] Table 9 is a representative listing of signal proteins or peptides which may be incorporated for encoding by the polynucleotides, primary constructs or mmRNA of the invention.

Table 9. Signal Peptides

ID	Description	NUCLEOTIDE SEQUENCE (5'-3')	SEQ ID NO.	ENCODED PEPTIDE	SEQ ID NO.
SS-001	a-1-antitrypsin	ATGATGCCATCCTCAGTCTCA TGGGGTATTTTGCTCTTGCGG GGTCTGTGCTGTCTCGTGCCG GTGTCGCTCGCA	36	MMPSSVSW GILLAGLCC LVPVSLA	98
SS-002	G-CSF	ATGGCCGGACCGGCGACTCAG TCGCCCATGAACTCATGGCC CTGCAGTTGTTGCTTTGGCAC TCAGCCCTCTGGACCGTCCAA GAGGCG	37	MAGPATQS PMKLMALQ LLLWHSAL WTVQEA	99
SS-003	Factor IX	ATGCAGAGAGTGAACATGATT ATGGCCGAGTCCCCATCGCTC ATCACAATCTGCCTGCTTGGT ACCTGCTTTCCGCCGAATGCA CTGTCTTTCTGGATCACGAGA ATGCGAATAAGATCTTGAACC GACCCAAACGG	38	MQRVNMIM AESPSLITIC LLGYLLSAE CTVFLDHEN ANKILNRPK R	100
SS-004	Prolactin	ATGAAAGGATCATTGCTGTTG CTCCTCGTGTCGAACCTTCTG CTTTGCCAGTCCGTAGCCCCC	39	MKGSLLLLL VSNLLCQS VAP	101
SS-005	Albumin	ATGAAATGGGTGACGTTTCATC TCACTGTTGTTTTTGTTCCTCGT CCGCCTACTCCAGGGGAGTAT TCCGCCGA	40	MKWVTFISL LFLFSSAYS RG VFRR	102
SS-006	HMMSP38	ATGTGGTGGCGGCTCTGGTGG CTGCTCCTGTTGCTCCTCTTGC	41	MWWRLWW LLLLLLLLP	103

		TGTGGCCCATGGTGTGGGCA		MWA	
MLS-001	ornithine carbamoyltransferase	TGCTCTTTAACCTCCGCATCCT GTTGAATAACGCTGCGTTCCG AAATGGGCATAACTTCATGGT ACGCAACTTCAGATGCGGCCA GCCACTCCAG	42	MLFNLRILL NNAAFRNG HNFMVRNF RCGQPLQ	104
MLS-002	Cytochrome C Oxidase subunit 8A	ATGTCCGTCCTTGACACCCCTG CTCTTGAGAGGGCTGACGGGG TCCGCTAGACGCCTGCCGGTA CCGCGAGCGAAGATCCACTCC CTG	43	MSVLTPLLL RGLTGSARR LPVPRAKIH SL	105
MLS-003	Cytochrome C Oxidase subunit 8A	ATGAGCGTGCTCACTCCGTTG CTTCTTCGAGGGCTTACGGGA TCGGCTCGGAGGTTGCCCGTC CCGAGAGCGAAGATCCATTCTG TTG	44	MSVLTPLLL RGLTGSARR LPVPRAKIH SL	106
SS-007	Type III, bacterial	TGACAAAAATAACTTTATCTC CCCAGAAATTTAGAAATCCAAA AACAGGAAACCACACTACTA AAAGAAAAATCAACCGAGAA AAATTCTTTAGCAAAAAGTAT TCTCGCAGTAAAAATCACTTC ATCGAATTAAGGTCAAAATTA TCGGAACGTTTTATTTTCGCAT AAGAACACT	45	MVTKITLSP QNFRIOKQE TLLKEKST EKNSLAKSI LAVKNHFIE LRSKLSERFI SHKNT	107
SS-008	Viral	ATGCTGAGCTTTGTGGATACC CGCACCTGCTGCTGCTGGCG GTGACCAGCTGCCTGGCGACC TGCCAG	46	MLSFVDTRT LLLAVTSC LATCQ	108
SS-009	viral	ATGGGCAGCAGCCAGGCGCC GCGCATGGGCAGCGTGGGCG GCCATGGCCTGATGGCGCTGC TGATGGCGGGCCTGATTCTGC CGGGCATTCTGGCG	47	MGSSQAPR MGSVGGHG LMALLMAG LILPGILA	109
SS-010	Viral	ATGGCGGGCATTTTTTATTTTC TGTTTAGCTTTCTGTTTGGCAT TTGCGAT	48	MAGIFYFLF SFLFGICD	110
SS-011	Viral	ATGGAAAACCGCCTGCTGCGC GTGTTTCTGGTGTGGGCGGCG CTGACCATGGATGGCGCGAGC GCG	49	MENRLLRV FLVWAALT MDGASA	111
SS-012	Viral	ATGGCGCGCCAGGGCTGCTTT GGCAGCTATCAGGTGATTAGC CTGTTTACCTTTGCGATTGGC GTGAACCTGTGCCTGGGC	50	MARQGCFC SYQVISLFTF AIGVNLCLG	112
SS-013	<i>Bacillus</i>	ATGAGCCGCTGCCGGTGCTG CTGCTGCTGCAGCTGCTGGTG CGCCCGGGCCTGCAG	51	MSRLPVLLL LQLLVRPGL Q	113
SS-014	<i>Bacillus</i>	ATGAAACAGCAGAAACGCCT GTATGCGCGCCTGCTGACCT	52	MKQQKRLY ARLLTLLFA	114

		GCTGTTTGCGCTGATTTTTCTG CTGCCGCATAGCAGCGCGAGC GCG		LIFLLPHSSA SA	
SS-015	Secretion signal	ATGGCGACGCCGCTGCCTCCG CCCTCCCCGCGGCACCTGCGG CTGCTGCGGCTGCTGCTCTCC GCCCTCGTCCTCGGC	53	MATPLPPPS PRHLRLLRL LLSG	115
SS-016	Secretion signal	ATGAAGGCTCCGGGTCGGCTC GTGCTCATCATCCTGTGCTCC GTGGTCTTCTCT	54	MKAPGRLV LIILCSVVFS	116
SS-017	Secretion signal	ATGCTTCAGCTTTGGAACTT GTTCTCCTGTGCGGCGTGCTC ACT	55	MLQLWKLL CGVLT	117
SS-018	Secretion signal	ATGCTTTATCTCCAGGGTTGG AGCATGCCTGCTGTGGCA	56	MLYLQGWS MPAVA	118
SS-019	Secretion signal	ATGGATAACGTGCAGCCGAA AATAAAACATCGCCCCTTCTG CTTCAGTGTGAAAGGCCACGT GAAGATGCTGCGGCTGGATAT TATCAACTCACTGGTAACAAC AGTATTCATGCTCATCGTATC TGTGTTGGCACTGATACCA	57	MDNVQPKI KHRPFCFSV KGHVKMLR LDIINSLVTT VFMLIVSVL ALIP	119
SS-020	Secretion signal	ATGCCCTGCCTAGACCAACAG CTCACTGTTTCATGCCCTACCCT GCCCTGCCCAGCCCTCCTCTC TGGCCTTCTGCCAAGTGGGGT TCTTAACAGCA	58	MPCLDQQL TVHALPCPA QPSSLAFCQ VGFLTA	120
SS-021	Secretion signal	ATGAAAACCTTGTTCAATCCA GCCCCTGCCATTGCTGACCTG GATCCCCAGTTCTACACCCTC TCAGATGTGTTCTGCTGCAAT GAAAGTGAGGCTGAGATTTTA ACTGGCCTCACGGTGGGCAGC GCTGCAGATGCT	59	MKTLEFPAP AIADLDPQF YTLSDFCC NESEAEILT GLTVGSAA DA	121
SS-022	Secretion signal	ATGAAGCCTCTCCTTGTTGTG TTTGTCTTTCTTTTCCTTTGGG ATCCAGTGCTGGCA	60	MKPLLVVF VFLFLWDPV LA	122
SS-023	Secretion signal	ATGTCCTGTTCCCTAAAGTTT ACTTTGATTGTAATTTTTTTTT ACTGTTGGCTTTCATCCAGC	61	MSCSLKFTL IVIFFTCTLS SS	123
SS-024	Secretion signal	ATGGTTCTTACTAAACCTCTTC AAAGAAATGGCAGCATGATG AGCTTTGAAAATGTGAAAGAA AAGAGCAGAGAAGGAGGGCC CCATGCACACACACCCGAAGA AGAATTGTGTTTCGTGGTAAC ACACTACCCTCAGGTTTCAGAC CACACTCAACCTGTTTTTCCAT ATATTCAAGGTTCTTACTCAA CCACTTTCCTTCTGTGGGGT	62	MVLTKPLQ RNGSMMSF ENVKEKSRE GGPHAHTPE EELCFVVTH TPQVQTTLN LFFHIFKVL TQPLSLLWG	124

SS-025	Secretion signal	ATGGCCACCCCGCCATTCCGG CTGATAAGGAAGATGTTTTCC TTCAAGGTGAGCAGATGGATG GGGCTTGCCTGCTTCCGGTCC CTGGCGGCATCC	63	MATPPFRLI RKMFSEKVS RWMGLACF RSLAAS	125
SS-026	Secretion signal	ATGAGCTTTTTTCCAACCTCCTG ATGAAAAGGAAGGAACATCAT TCCCTTGGTGGTGTTCATGAC TGTGGCGGCGGGTGGAGCCTC ATCT	64	MSFFQLLM KRKELIPLV VFMTVAAG GASS	126
SS-027	Secretion signal	ATGGTCTCAGCTCTGCGGGGA GCACCCCTGATCAGGGTGCAC TCAAGCCCTGTTTCTTCTCCTT CTGTGAGTGGACCACGGAGGC TGGTGAGCTGCCTGTCATCCC AAAGCTCAGCTCTGAGC	65	MVSALRGA PLIRVHSSPV SSPSVSGPA ALVSLSSQ SSALS	127
SS-028	Secretion signal	ATGATGGGGTCCCCAGTGAGT CATCTGCTGGCCGGCTTCTGT GTGTGGGTCGTCTTGGGC	66	MMGSPVSH LLAGFCVW VVLG	128
SS-029	Secretion signal	ATGGCAAGCATGGCTGCCGTG CTCACCTGGGCTCTGGCTCTT CTTTCAGCGTTTTTCGGCCACC CAGGCA	67	MASMAAVL TWALALLS AFSATQA	129
SS-030	Secretion signal	ATGGTGCTCATGTGGACCAGT GGTGACGCCTTCAAGACGGCC TACTTCTGCTGAAGGGTGCC CCTCTGCAGTTCTCCGTGTGC GGCCTGCTGCAGGTGCTGGTG GACCTGGCCATCCTGGGGCAG GCCTACGCC	68	MVLMWTSG DAFKTAYFL LKGAPLQFS VCGLLQVL VDLAILGQA TA	130
SS-031	Secretion signal	ATGGATTTTGTGCTGAGGCC ATCGGAGGCGTCTGCGGTGTT GCTGTGGGCTACCCCTGGAC ACGGTGAAGGTCAGGATCCA GACGGAGCCAAAGTACACAG GCATCTGGCACTGCGTCCGGG ATACGTATCACCGAGAGCGCG TGTGGG GCTTCTACCGGGGCCTCTCGC TGCCCGTGTGCACGGTGTCCC TGGTATCTTCC	69	MDFVAGAI GGVCGVAV GYPLDTVK VRIQTEPLY TGIWHCVR DTYHRERV WGFYRGLS LPVCTVSLV SS	131
SS-032	Secretion signal	ATGGAGAAGCCCCTCTTCCCA TTAGTGCCTTTGCATTGGTTTG GCTTTGGCTACACAGCACTGG TTGTTTCTGGTGGGATCGTTG GCTATGTAAAAACAGGCAGC GTGCCGTCCCTGGCTGCAGGG CTGCTCTTCGGCAGTCTAGCC	70	MEKPLFPLV PLHWFGFG YTALVVS GIVGYVKTG SVPSLAAGL LFGSLA	132
SS-033	Secretion signal	ATGGGTCTGCTCCTTCCCCTG GCACTCTGCATCCTAGTCCTG	71	MGLLLPLAL CILVLC	133

		TGC			
SS-034	Secretion signal	ATGGGGATCCAGACGAGCCCC GTCCTGCTGGCCTCCCTGGGG GTGGGGCTGGTCACTCTGCTC GGCCTGGCTGTGGGC	72	MGIQTSPVL LASLGVGLV TLLGLAVG	134
SS-035	Secretion signal	ATGTGCGACCTGCTACTACTG GGCCTGATTGGGGGCCTGACT CTCTTACTGCTGCTGACGCTG CTAGCCTTTGCC	73	MSDLLLLGL IGGLTLLLL LTLLAFA	135
SS-036	Secretion signal	ATGGAGACTGTGGTGATTGTT GCCATAGGTGTGCTGGCCACC ATGTTTCTGGCTTCGTTTGAG CCTTGGTGCTGGTTTGAGGC AG	74	METVVIVAI GVLATIFLA SFAALVLC RQ	136
SS-037	Secretion signal	ATGCGCGGCTCTGTGGAGTGC ACCTGGGGTTGGGGGCACTGT GCCCCAGCCCCCTGCTCCTT TGGACTCTACTTCTGTTTGCA GCCCCATTGGCCTGCTGGGG	75	MAGSVECT WGWGHCAP SPLLLWTL LFAAPFGLL G	137
SS-038	Secretion signal	ATGATGCCGTCCCGTACCAAC CTGGCTACTGGAATCCCCAGT AGTAAAGTGAAATATTCAAGG CTCTCCAGCACAGACGATGGC TACATTGACCTTCAGTTTAAG AAAACCCCTCCTAAGATCCCT TATAAGGCCATCGCACTTGCC ACTGTGCTGTTTTTGATTGGC GCC	76	MMPSPvTNL ATGIPSSKV KYSRLSSTD DGYIDLQFK KTPPKIPYK AIALATVLF LIGA	138
SS-039	Secretion signal	ATGGCCCTGCCCCAGATGTGT GACGGGAGCCACTTGGCCTCC ACCCTCCGCTATTGCATGACA GTCAGCGGCACAGTGGTTCTG GTGGCCGGGACGCTCTGCTTC GCT	77	MALPQMCD GSHLASTLR YCMTVSGT VVLVAGTL CFA	139
SS-041	Vrg-6	TGAAAAAGTGTTTCGTTGCTG CCGGCATCGGCGCTGCCGGAC TCATGCTCTCCAGCGCCGCCA	78	MKKWFVAA GIGAGLLML SSAA	140
SS-042	PhoA	ATGAAACAGAGCACCATTGCG CTGGCGCTGCTGCCGCTGCTG TTTACCCCGGTGACCAAAGCG	79	MKQSTIALA LLPLLFTPV TKA	141
SS-043	OmpA	ATGAAAAAAACCGCGATTGC GATTGCGGTGGCGCTGGCGGG CTTTGCGACCGTGGCGCAGGC G	80	MKKTAIAIA VALAGFAT VAQA	142
SS-044	STI	ATGAAAAAACTGATGCTGGCG ATTTTTTTTAGCGTGCTGAGCT TTCCGAGCTTTAGCCAGAGC	81	MKKLMLAI FFSVLSFSPF SQS	143
SS-045	STII	ATGAAAAAAAACATTGCGTTT CTGCTGGCGAGCATGTTTGTG TTTAGCATTGCGACCAACGCG	82	MKKNIAFL ASMFVFSIA TNAYA	144

		TATGCG			
SS-046	Amylase	ATGTTTGCAGAAACGCTTTAAA ACCAGCCTGCTGCCGCTGTTT GCGGGCTTTCTGCTGCTGTTT ATCTGGTGCTGGCGGGCCCGG CGGCGGCGAGC	83	MFAKRFKTS LLPLFAGFL LLFHLVLAG PAAAS	145
SS-047	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCGCGGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	84	MRFPSIFTA VLFAASSAL A	146
SS-048	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCACCGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	85	MRFPSIFTT VLFAASSAL A	147
SS-049	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCAGCGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	86	MRFPSIFTSV LFAASSALA	148
SS-050	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCCATGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	87	MRFPSIFTH VLFAASSAL A	149
SS-051	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCATTGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	88	MRFPSIFTIV LFAASSALA	150
SS-052	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCTTTGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	89	MRFPSIFTFV LFAASSALA	151
SS-053	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCGAAGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	90	MRFPSIFTE VLFAASSAL A	152
SS-054	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCGGCGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	91	MRFPSIFTG VLFAASSAL A	153
SS-055	Endoglucanase V	ATGCGTTCCTCCCCCTCCTCC GCTCCGCCGTTGTGGCCGCC TGCCGGTGTTGGCCCTTGCC	92	MRSSPLLRS AVVAALPV LALA	154
SS-056	Secretion signal	ATGGGCGCGGCGGCCGTGCGC TGGCACTTGTCGTGCTGCTG GCCCTGGGCACACGCGGGCG GCTG	93	MGAAAVR WHLCVLLA LGTRGRL	155
SS-057	Fungal	ATGAGGAGCTCCCTTGTGCTG TTCTTTGTCTCTGCGTGGACG GCCTTGGCCAG	94	MRSSLVLF VSAWTALA	156
SS-058	Fibronectin	ATGCTCAGGGGTCCGGGACCC GGGCGGCTGCTGCTGCTAGCA GTCCTGTGCCTGGGGACATCG GTGCGCTGCACCGAAACCGGG AAGAGCAAGAGG	95	MLRGPGPG RLLLAVLC LGTSVRCTE TGKSKR	157
SS-059	Fibronectin	ATGCTTAGGGGTCCGGGGCCC GGGCTGCTGCTGCTGGCCGTC CAGCTGGGGACAGCGGTGCCC TCCACG	96	MLRGPGPG LLLLAVQCL GTAVPSTGA	158
SS-	Fibronectin	ATGCGCCGGGGGGCCCTGACC	97	MRRGALTG	159

060		GGGCTGCTCCTGGTCCTGTGC CTGAGTGTGTGCTACGTGCA GCCCCCTCTGCAACAAGCAAG AAGCGCAGG		LLVLCLSV VLRAAPSAT SKKRR	

[00335] In table 9, SS is secretion signal and MLS is mitochondrial leader signal. The primary constructs or mmRNA of the present invention may be designed to encode any of the signal peptide sequences of SEQ ID NOs 98-159, or fragments or variants thereof. These sequences may be included at the beginning of the polypeptide coding region, in the middle or at the terminus or alternatively into a flanking region. Further, any of the polynucleotide primary constructs of the present invention may also comprise one or more of the sequences defined by SEQ ID NOs 36-97. These may be in the first region or either flanking region.

[00336] Additional signal peptide sequences which may be utilized in the present invention include those taught in, for example, databases such as those found at <http://www.signalpeptide.de/> or <http://proline.bic.nus.edu.sg/spdb/>. Those described in US Patents 8,124,379; 7,413,875 and 7,385,034 are also within the scope of the invention and the contents of each are incorporated herein by reference in their entirety.

[00337] In one embodiment, the modified nucleic acid molecules may include a nucleic acid sequence encoding a nuclear localization signal (NLS) and/or a nuclear export signal (NES). In one aspect, a modified nucleic acid molecules may include a nucleic acid sequence encoding a nuclear localization signal (NLS). The modified nucleic acid molecules encoding a NLS would be able to traffic a polypeptide into the nucleus and deliver a survival or death signal to the nuclear microenvironment. In another aspect, the modified nucleic acid molecules may include a nucleic acid sequence encoding a nuclear export signal such as NES1 and/or NES2. As a nonlimiting example, the modified nucleic acid molecules may encode a NES1, NES2 and a NLS signal and an oncology related polypeptide or a scrambled sequence which is not translatable in order to interact with HIF1-alpha to alter the transcriptome of the cancer cells.

Target Selection

[00338] According to the present invention, the primary constructs comprise at least a first region of linked nucleosides encoding at least one polypeptide of interest. The polypeptides of interest or "targets" or proteins and peptides of the present invention are

listed in U.S. Provisional Patent Application No 61/618,862, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Biologies; U.S. Provisional Patent Application No 61/681,645, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Biologies; U.S. Provisional Patent Application No 61/737,130, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Biologies; U.S. Provisional Patent Application No 61/618,866, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional Patent Application No 61/681,647, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional Patent Application No 61/737,134, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional Patent Application No 61/618,868, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/681,648, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/737,135, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/618,870, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/681,649, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/737,139, filed December 14, 2012, Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/618,873, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/681,650, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/737,147, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/618,878, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins; U.S. Provisional Patent Application No 61/681,654, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins; U.S. Provisional Patent Application No 61/737,152, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Plasma

Membrane Proteins; U.S. Provisional Patent Application No 61/618,885, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/681,658, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/737,155, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/618,896, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/668,157, filed July 5, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/681,661, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/737,160, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/618,911, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/681,667, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/737,168, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/618,922, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/681,675, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/737,174, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/618,935, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/681,687, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,184, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/618,945, filed April 2, 2012, entitled Modified

Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/681,696, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,191, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/618,953, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/681,704, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,203, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/753,661, entitled Polynucleotides For The Alteration Of Cellular Phenotypes And Microenvironments; International Application No PCT/US2013/030062, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Biologies and Proteins Associated with Human Disease; International Application No PCT/US2013/030063, filed March 9, 2013, entitled Modified Polynucleotides; International Application No. PCT/US2013/030064, entitled Modified Polynucleotides for the Production of Secreted Proteins; International Application No PCT/US2013/030059, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Membrane Proteins; International Application No. PCT/US2013/030066, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; International Application No. PCT/US2013/030067, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Nuclear Proteins; International Application No. PCT/US2013/030060, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins; International Application No. PCT/US2013/030061, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; International Application No. PCT/US2013/030068, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; International Application No. PCT/US2013/030070, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides; and International Application No.

PCT/US20 13/03 1821, filed March 15, 2013, entitled In Vivo Production of Proteins, the contents of each of which are herein incorporated by reference in their entireties.

Protein Cleavage Signals and Sites

[00339] In one embodiment, the polypeptides of the present invention may include at least one protein cleavage signal containing at least one protein cleavage site. The protein cleavage site may be located at the N-terminus, the C-terminus, at any space between the N- and the C- termini such as, but not limited to, half-way between the N- and C-termini, between the N-terminus and the half way point, between the half way point and the C-terminus, and combinations thereof.

[00340] The polypeptides of the present invention may include, but is not limited to, a proprotein convertase (or prohormone convertase), thrombin or Factor Xa protein cleavage signal. Proprotein convertases are a family of nine proteinases, comprising seven basic amino acid-specific subtilisin-like serine proteinases related to yeast kexin, known as prohormone convertase 1/3 (PC 1/3), PC2, furin, PC4, PC5/6, paired basic amino-acid cleaving enzyme 4 (PACE4) and PC7, and two other subtilases that cleave at non-basic residues, called subtilisin kexin isozyme 1 (SKI-1) and proprotein convertase subtilisin kexin 9 (PCSK9). Non-limiting examples of protein cleavage signal amino acid sequences are listing in Table 10. In Table 10, "X" refers to any amino acid, "n" may be 0, 2, 4 or 6 amino acids and "*" refers to the protein cleavage site. In Table 10, SEQ ID NO: 162 refers to when n=4 and SEQ ID NO: 163 refers to when n=6.

Table 10. Protein Cleavage Site Sequences

Protein Cleavage Signal	Amino Acid Cleavage Sequence	SEQ ID NO
Proprotein convertase	R-X-X-R*	160
	R-X-K/R-R*	161
	K/R-X _n -K/R*	162 or 163
Thrombin	L-V-P-R*-G-S	164
	L-V-P-R*	165
	A/F/G/I/L/T/V/M-A/F/G/I/L/T/V/W/A-P-R*	166
Factor Xa	I-E-G-R*	167
	I-D-G-R*	168
	A-E-G-R*	169
	A/F/G/I/L/T/V/M-D/E-G-R*	170

[00341] In one embodiment, the primary constructs, modified nucleic acids and the mmRNA of the present invention may be engineered such that the primary construct, modified nucleic acid or mmRNA contains at least one encoded protein cleavage signal. The encoded protein cleavage signal may be located before the start codon, after the start codon, before the coding region, within the coding region such as, but not limited to, half way in the coding region, between the start codon and the half way point, between the half way point and the stop codon, after the coding region, before the stop codon, between two stop codons, after the stop codon and combinations thereof.

[00342] In one embodiment, the primary constructs, modified nucleic acids or mmRNA of the present invention may include at least one encoded protein cleavage signal containing at least one protein cleavage site. The encoded protein cleavage signal may include, but is not limited to, a proprotein convertase (or prohormone convertase), thrombin and/or Factor Xa protein cleavage signal. One of skill in the art may use Table 5 above or other known methods to determine the appropriate encoded protein cleavage signal to include in the primary constructs, modified nucleic acids or mmRNA of the present invention. For example, starting with the signal of Table 10 and considering the codons of Table 5 one can design a signal for the primary construct which can produce a protein signal in the resulting polypeptide.

[00343] In one embodiment, the polypeptides of the present invention include at least one protein cleavage signal and/or site.

[00344] As a non-limiting example, U.S. Pat. No. 7,374,930 and U.S. Pub. No. 20090227660, herein incorporated by reference in their entireties, use a furin cleavage site to cleave the N-terminal methionine of GLP-1 in the expression product from the Golgi apparatus of the cells. In one embodiment, the polypeptides of the present invention include at least one protein cleavage signal and/or site with the proviso that the polypeptide is not GLP-1.

[00345] In one embodiment, the primary constructs, modified nucleic acids or mmRNA of the present invention includes at least one encoded protein cleavage signal and/or site.

[00346] In one embodiment, the primary constructs, modified nucleic acid or mmRNA of the present invention includes at least one encoded protein cleavage signal and/or site

with the proviso that the primary construct, modified nucleic acid or mmRNA does not encode GLP-1.

[00347] In one embodiment, the primary constructs, modified nucleic acid or mmRNA of the present invention may include more than one coding region. Where multiple coding regions are present in the primary construct, modified nucleic acid or mmRNA of the present invention, the multiple coding regions may be separated by encoded protein cleavage sites. As a non-limiting example, the primary construct, modified nucleic acid or mmRNA may be signed in an ordered pattern. On such pattern follows AXBY form where A and B are coding regions which may be the same or different coding regions and/or may encode the same or different polypeptides, and X and Y are encoded protein cleavage signals which may encode the same or different protein cleavage signals. A second such pattern follows the form AXBYZ where A and B are coding regions which may be the same or different coding regions and/or may encode the same or different polypeptides, and X, Y and Z are encoded protein cleavage signals which may encode the same or different protein cleavage signals. A third pattern follows the form ABXCY where A, B and C are coding regions which may be the same or different coding regions and/or may encode the same or different polypeptides, and X and Y are encoded protein cleavage signals which may encode the same or different protein cleavage signals.

[00348] In one embodiment, the polypeptides, primary constructs, modified nucleic acids and mmRNA can also contain sequences that encode protein cleavage sites so that the polypeptides, primary constructs, modified nucleic acids and mmRNA can be released from a carrier region or a fusion partner by treatment with a specific protease for said protein cleavage site.

[00349] Table 11 is a non-exhaustive listing of miRs and miR binding sites (miR BS) and their sequences which may be used with the present invention.

Table 11. Mirs and mir binding sites

microRNA	mir SEQ ID	MIR BS SEQ ID	microRNA	mir SEQ ID	MIR BS SEQ ID
hsa-let-7a-2-3p	171	1192	hsa-miR-4471	2213	3234

hsa-let-7a-3p	172	1193	hsa-miR-4472	2214	3235
hsa-let-7a-5p	173	1194	hsa-miR-4473	2215	3236
hsa-let-7b-3p	174	1195	hsa-miR-4474-3p	2216	3237
hsa-let-7b-5p	175	1196	hsa-miR-4474-5p	2217	3238
hsa-let-7c	176	1197	hsa-miR-4475	2218	3239
hsa-let-7d-3p	177	1198	hsa-miR-4476	2219	3240
hsa-let-7d-5p	178	1199	hsa-miR-4477a	2220	3241
hsa-let-7e-3p	179	1200	hsa-miR-4477b	2221	3242
hsa-let-7e-5p	180	1201	hsa-miR-4478	2222	3243
hsa-let-7f-1-3p	181	1202	hsa-miR-4479	2223	3244
hsa-let-7f-2-3p	182	1203	hsa-miR-448	2224	3245
hsa-let-7f-5p	183	1204	hsa-miR-4480	2225	3246
hsa-let-7g-3p	184	1205	hsa-miR-448 1	2226	3247
hsa-let-7g-5p	185	1206	hsa-miR-4482-3p	2227	3248
hsa-let-7i-3p	186	1207	hsa-miR-4482-5p	2228	3249
hsa-let-7i-5p	187	1208	hsa-miR-4483	2229	3250
hsa-miR- 1	188	1209	hsa-miR-4484	2230	3251
hsa-miR- 100-3p	189	1210	hsa-miR-4485	2231	3252
hsa-miR- 100-5p	190	121 1	hsa-miR-448 6	2232	3253
hsa-miR- 10 l-3p	191	1212	hsa-miR-4487	2233	3254
hsa-miR- 10 l-5p	192	1213	hsa-miR-4488	2234	3255
hsa-miR- 103a-2-5p	193	1214	hsa-miR-4489	2235	3256
hsa-miR- 103a-3p	194	1215	hsa-miR-4490	2236	3257
hsa-miR- 103b	195	1216	hsa-miR-4491	2237	3258
hsa-miR- 105-3p	196	1217	hsa-miR-4492	2238	3259
hsa-miR- 105-5p	197	1218	hsa-miR-4493	2239	3260
hsa-miR- 106a-3p	198	1219	hsa-miR-4494	2240	3261
hsa-miR- 106a-5p	199	1220	hsa-miR-4495	2241	3262
hsa-miR- 106b-3p	200	1221	hsa-miR-4496	2242	3263
hsa-miR- 106b-5p	201	1222	hsa-miR-4497	2243	3264
hsa-miR- 107	202	1223	hsa-miR-4498	2244	3265
hsa-miR- 10a-3p	203	1224	hsa-miR-4499	2245	3266
hsa-miR- 10a-5p	204	1225	hsa-miR-449a	2246	3267

hsa-miR-10b-3p	205	1226	hsa-miR-449b-3p	2247	3268
hsa-miR-10b-5p	206	1227	hsa-miR-449b-5p	2248	3269
hsa-miR-1 178-3p	207	1228	hsa-miR-449c-3p	2249	3270
hsa-miR-1 178-5p	208	1229	hsa-miR-449c-5p	2250	3271
hsa-miR-1179	209	1230	hsa-miR-4500	2251	3272
hsa-miR-1180	210	1231	hsa-miR-4501	2252	3273
hsa-miR-1181	2 11	1232	hsa-miR-4502	2253	3274
hsa-miR-1182	212	1233	hsa-miR-4503	2254	3275
hsa-miR-1183	213	1234	hsa-miR-4504	2255	3276
hsa-miR-1184	214	1235	hsa-miR-4505	2256	3277
hsa-miR-1185-l-3p	215	1236	hsa-miR-4506	2257	3278
hsa-miR-1185-2-3p	216	1237	hsa-miR-4507	2258	3279
hsa-miR-1185-5p	217	1238	hsa-miR-4508	2259	3280
hsa-miR-1193	218	1239	hsa-miR-4509	2260	3281
hsa-miR-1 197	219	1240	hsa-miR-450a-3p	2261	3282
hsa-miR-1200	220	1241	hsa-miR-450a-5p	2262	3283
hsa-miR-1202	221	1242	hsa-miR-450b-3p	2263	3284
hsa-miR-1203	222	1243	hsa-miR-450b-5p	2264	3285
hsa-miR-1204	223	1244	hsa-miR-4510	2265	3286
hsa-miR-1205	224	1245	hsa-miR-451 1	2266	3287
hsa-miR-1206	225	1246	hsa-miR-4512	2267	3288
hsa-miR-1207-3p	226	1247	hsa-miR-4513	2268	3289
hsa-miR-1207-5p	227	1248	hsa-miR-4514	2269	3290
hsa-miR-1208	228	1249	hsa-miR-4515	2270	3291
hsa-miR-122-3p	229	1250	hsa-miR-4516	2271	3292
hsa-miR-1224-3p	230	1251	hsa-miR-4517	2272	3293
hsa-miR-1224-5p	231	1252	hsa-miR-4518	2273	3294
hsa-miR-1225-3p	232	1253	hsa-miR-4519	2274	3295
hsa-miR-1225-5p	233	1254	hsa-miR-45 1a	2275	3296
hsa-miR-122-5p	234	1255	hsa-miR-451b	2276	3297
hsa-miR-1226-3p	235	1256	hsa-miR-4520a-3p	2277	3298
hsa-miR-1226-5p	236	1257	hsa-miR-4520a-5p	2278	3299
hsa-miR-1227-3p	237	1258	hsa-miR-4520b-3p	2279	3300

hsa-miR- 1227-5p	238	1259	hsa-miR-4520b-5p	2280	3301
hsa-miR- 1228-3p	239	1260	hsa-miR-4521	2281	3302
hsa-miR- 1228-5p	240	1261	hsa-miR-4522	2282	3303
hsa-miR- 1229-3p	241	1262	hsa-miR-4523	2283	3304
hsa-miR- 1229-5p	242	1263	hsa-miR-452-3p	2284	3305
hsa-miR- 1231	243	1264	hsa-miR-4524a-3p	2285	3306
hsa-miR- 1233- 1-5p	244	1265	hsa-miR-4524a-5p	2286	3307
hsa-miR- 1233-3p	245	1266	hsa-miR-4524b-3p	2287	3308
hsa-miR- 1234-3p	246	1267	hsa-miR-4524b-5p	2288	3309
hsa-miR- 1234-5p	247	1268	hsa-miR-4525	2289	3310
hsa-miR- 1236-3p	248	1269	hsa-miR-452-5p	2290	331 1
hsa-miR- 1236-5p	249	1270	hsa-miR-4526	2291	3312
hsa-miR- 1237-3p	250	1271	hsa-miR-4527	2292	3313
hsa-miR- 1237-5p	251	1272	hsa-miR-4528	2293	3314
hsa-miR- 1238-3p	252	1273	hsa-miR-4529-3p	2294	3315
hsa-miR- 1238-5p	253	1274	hsa-miR-4529-5p	2295	3316
hsa-miR- 1243	254	1275	hsa-miR-4530	2296	3317
hsa-miR- 124-3p	255	1276	hsa-miR-453 1	2297	3318
hsa-miR- 1244	256	1277	hsa-miR-4532	2298	3319
hsa-miR- 1245a	257	1278	hsa-miR-4533	2299	3320
hsa-miR- 1245b-3p	258	1279	hsa-miR-4534	2300	3321
hsa-miR- 1245b-5p	259	1280	hsa-miR-4535	2301	3322
hsa-miR- 124-5p	260	1281	hsa-miR-4536-3p	2302	3323
hsa-miR- 1246	261	1282	hsa-miR-4536-5p	2303	3324
hsa-miR- 1247-3p	262	1283	hsa-miR-4537	2304	3325
hsa-miR- 1247-5p	263	1284	hsa-miR-453 8	2305	3326
hsa-miR- 1248	264	1285	hsa-miR-4539	2306	3327
hsa-miR- 1249	265	1286	hsa-miR-4540	2307	3328
hsa-miR- 1250	266	1287	hsa-miR-454-3p	2308	3329
hsa-miR- 1251	267	1288	hsa-miR-454-5p	2309	3330
hsa-miR- 1252	268	1289	hsa-miR-455-3p	2310	3331
hsa-miR- 1253	269	1290	hsa-miR-455-5p	231 1	3332
hsa-miR- 1254	270	1291	hsa-miR-4632-3p	2312	3333

hsa-miR- 1255a	271	1292	hsa-miR-4632-5p	2313	3334
hsa-miR- 1255b-2-3p	272	1293	hsa-miR-4633-3p	2314	3335
hsa-miR- 1255b-5p	273	1294	hsa-miR-463 3-5p	2315	3336
hsa-miR- 1256	274	1295	hsa-miR-4634	2316	3337
hsa-miR- 1257	275	1296	hsa-miR-463 5	2317	3338
hsa-miR- 1258	276	1297	hsa-miR-463 6	2318	3339
hsa-miR- 125a-3p	277	1298	hsa-miR-463 7	2319	3340
hsa-miR- 125a-5p	278	1299	hsa-miR-463 8-3p	2320	3341
hsa-miR- 125b- 1-3p	279	1300	hsa-miR-463 8-5p	2321	3342
hsa-miR- 125b-2-3p	280	1301	hsa-miR-463 9-3p	2322	3343
hsa-miR- 125b-5p	281	1302	hsa-miR-463 9-5p	2323	3344
hsa-miR- 1260a	282	1303	hsa-miR-4640-3p	2324	3345
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hsa-miR- 1261	284	1305	hsa-miR-4641	2326	3347
hsa-miR- 1262	285	1306	hsa-miR-4642	2327	3348
hsa-miR- 1263	286	1307	hsa-miR-4643	2328	3349
hsa-miR- 126-3p	287	1308	hsa-miR-4644	2329	3350
hsa-miR- 1264	288	1309	hsa-miR-4645-3p	2330	3351
hsa-miR- 1265	289	1310	hsa-miR-4645-5p	2331	3352
hsa-miR- 126-5p	290	131 1	hsa-miR-4646-3p	2332	3353
hsa-miR- 1266	291	1312	hsa-miR-4646-5p	2333	3354
hsa-miR- 1267	292	1313	hsa-miR-4647	2334	3355
hsa-miR- 1268a	293	1314	hsa-miR-4648	2335	3356
hsa-miR- 1268b	294	1315	hsa-miR-4649-3p	2336	3357
hsa-miR- 1269a	295	1316	hsa-miR-4649-5p	2337	3358
hsa-miR- 1269b	296	1317	hsa-miR-4650-3p	2338	3359
hsa-miR- 1270	297	1318	hsa-miR-4650-5p	2339	3360
hsa-miR- 1271-3p	298	1319	hsa-miR-465 1	2340	3361
hsa-miR- 1271-5p	299	1320	hsa-miR-4652-3p	2341	3362
hsa-miR- 1272	300	1321	hsa-miR-4652-5p	2342	3363
hsa-miR- 1273 a	301	1322	hsa-miR-4653-3p	2343	3364
hsa-miR- 1273c	302	1323	hsa-miR-4653-5p	2344	3365
hsa-miR- 1273 d	303	1324	hsa-miR-4654	2345	3366

hsa-miR- 1273e	304	1325	hsa-miR-4655-3p	2346	3367
hsa-miR- 1273 f	305	1326	hsa-miR-4655-5p	2347	3368
hsa-miR- 1273g-3p	306	1327	hsa-miR-4656	2348	3369
hsa-miR- 1273g-5p	307	1328	hsa-miR-4657	2349	3370
hsa-miR- 127-3p	308	1329	hsa-miR-4658	2350	3371
hsa-miR- 1275	309	1330	hsa-miR-4659a-3p	2351	3372
hsa-miR- 127-5p	310	1331	hsa-miR-4659a-5p	2352	3373
hsa-miR- 1276	311	1332	hsa-miR-4659b-3p	2353	3374
hsa-miR- 1277-3p	312	1333	hsa-miR-4659b-5p	2354	3375
hsa-miR- 1277-5p	313	1334	hsa-miR-466	2355	3376
hsa-miR- 1278	314	1335	hsa-miR-4660	2356	3377
hsa-miR- 1279	315	1336	hsa-miR-466 1-3p	2357	3378
hsa-miR- 128	316	1337	hsa-miR-466 1-5p	2358	3379
hsa-miR- 1281	317	1338	hsa-miR-4662a-3p	2359	3380
hsa-miR- 1282	318	1339	hsa-miR-4662a-5p	2360	3381
hsa-miR- 1283	319	1340	hsa-miR-4662b	2361	3382
hsa-miR- 1284	320	1341	hsa-miR-4663	2362	3383
hsa-miR- 1285-3p	321	1342	hsa-miR-4664-3p	2363	3384
hsa-miR- 1285-5p	322	1343	hsa-miR-4664-5p	2364	3385
hsa-miR- 1286	323	1344	hsa-miR-4665-3p	2365	3386
hsa-miR- 1287	324	1345	hsa-miR-4665-5p	2366	3387
hsa-miR- 1288	325	1346	hsa-miR-4666a-3p	2367	3388
hsa-miR- 1289	326	1347	hsa-miR-4666a-5p	2368	3389
hsa-miR- 1290	327	1348	hsa-miR-4666b	2369	3390
hsa-miR- 1291	328	1349	hsa-miR-4667-3p	2370	3391
hsa-miR- 129- 1-3p	329	1350	hsa-miR-4667-5p	2371	3392
hsa-miR- 1292-3p	330	1351	hsa-miR-4668-3p	2372	3393
hsa-miR- 129-2-3p	331	1352	hsa-miR-4668-5p	2373	3394
hsa-miR- 1292-5p	332	1353	hsa-miR-4669	2374	3395
hsa-miR- 1293	333	1354	hsa-miR-4670-3p	2375	3396
hsa-miR- 1294	334	1355	hsa-miR-4670-5p	2376	3397
hsa-miR- 1295a	335	1356	hsa-miR-4671-3p	2377	3398
hsa-miR- 1295b-3p	336	1357	hsa-miR-4671-5p	2378	3399

hsa-miR-1295b-5p	337	1358	hsa-miR-4672	2379	3400
hsa-miR-129-5p	338	1359	hsa-miR-4673	2380	3401
hsa-miR-1296	339	1360	hsa-miR-4674	2381	3402
hsa-miR-1297	340	1361	hsa-miR-4675	2382	3403
hsa-miR-1298	341	1362	hsa-miR-4676-3p	2383	3404
hsa-miR-1299	342	1363	hsa-miR-4676-5p	2384	3405
hsa-miR-1301	343	1364	hsa-miR-4677-3p	2385	3406
hsa-miR-1302	344	1365	hsa-miR-4677-5p	2386	3407
hsa-miR-1303	345	1366	hsa-miR-4678	2387	3408
hsa-miR-1304-3p	346	1367	hsa-miR-4679	2388	3409
hsa-miR-1304-5p	347	1368	hsa-miR-4680-3p	2389	3410
hsa-miR-1305	348	1369	hsa-miR-4680-5p	2390	341 1
hsa-miR-1306-3p	349	1370	hsa-miR-4681	2391	3412
hsa-miR-1306-5p	350	1371	hsa-miR-4682	2392	3413
hsa-miR-1307-3p	351	1372	hsa-miR-4683	2393	3414
hsa-miR-1307-5p	352	1373	hsa-miR-4684-3p	2394	3415
hsa-miR-130a-3p	353	1374	hsa-miR-4684-5p	2395	3416
hsa-miR-130a-5p	354	1375	hsa-miR-4685-3p	2396	3417
hsa-miR-130b-3p	355	1376	hsa-miR-4685-5p	2397	3418
hsa-miR-130b-5p	356	1377	hsa-miR-4686	2398	3419
hsa-miR-1321	357	1378	hsa-miR-4687-3p	2399	3420
hsa-miR-1322	358	1379	hsa-miR-4687-5p	2400	3421
hsa-miR-1323	359	1380	hsa-miR-4688	2401	3422
hsa-miR-132-3p	360	1381	hsa-miR-4689	2402	3423
hsa-miR-1324	361	1382	hsa-miR-4690-3p	2403	3424
hsa-miR-132-5p	362	1383	hsa-miR-4690-5p	2404	3425
hsa-miR-133a	363	1384	hsa-miR-4691-3p	2405	3426
hsa-miR-133b	364	1385	hsa-miR-4691-5p	2406	3427
hsa-miR-134	365	1386	hsa-miR-4692	2407	3428
hsa-miR-1343	366	1387	hsa-miR-4693-3p	2408	3429
hsa-miR-135a-3p	367	1388	hsa-miR-4693-5p	2409	3430
hsa-miR-135a-5p	368	1389	hsa-miR-4694-3p	2410	3431
hsa-miR-135b-3p	369	1390	hsa-miR-4694-5p	241 1	3432

hsa-miR-135b-5p	370	1391	hsa-miR-4695-3p	2412	3433
hsa-miR-136-3p	371	1392	hsa-miR-4695-5p	2413	3434
hsa-miR-136-5p	372	1393	hsa-miR-4696	2414	3435
hsa-miR-137	373	1394	hsa-miR-4697-3p	2415	3436
hsa-miR-138-l-3p	374	1395	hsa-miR-4697-5p	2416	3437
hsa-miR-138-2-3p	375	1396	hsa-miR-4698	2417	3438
hsa-miR-138-5p	376	1397	hsa-miR-4699-3p	2418	3439
hsa-miR-139-3p	377	1398	hsa-miR-4699-5p	2419	3440
hsa-miR-139-5p	378	1399	hsa-miR-4700-3p	2420	3441
hsa-miR-140-3p	379	1400	hsa-miR-4700-5p	2421	3442
hsa-miR-140-5p	380	1401	hsa-miR-4701-3p	2422	3443
hsa-miR-141-3p	381	1402	hsa-miR-4701-5p	2423	3444
hsa-miR-141-5p	382	1403	hsa-miR-4703-3p	2424	3445
hsa-miR-142-3p	383	1404	hsa-miR-4703-5p	2425	3446
hsa-miR-142-5p	384	1405	hsa-miR-4704-3p	2426	3447
hsa-miR-143-3p	385	1406	hsa-miR-4704-5p	2427	3448
hsa-miR-143-5p	386	1407	hsa-miR-4705	2428	3449
hsa-miR-144-3p	387	1408	hsa-miR-4706	2429	3450
hsa-miR-144-5p	388	1409	hsa-miR-4707-3p	2430	3451
hsa-miR-145-3p	389	1410	hsa-miR-4707-5p	2431	3452
hsa-miR-145-5p	390	141 1	hsa-miR-4708-3p	2432	3453
hsa-miR-1468	391	1412	hsa-miR-4708-5p	2433	3454
hsa-miR-1469	392	1413	hsa-miR-4709-3p	2434	3455
hsa-miR-146a-3p	393	1414	hsa-miR-4709-5p	2435	3456
hsa-miR-146a-5p	394	1415	hsa-miR-4710	2436	3457
hsa-miR-146b-3p	395	1416	hsa-miR-471 1-3p	2437	3458
hsa-miR-146b-5p	396	1417	hsa-miR-471 1-5p	2438	3459
hsa-miR-1470	397	1418	hsa-miR-4712-3p	2439	3460
hsa-miR-1471	398	1419	hsa-miR-4712-5p	2440	3461
hsa-miR-147a	399	1420	hsa-miR-4713-3p	2441	3462
hsa-miR-147b	400	1421	hsa-miR-4713-5p	2442	3463
hsa-miR-148a-3p	401	1422	hsa-miR-4714-3p	2443	3464
hsa-miR-148a-5p	402	1423	hsa-miR-4714-5p	2444	3465

hsa-miR- 148b-3p	403	1424	hsa-miR-4715-3p	2445	3466
hsa-miR- 148b-5p	404	1425	hsa-miR-4715-5p	2446	3467
hsa-miR- 149-3p	405	1426	hsa-miR-4716-3p	2447	3468
hsa-miR- 149-5p	406	1427	hsa-miR-4716-5p	2448	3469
hsa-miR- 150-3p	407	1428	hsa-miR-4717-3p	2449	3470
hsa-miR- 150-5p	408	1429	hsa-miR-4717-5p	2450	3471
hsa-miR- 151 a-3p	409	1430	hsa-miR-4718	2451	3472
hsa-miR- 151 a-5p	410	1431	hsa-miR-4719	2452	3473
hsa-miR- 151 b	411	1432	hsa-miR-4720-3p	2453	3474
hsa-miR- 152	412	1433	hsa-miR-4720-5p	2454	3475
hsa-miR- 153	413	1434	hsa-miR-4721	2455	3476
hsa-miR- 1537	414	1435	hsa-miR-4722-3p	2456	3477
hsa-miR- 1538	415	1436	hsa-miR-4722-5p	2457	3478
hsa-miR- 1539	416	1437	hsa-miR-4723-3p	2458	3479
hsa-miR- 154-3p	417	1438	hsa-miR-4723-5p	2459	3480
hsa-miR- 154-5p	418	1439	hsa-miR-4724-3p	2460	3481
hsa-miR- 155-3p	419	1440	hsa-miR-4724-5p	2461	3482
hsa-miR- 155-5p	420	1441	hsa-miR-4725-3p	2462	3483
hsa-miR- 1587	421	1442	hsa-miR-4725-5p	2463	3484
hsa-miR- 15a-3p	422	1443	hsa-miR-4726-3p	2464	3485
hsa-miR- 15a-5p	423	1444	hsa-miR-4726-5p	2465	3486
hsa-miR- 15b-3p	424	1445	hsa-miR-4727-3p	2466	3487
hsa-miR- 15b-5p	425	1446	hsa-miR-4727-5p	2467	3488
hsa-miR- 16-1-3p	426	1447	hsa-miR-4728-3p	2468	3489
hsa-miR- 16-2-3p	427	1448	hsa-miR-4728-5p	2469	3490
hsa-miR- 16-5p	428	1449	hsa-miR-4729	2470	3491
hsa-miR- 17-3p	429	1450	hsa-miR-4730	2471	3492
hsa-miR- 17-5p	430	1451	hsa-miR-4731-3p	2472	3493
hsa-miR- 181 a-2-3p	431	1452	hsa-miR-4731-5p	2473	3494
hsa-miR- 181 a-3p	432	1453	hsa-miR-4732-3p	2474	3495
hsa-miR- 181 a-5p	433	1454	hsa-miR-4732-5p	2475	3496
hsa-miR- 181b-3p	434	1455	hsa-miR-4733-3p	2476	3497
hsa-miR- 181b-5p	435	1456	hsa-miR-4733-5p	2477	3498

hsa-miR-181c-3p	436	1457	hsa-miR-4734	2478	3499
hsa-miR-181c-5p	437	1458	hsa-miR-4735-3p	2479	3500
hsa-miR-181d	438	1459	hsa-miR-4735-5p	2480	3501
hsa-miR-182-3p	439	1460	hsa-miR-4736	2481	3502
hsa-miR-1825	440	1461	hsa-miR-4737	2482	3503
hsa-miR-182-5p	441	1462	hsa-miR-4738-3p	2483	3504
hsa-miR-1827	442	1463	hsa-miR-4738-5p	2484	3505
hsa-miR-183-3p	443	1464	hsa-miR-4739	2485	3506
hsa-miR-183-5p	444	1465	hsa-miR-4740-3p	2486	3507
hsa-miR-184	445	1466	hsa-miR-4740-5p	2487	3508
hsa-miR-185-3p	446	1467	hsa-miR-4741	2488	3509
hsa-miR-185-5p	447	1468	hsa-miR-4742-3p	2489	3510
hsa-miR-186-3p	448	1469	hsa-miR-4742-5p	2490	351 1
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hsa-miR-187-3p	450	1471	hsa-miR-4743-5p	2492	3513
hsa-miR-187-5p	451	1472	hsa-miR-4744	2493	3514
hsa-miR-188-3p	452	1473	hsa-miR-4745-3p	2494	3515
hsa-miR-188-5p	453	1474	hsa-miR-4745-5p	2495	3516
hsa-miR-18a-3p	454	1475	hsa-miR-4746-3p	2496	3517
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hsa-miR-1908	458	1479	hsa-miR-4748	2500	3521
hsa-miR-1909-3p	459	1480	hsa-miR-4749-3p	2501	3522
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hsa-miR-190a	461	1482	hsa-miR-4750-3p	2503	3524
hsa-miR-190b	462	1483	hsa-miR-4750-5p	2504	3525
hsa-miR-1910	463	1484	hsa-miR-4751	2505	3526
hsa-miR-191 1-3p	464	1485	hsa-miR-4752	2506	3527
hsa-miR-191 1-5p	465	1486	hsa-miR-4753-3p	2507	3528
hsa-miR-1912	466	1487	hsa-miR-4753-5p	2508	3529
hsa-miR-1913	467	1488	hsa-miR-4754	2509	3530
hsa-miR-191-3p	468	1489	hsa-miR-4755-3p	2510	3531

hsa-miR-1914-3p	469	1490	hsa-miR-4755-5p	251 1	3532
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hsa-miR-1915-3p	471	1492	hsa-miR-4756-5p	2513	3534
hsa-miR-1915-5p	472	1493	hsa-miR-4757-3p	2514	3535
hsa-miR-191-5p	473	1494	hsa-miR-4757-5p	2515	3536
hsa-miR-192-3p	474	1495	hsa-miR-4758-3p	2516	3537
hsa-miR-192-5p	475	1496	hsa-miR-4758-5p	2517	3538
hsa-miR-193a-3p	476	1497	hsa-miR-4759	2518	3539
hsa-miR-193a-5p	477	1498	hsa-miR-4760-3p	2519	3540
hsa-miR-193b-3p	478	1499	hsa-miR-4760-5p	2520	3541
hsa-miR-193b-5p	479	1500	hsa-miR-4761-3p	2521	3542
hsa-miR-194-3p	480	1501	hsa-miR-4761-5p	2522	3543
hsa-miR-194-5p	481	1502	hsa-miR-4762-3p	2523	3544
hsa-miR-195-3p	482	1503	hsa-miR-4762-5p	2524	3545
hsa-miR-195-5p	483	1504	hsa-miR-4763-3p	2525	3546
hsa-miR- 196a-3p	484	1505	hsa-miR-4763-5p	2526	3547
hsa-miR- 196a-5p	485	1506	hsa-miR-4764-3p	2527	3548
hsa-miR- 196b-3p	486	1507	hsa-miR-4764-5p	2528	3549
hsa-miR- 196b-5p	487	1508	hsa-miR-4765	2529	3550
hsa-miR- 1972	488	1509	hsa-miR-4766-3p	2530	3551
hsa-miR- 1973	489	1510	hsa-miR-4766-5p	2531	3552
hsa-miR- 197-3p	490	151 1	hsa-miR-4767	2532	3553
hsa-miR- 197-5p	491	1512	hsa-miR-4768-3p	2533	3554
hsa-miR- 1976	492	1513	hsa-miR-4768-5p	2534	3555
hsa-miR- 198	493	1514	hsa-miR-4769-3p	2535	3556
hsa-miR- 199a-3p	494	1515	hsa-miR-4769-5p	2536	3557
hsa-miR- 199a-5p	495	1516	hsa-miR-4770	2537	3558
hsa-miR- 199b-3p	496	1517	hsa-miR-4771	2538	3559
hsa-miR- 199b-5p	497	1518	hsa-miR-4772-3p	2539	3560
hsa-miR- 19a-3p	498	1519	hsa-miR-4772-5p	2540	3561
hsa-miR- 19a-5p	499	1520	hsa-miR-4773	2541	3562
hsa-miR- 19b- 1-5p	500	1521	hsa-miR-4774-3p	2542	3563
hsa-miR- 19b-2-5p	501	1522	hsa-miR-4774-5p	2543	3564

hsa-miR-19b-3p	502	1523	hsa-miR-4775	2544	3565
hsa-miR-200a-3p	503	1524	hsa-miR-4776-3p	2545	3566
hsa-miR-200a-5p	504	1525	hsa-miR-4776-5p	2546	3567
hsa-miR-200b-3p	505	1526	hsa-miR-4777-3p	2547	3568
hsa-miR-200b-5p	506	1527	hsa-miR-4777-5p	2548	3569
hsa-miR-200c-3p	507	1528	hsa-miR-4778-3p	2549	3570
hsa-miR-200c-5p	508	1529	hsa-miR-4778-5p	2550	3571
hsa-miR-202-3p	509	1530	hsa-miR-4779	2551	3572
hsa-miR-202-5p	510	1531	hsa-miR-4780	2552	3573
hsa-miR-203a	511	1532	hsa-miR-4781-3p	2553	3574
hsa-miR-203b-3p	512	1533	hsa-miR-4781-5p	2554	3575
hsa-miR-203b-5p	513	1534	hsa-miR-4782-3p	2555	3576
hsa-miR-204-3p	514	1535	hsa-miR-4782-5p	2556	3577
hsa-miR-204-5p	515	1536	hsa-miR-4783-3p	2557	3578
hsa-miR-2052	516	1537	hsa-miR-4783-5p	2558	3579
hsa-miR-2053	517	1538	hsa-miR-4784	2559	3580
hsa-miR-205-3p	518	1539	hsa-miR-4785	2560	3581
hsa-miR-2054	519	1540	hsa-miR-4786-3p	2561	3582
hsa-miR-205-5p	520	1541	hsa-miR-4786-5p	2562	3583
hsa-miR-206	521	1542	hsa-miR-4787-3p	2563	3584
hsa-miR-208a	522	1543	hsa-miR-4787-5p	2564	3585
hsa-miR-208b	523	1544	hsa-miR-4788	2565	3586
hsa-miR-20a-3p	524	1545	hsa-miR-4789-3p	2566	3587
hsa-miR-20a-5p	525	1546	hsa-miR-4789-5p	2567	3588
hsa-miR-20b-3p	526	1547	hsa-miR-4790-3p	2568	3589
hsa-miR-20b-5p	527	1548	hsa-miR-4790-5p	2569	3590
hsa-miR-210	528	1549	hsa-miR-4791	2570	3591
hsa-miR-21 10	529	1550	hsa-miR-4792	2571	3592
hsa-miR-21 13	530	1551	hsa-miR-4793-3p	2572	3593
hsa-miR-21 1-3p	531	1552	hsa-miR-4793-5p	2573	3594
hsa-miR-21 14-3p	532	1553	hsa-miR-4794	2574	3595
hsa-miR-21 14-5p	533	1554	hsa-miR-4795-3p	2575	3596
hsa-miR-21 15-3p	534	1555	hsa-miR-4795-5p	2576	3597

hsa-miR-21 15-5p	535	1556	hsa-miR-4796-3p	2577	3598
hsa-miR-21 1-5p	536	1557	hsa-miR-4796-5p	2578	3599
hsa-miR-21 16-3p	537	1558	hsa-miR-4797-3p	2579	3600
hsa-miR-21 16-5p	538	1559	hsa-miR-4797-5p	2580	3601
hsa-miR-21 17	539	1560	hsa-miR-4798-3p	2581	3602
hsa-miR-21 2-3p	540	1561	hsa-miR-4798-5p	2582	3603
hsa-miR-21 2-5p	541	1562	hsa-miR-4799-3p	2583	3604
hsa-miR-21 -3p	542	1563	hsa-miR-4799-5p	2584	3605
hsa-miR-21 4-3p	543	1564	hsa-miR-4800-3p	2585	3606
hsa-miR-21 4-5p	544	1565	hsa-miR-4800-5p	2586	3607
hsa-miR-21 5	545	1566	hsa-miR-4801	2587	3608
hsa-miR-21 -5p	546	1567	hsa-miR-4802-3p	2588	3609
hsa-miR-21 6a-3p	547	1568	hsa-miR-4802-5p	2589	3610
hsa-miR-2 16a-5p	548	1569	hsa-miR-4803	2590	361 1
hsa-miR-21 6b	549	1570	hsa-miR-4804-3p	2591	3612
hsa-miR-2 17	550	1571	hsa-miR-4804-5p	2592	3613
hsa-miR-21 8-1-3p	551	1572	hsa-miR-483-3p	2593	3614
hsa-miR-21 8-2-3p	552	1573	hsa-miR-483-5p	2594	3615
hsa-miR-21 8-5p	553	1574	hsa-miR-484	2595	3616
hsa-miR-21 9-1-3p	554	1575	hsa-miR-485-3p	2596	3617
hsa-miR-2 19-2-3p	555	1576	hsa-miR-485-5p	2597	3618
hsa-miR-2 19-5p	556	1577	hsa-miR-486-3p	2598	3619
hsa-miR-221-3p	557	1578	hsa-miR-486-5p	2599	3620
hsa-miR-221-5p	558	1579	hsa-miR-487a	2600	3621
hsa-miR-222-3p	559	1580	hsa-miR-487b	2601	3622
hsa-miR-222-5p	560	1581	hsa-miR-488-3p	2602	3623
hsa-miR-223-3p	561	1582	hsa-miR-488-5p	2603	3624
hsa-miR-223-5p	562	1583	hsa-miR-489	2604	3625
hsa-miR-22-3p	563	1584	hsa-miR-490-3p	2605	3626
hsa-miR-224-3p	564	1585	hsa-miR-490-5p	2606	3627
hsa-miR-224-5p	565	1586	hsa-miR-491-3p	2607	3628
hsa-miR-22-5p	566	1587	hsa-miR-491-5p	2608	3629
hsa-miR-2276	567	1588	hsa-miR-492	2609	3630

hsa-miR-2277-3p	568	1589	hsa-miR-493-3p	2610	3631
hsa-miR-2277-5p	569	1590	hsa-miR-493-5p	2611	3632
hsa-miR-2278	570	1591	hsa-miR-494	2612	3633
hsa-miR-2355-3p	571	1592	hsa-miR-495-3p	2613	3634
hsa-miR-2355-5p	572	1593	hsa-miR-495-5p	2614	3635
hsa-miR-2392	573	1594	hsa-miR-496	2615	3636
hsa-miR-23a-3p	574	1595	hsa-miR-497-3p	2616	3637
hsa-miR-23a-5p	575	1596	hsa-miR-497-5p	2617	3638
hsa-miR-23b-3p	576	1597	hsa-miR-498	2618	3639
hsa-miR-23b-5p	577	1598	hsa-miR-4999-3p	2619	3640
hsa-miR-23c	578	1599	hsa-miR-4999-5p	2620	3641
hsa-miR-24-1-5p	579	1600	hsa-miR-499a-3p	2621	3642
hsa-miR-24-2-5p	580	1601	hsa-miR-499a-5p	2622	3643
hsa-miR-24-3p	581	1602	hsa-miR-499b-3p	2623	3644
hsa-miR-2467-3p	582	1603	hsa-miR-499b-5p	2624	3645
hsa-miR-2467-5p	583	1604	hsa-miR-5000-3p	2625	3646
hsa-miR-25-3p	584	1605	hsa-miR-5000-5p	2626	3647
hsa-miR-25-5p	585	1606	hsa-miR-5001-3p	2627	3648
hsa-miR-2681-3p	586	1607	hsa-miR-5001-5p	2628	3649
hsa-miR-2681-5p	587	1608	hsa-miR-5002-3p	2629	3650
hsa-miR-2682-3p	588	1609	hsa-miR-5002-5p	2630	3651
hsa-miR-2682-5p	589	1610	hsa-miR-5003-3p	2631	3652
hsa-miR-26a-1-3p	590	1611	hsa-miR-5003-5p	2632	3653
hsa-miR-26a-2-3p	591	1612	hsa-miR-5004-3p	2633	3654
hsa-miR-26a-5p	592	1613	hsa-miR-5004-5p	2634	3655
hsa-miR-26b-3p	593	1614	hsa-miR-5006-3p	2635	3656
hsa-miR-26b-5p	594	1615	hsa-miR-5006-5p	2636	3657
hsa-miR-27a-3p	595	1616	hsa-miR-5007-3p	2637	3658
hsa-miR-27a-5p	596	1617	hsa-miR-5007-5p	2638	3659
hsa-miR-27b-3p	597	1618	hsa-miR-5008-3p	2639	3660
hsa-miR-27b-5p	598	1619	hsa-miR-5008-5p	2640	3661
hsa-miR-28-3p	599	1620	hsa-miR-5009-3p	2641	3662
hsa-miR-28-5p	600	1621	hsa-miR-5009-5p	2642	3663

hsa-miR-2861	601	1622	hsa-miR-500a-3p	2643	3664
hsa-miR-2909	602	1623	hsa-miR-500a-5p	2644	3665
hsa-miR-296-3p	603	1624	hsa-miR-500b	2645	3666
hsa-miR-2964a-3p	604	1625	hsa-miR-5010-3p	2646	3667
hsa-miR-2964a-5p	605	1626	hsa-miR-5010-5p	2647	3668
hsa-miR-296-5p	606	1627	hsa-miR-501 1-3p	2648	3669
hsa-miR-297	607	1628	hsa-miR-501 1-5p	2649	3670
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hsa-miR-299-3p	609	1630	hsa-miR-501-5p	2651	3672
hsa-miR-299-5p	610	1631	hsa-miR-502-3p	2652	3673
hsa-miR-29a-3p	611	1632	hsa-miR-502-5p	2653	3674
hsa-miR-29a-5p	612	1633	hsa-miR-503-3p	2654	3675
hsa-miR-29b-l-5p	613	1634	hsa-miR-503-5p	2655	3676
hsa-miR-29b-2-5p	614	1635	hsa-miR-504	2656	3677
hsa-miR-29b-3p	615	1636	hsa-miR-5047	2657	3678
hsa-miR-29c-3p	616	1637	hsa-miR-505-3p	2658	3679
hsa-miR-29c-5p	617	1638	hsa-miR-505-5p	2659	3680
hsa-miR-300	618	1639	hsa-miR-506-3p	2660	3681
hsa-miR-301a-3p	619	1640	hsa-miR-506-5p	2661	3682
hsa-miR-301a-5p	620	1641	hsa-miR-507	2662	3683
hsa-miR-301b	621	1642	hsa-miR-508-3p	2663	3684
hsa-miR-302a-3p	622	1643	hsa-miR-508-5p	2664	3685
hsa-miR-302a-5p	623	1644	hsa-miR-5087	2665	3686
hsa-miR-302b-3p	624	1645	hsa-miR-5088	2666	3687
hsa-miR-302b-5p	625	1646	hsa-miR-5089-3p	2667	3688
hsa-miR-302c-3p	626	1647	hsa-miR-5089-5p	2668	3689
hsa-miR-302c-5p	627	1648	hsa-miR-5090	2669	3690
hsa-miR-302d-3p	628	1649	hsa-miR-5091	2670	3691
hsa-miR-302d-5p	629	1650	hsa-miR-5092	2671	3692
hsa-miR-302e	630	1651	hsa-miR-5093	2672	3693
hsa-miR-302f	631	1652	hsa-miR-509-3-5p	2673	3694
hsa-miR-3064-3p	632	1653	hsa-miR-509-3p	2674	3695
hsa-miR-3064-5p	633	1654	hsa-miR-5094	2675	3696

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hsa-miR-3065-5p	635	1656	hsa-miR-509-5p	2677	3698
hsa-miR-3074-3p	636	1657	hsa-miR-5096	2678	3699
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hsa-miR-30a-5p	639	1660	hsa-miR-5 11	2681	3702
hsa-miR-30b-3p	640	1661	hsa-miR-512-3p	2682	3703
hsa-miR-30b-5p	641	1662	hsa-miR-5 12-5p	2683	3704
hsa-miR-30c-l-3p	642	1663	hsa-miR-5 13a-3p	2684	3705
hsa-miR-30c-2-3p	643	1664	hsa-miR-5 13a-5p	2685	3706
hsa-miR-30c-5p	644	1665	hsa-miR-5 13b	2686	3707
hsa-miR-30d-3p	645	1666	hsa-miR-5 13c-3p	2687	3708
hsa-miR-30d-5p	646	1667	hsa-miR-5 13c-5p	2688	3709
hsa-miR-30e-3p	647	1668	hsa-miR-5 14a-3p	2689	3710
hsa-miR-30e-5p	648	1669	hsa-miR-5 14a-5p	2690	371 1
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hsa-miR-31 16	650	1671	hsa-miR-5 14b-5p	2692	3713
hsa-miR-31 17-3p	651	1672	hsa-miR-5 15-3p	2693	3714
hsa-miR-31 17-5p	652	1673	hsa-miR-5 15-5p	2694	3715
hsa-miR-31 18	653	1674	hsa-miR-5 16a-3p	2695	3716
hsa-miR-31 19	654	1675	hsa-miR-5 16a-5p	2696	3717
hsa-miR-31 20-3p	655	1676	hsa-miR-5 16b-3p	2697	3718
hsa-miR-31 20-5p	656	1677	hsa-miR-5 16b-5p	2698	3719
hsa-miR-3121-3p	657	1678	hsa-miR-5 17-5p	2699	3720
hsa-miR-3121-5p	658	1679	hsa-miR-5 17a-3p	2700	3721
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hsa-miR-3123	660	1681	hsa-miR-5 17c-3p	2702	3723
hsa-miR-31 24-3p	661	1682	hsa-miR-5 186	2703	3724
hsa-miR-31 24-5p	662	1683	hsa-miR-5 187-3p	2704	3725
hsa-miR-3125	663	1684	hsa-miR-5 187-5p	2705	3726
hsa-miR-31 26-3p	664	1685	hsa-miR-5 188	2706	3727
hsa-miR-31 26-5p	665	1686	hsa-miR-5 189	2707	3728
hsa-miR-31 27-3p	666	1687	hsa-miR-5 18a-3p	2708	3729

hsa-miR-3 127-5p	667	1688	hsa-miR-518a-5p	2709	3730
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hsa-miR-3 129-5p	670	1691	hsa-miR-518c-5p	2712	3733
hsa-miR-3 130-3p	671	1692	hsa-miR-518d-3p	2713	3734
hsa-miR-3 130-5p	672	1693	hsa-miR-518d-5p	2714	3735
hsa-miR-3 131	673	1694	hsa-miR-518e-3p	2715	3736
hsa-miR-3 132	674	1695	hsa-miR-518e-5p	2716	3737
hsa-miR-3 133	675	1696	hsa-miR-518f-3p	2717	3738
hsa-miR-3 134	676	1697	hsa-miR-518f-5p	2718	3739
hsa-miR-3 135a	677	1698	hsa-miR-5190	2719	3740
hsa-miR-3 135b	678	1699	hsa-miR-5191	2720	3741
hsa-miR-3 136-3p	679	1700	hsa-miR-5192	2721	3742
hsa-miR-3 136-5p	680	1701	hsa-miR-5193	2722	3743
hsa-miR-3 137	681	1702	hsa-miR-5194	2723	3744
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hsa-miR-3 140-5p	686	1707	hsa-miR-5197-3p	2728	3749
hsa-miR-3 141	687	1708	hsa-miR-5197-5p	2729	3750
hsa-miR-3 142	688	1709	hsa-miR-519a-3p	2730	3751
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hsa-miR-3 145-5p	693	1714	hsa-miR-519c-5p	2735	3756
hsa-miR-3 146	694	1715	hsa-miR-519d	2736	3757
hsa-miR-3 147	695	1716	hsa-miR-519e-3p	2737	3758
hsa-miR-3 148	696	1717	hsa-miR-519e-5p	2738	3759
hsa-miR-3 149	697	1718	hsa-miR-520a-3p	2739	3760
hsa-miR-3 150a-3p	698	1719	hsa-miR-520a-5p	2740	3761
hsa-miR-3 150a-5p	699	1720	hsa-miR-520b	2741	3762

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hsa-miR-3 150b-5p	701	1722	hsa-miR-520c-5p	2743	3764
hsa-miR-3 151	702	1723	hsa-miR-520d-3p	2744	3765
hsa-miR-3 152-3p	703	1724	hsa-miR-520d-5p	2745	3766
hsa-miR-3 152-5p	704	1725	hsa-miR-520e	2746	3767
hsa-miR-3 153	705	1726	hsa-miR-520f	2747	3768
hsa-miR-3 154	706	1727	hsa-miR-520g	2748	3769
hsa-miR-3 155a	707	1728	hsa-miR-520h	2749	3770
hsa-miR-3 155b	708	1729	hsa-miR-52 1	2750	3771
hsa-miR-3 156-3p	709	1730	hsa-miR-522-3p	2751	3772
hsa-miR-3 156-5p	710	1731	hsa-miR-522-5p	2752	3773
hsa-miR-3 157-3p	711	1732	hsa-miR-523-3p	2753	3774
hsa-miR-3 157-5p	712	1733	hsa-miR-523-5p	2754	3775
hsa-miR-3 158-3p	713	1734	hsa-miR-524-3p	2755	3776
hsa-miR-3 158-5p	714	1735	hsa-miR-524-5p	2756	3777
hsa-miR-3 159	715	1736	hsa-miR-525-3p	2757	3778
hsa-miR-3 159-5p	716	1737	hsa-miR-525-5p	2758	3779
hsa-miR-3 160-3p	717	1738	hsa-miR-526a	2759	3780
hsa-miR-3 160-5p	718	1739	hsa-miR-526b-3p	2760	3781
hsa-miR-3 161	719	1740	hsa-miR-526b-5p	2761	3782
hsa-miR-3 162-3p	720	1741	hsa-miR-527	2762	3783
hsa-miR-3 162-5p	721	1742	hsa-miR-532-3p	2763	3784
hsa-miR-3 163	722	1743	hsa-miR-532-5p	2764	3785
hsa-miR-3 164	723	1744	hsa-miR-539-3p	2765	3786
hsa-miR-3 165	724	1745	hsa-miR-539-5p	2766	3787
hsa-miR-3 166	725	1746	hsa-miR-541-3p	2767	3788
hsa-miR-3 167	726	1747	hsa-miR-541-5p	2768	3789
hsa-miR-3 168	727	1748	hsa-miR-542-3p	2769	3790
hsa-miR-3 169	728	1749	hsa-miR-542-5p	2770	3791
hsa-miR-3 170	729	1750	hsa-miR-543	2771	3792
hsa-miR-3 171	730	1751	hsa-miR-544a	2772	3793
hsa-miR-3 173-3p	731	1752	hsa-miR-544b	2773	3794
hsa-miR-3 173-5p	732	1753	hsa-miR-545-3p	2774	3795

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hsa-miR-3175	734	1755	hsa-miR-548	2776	3797
hsa-miR-3176	735	1756	hsa-miR-548-3p	2777	3798
hsa-miR-3177-3p	736	1757	hsa-miR-548-5p	2778	3799
hsa-miR-3177-5p	737	1758	hsa-miR-548a	2779	3800
hsa-miR-3178	738	1759	hsa-miR-548a-3p	2780	3801
hsa-miR-3179	739	1760	hsa-miR-548a-5p	2781	3802
hsa-miR-3180	740	1761	hsa-miR-548aa	2782	3803
hsa-miR-3180-3p	741	1762	hsa-miR-548ab	2783	3804
hsa-miR-3180-5p	742	1763	hsa-miR-548ac	2784	3805
hsa-miR-3181	743	1764	hsa-miR-548ad	2785	3806
hsa-miR-3182	744	1765	hsa-miR-548ae	2786	3807
hsa-miR-3183	745	1766	hsa-miR-548ag	2787	3808
hsa-miR-3184-3p	746	1767	hsa-miR-548ah-3p	2788	3809
hsa-miR-3184-5p	747	1768	hsa-miR-548ah-5p	2789	3810
hsa-miR-3185	748	1769	hsa-miR-548ai	2790	381 1
hsa-miR-3186-3p	749	1770	hsa-miR-548aj-3p	2791	3812
hsa-miR-3186-5p	750	1771	hsa-miR-548aj-5p	2792	3813
hsa-miR-3187-3p	751	1772	hsa-miR-548ak	2793	3814
hsa-miR-3187-5p	752	1773	hsa-miR-548al	2794	3815
hsa-miR-3188	753	1774	hsa-miR-548am-3p	2795	3816
hsa-miR-3189-3p	754	1775	hsa-miR-548am-5p	2796	3817
hsa-miR-3189-5p	755	1776	hsa-miR-548an	2797	3818
hsa-miR-3190-3p	756	1777	hsa-miR-548ao-3p	2798	3819
hsa-miR-3190-5p	757	1778	hsa-miR-548ao-5p	2799	3820
hsa-miR-3191-3p	758	1779	hsa-miR-548ap-3p	2800	3821
hsa-miR-3191-5p	759	1780	hsa-miR-548ap-5p	2801	3822
hsa-miR-3192	760	1781	hsa-miR-548aq-3p	2802	3823
hsa-miR-3193	761	1782	hsa-miR-548aq-5p	2803	3824
hsa-miR-3194-3p	762	1783	hsa-miR-548ar-3p	2804	3825
hsa-miR-3194-5p	763	1784	hsa-miR-548ar-5p	2805	3826
hsa-miR-3195	764	1785	hsa-miR-548as-3p	2806	3827
hsa-miR-3196	765	1786	hsa-miR-548as-5p	2807	3828

hsa-miR-3197	766	1787	hsa-miR-548at-3p	2808	3829
hsa-miR-3198	767	1788	hsa-miR-548at-5p	2809	3830
hsa-miR-3199	768	1789	hsa-miR-548au-3p	2810	3831
hsa-miR-3200-3p	769	1790	hsa-miR-548au-5p	281 1	3832
hsa-miR-3200-5p	770	1791	hsa-miR-548av-3p	2812	3833
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hsa-miR-3202	772	1793	hsa-miR-548aw	2814	3835
hsa-miR-320a	773	1794	hsa-miR-548ay-3p	2815	3836
hsa-miR-320b	774	1795	hsa-miR-548ay-5p	2816	3837
hsa-miR-320c	775	1796	hsa-miR-548az-3p	2817	3838
hsa-miR-320d	776	1797	hsa-miR-548az-5p	2818	3839
hsa-miR-320e	777	1798	hsa-miR-548b-3p	2819	3840
hsa-miR-323a-3p	778	1799	hsa-miR-548b-5p	2820	3841
hsa-miR-323a-5p	779	1800	hsa-miR-548c-3p	2821	3842
hsa-miR-323b-3p	780	1801	hsa-miR-548c-5p	2822	3843
hsa-miR-323b-5p	781	1802	hsa-miR-548d-3p	2823	3844
hsa-miR-32-3p	782	1803	hsa-miR-548d-5p	2824	3845
hsa-miR-324-3p	783	1804	hsa-miR-548e	2825	3846
hsa-miR-324-5p	784	1805	hsa-miR-548f	2826	3847
hsa-miR-325	785	1806	hsa-miR-548g-3p	2827	3848
hsa-miR-32-5p	786	1807	hsa-miR-548g-5p	2828	3849
hsa-miR-326	787	1808	hsa-miR-548h-3p	2829	3850
hsa-miR-328	788	1809	hsa-miR-548h-5p	2830	3851
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hsa-miR-331-5p	793	1814	hsa-miR-548m	2835	3856
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hsa-miR-335-5p	795	1816	hsa-miR-548o-3p	2837	3858
hsa-miR-337-3p	796	1817	hsa-miR-548o-5p	2838	3859
hsa-miR-337-5p	797	1818	hsa-miR-548p	2839	3860
hsa-miR-338-3p	798	1819	hsa-miR-548q	2840	3861

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hsa-miR-339-3p	800	1821	hsa-miR-548t-3p	2842	3863
hsa-miR-339-5p	801	1822	hsa-miR-548t-5p	2843	3864
hsa-miR-33a-3p	802	1823	hsa-miR-548u	2844	3865
hsa-miR-33a-5p	803	1824	hsa-miR-548w	2845	3866
hsa-miR-33b-3p	804	1825	hsa-miR-548y	2846	3867
hsa-miR-33b-5p	805	1826	hsa-miR-548z	2847	3868
hsa-miR-340-3p	806	1827	hsa-miR-549a	2848	3869
hsa-miR-340-5p	807	1828	hsa-miR-550a-3-5p	2849	3870
hsa-miR-342-3p	808	1829	hsa-miR-550a-3p	2850	3871
hsa-miR-342-5p	809	1830	hsa-miR-550a-5p	2851	3872
hsa-miR-345-3p	810	1831	hsa-miR-550b-2-5p	2852	3873
hsa-miR-345-5p	811	1832	hsa-miR-550b-3p	2853	3874
hsa-miR-346	812	1833	hsa-miR-551a	2854	3875
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hsa-miR-34a-5p	814	1835	hsa-miR-551b-5p	2856	3877
hsa-miR-34b-3p	815	1836	hsa-miR-552	2857	3878
hsa-miR-34b-5p	816	1837	hsa-miR-553	2858	3879
hsa-miR-34c-3p	817	1838	hsa-miR-554	2859	3880
hsa-miR-34c-5p	818	1839	hsa-miR-555	2860	3881
hsa-miR-3529-3p	819	1840	hsa-miR-556-3p	2861	3882
hsa-miR-3529-5p	820	1841	hsa-miR-556-5p	2862	3883
hsa-miR-3591-3p	821	1842	hsa-miR-557	2863	3884
hsa-miR-3591-5p	822	1843	hsa-miR-5571-3p	2864	3885
hsa-miR-3605-3p	823	1844	hsa-miR-557 l-5p	2865	3886
hsa-miR-3605-5p	824	1845	hsa-miR-5572	2866	3887
hsa-miR-3606-3p	825	1846	hsa-miR-5579-3p	2867	3888
hsa-miR-3606-5p	826	1847	hsa-miR-5579-5p	2868	3889
hsa-miR-3607-3p	827	1848	hsa-miR-558	2869	3890
hsa-miR-3607-5p	828	1849	hsa-miR-5580-3p	2870	3891
hsa-miR-3609	829	1850	hsa-miR-5580-5p	2871	3892
hsa-miR-3610	830	1851	hsa-miR-558 l-3p	2872	3893
hsa-miR-361 1	831	1852	hsa-miR-558 l-5p	2873	3894

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hsa-miR-3613-3p	833	1854	hsa-miR-5582-5p	2875	3896
hsa-miR-3613-5p	834	1855	hsa-miR-5583-3p	2876	3897
hsa-miR-361-3p	835	1856	hsa-miR-5583-5p	2877	3898
hsa-miR-3614-3p	836	1857	hsa-miR-5584-3p	2878	3899
hsa-miR-3614-5p	837	1858	hsa-miR-5584-5p	2879	3900
hsa-miR-3615	838	1859	hsa-miR-5585-3p	2880	3901
hsa-miR-361-5p	839	1860	hsa-miR-5585-5p	2881	3902
hsa-miR-3616-3p	840	1861	hsa-miR-5586-3p	2882	3903
hsa-miR-3616-5p	841	1862	hsa-miR-5586-5p	2883	3904
hsa-miR-3617-3p	842	1863	hsa-miR-5587-3p	2884	3905
hsa-miR-3617-5p	843	1864	hsa-miR-5587-5p	2885	3906
hsa-miR-3618	844	1865	hsa-miR-5588-3p	2886	3907
hsa-miR-3619-3p	845	1866	hsa-miR-5588-5p	2887	3908
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hsa-miR-3620-3p	847	1868	hsa-miR-5589-5p	2889	3910
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hsa-miR-3622a-5p	851	1872	hsa-miR-559 1-3p	2893	3914
hsa-miR-3622b-3p	852	1873	hsa-miR-559 1-5p	2894	3915
hsa-miR-3622b-5p	853	1874	hsa-miR-561-3p	2895	3916
hsa-miR-362-3p	854	1875	hsa-miR-561-5p	2896	3917
hsa-miR-362-5p	855	1876	hsa-miR-562	2897	3918
hsa-miR-363-3p	856	1877	hsa-miR-563	2898	3919
hsa-miR-363-5p	857	1878	hsa-miR-564	2899	3920
hsa-miR-3646	858	1879	hsa-miR-566	2900	3921
hsa-miR-3648	859	1880	hsa-miR-567	2901	3922
hsa-miR-3649	860	1881	hsa-miR-568	2902	3923
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hsa-miR-3654	865	1886	hsa-miR-5683	2907	3928
hsa-miR-3655	866	1887	hsa-miR-5684	2908	3929
hsa-miR-3656	867	1888	hsa-miR-5685	2909	3930
hsa-miR-3657	868	1889	hsa-miR-5686	2910	3931
hsa-miR-3658	869	1890	hsa-miR-5687	291 1	3932
hsa-miR-3659	870	1891	hsa-miR-5688	2912	3933
hsa-miR-365a-3p	871	1892	hsa-miR-5689	2913	3934
hsa-miR-365a-5p	872	1893	hsa-miR-569	2914	3935
hsa-miR-365b-3p	873	1894	hsa-miR-5690	2915	3936
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hsa-miR-3660	875	1896	hsa-miR-5692a	2917	3938
hsa-miR-3661	876	1897	hsa-miR-5692b	2918	3939
hsa-miR-3662	877	1898	hsa-miR-5692c	2919	3940
hsa-miR-3663-3p	878	1899	hsa-miR-5693	2920	3941
hsa-miR-3663-5p	879	1900	hsa-miR-5694	2921	3942
hsa-miR-3664-3p	880	1901	hsa-miR-5695	2922	3943
hsa-miR-3664-5p	881	1902	hsa-miR-5696	2923	3944
hsa-miR-3665	882	1903	hsa-miR-5697	2924	3945
hsa-miR-3666	883	1904	hsa-miR-5698	2925	3946
hsa-miR-3667-3p	884	1905	hsa-miR-5699	2926	3947
hsa-miR-3667-5p	885	1906	hsa-miR-5700	2927	3948
hsa-miR-3668	886	1907	hsa-miR-5701	2928	3949
hsa-miR-3669	887	1908	hsa-miR-5702	2929	3950
hsa-miR-3670	888	1909	hsa-miR-5703	2930	3951
hsa-miR-3671	889	1910	hsa-miR-570-3p	2931	3952
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hsa-miR-367-3p	892	1913	hsa-miR-570-5p	2934	3955
hsa-miR-3674	893	1914	hsa-miR-5706	2935	3956
hsa-miR-3675-3p	894	1915	hsa-miR-5707	2936	3957
hsa-miR-3675-5p	895	1916	hsa-miR-5708	2937	3958
hsa-miR-367-5p	896	1917	hsa-miR-571	2938	3959
hsa-miR-3676-3p	897	1918	hsa-miR-572	2939	3960

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hsa-miR-3677-3p	899	1920	hsa-miR-5739	2941	3962
hsa-miR-3677-5p	900	1921	hsa-miR-574-3p	2942	3963
hsa-miR-3678-3p	901	1922	hsa-miR-574-5p	2943	3964
hsa-miR-3678-5p	902	1923	hsa-miR-575	2944	3965
hsa-miR-3679-3p	903	1924	hsa-miR-576-3p	2945	3966
hsa-miR-3679-5p	904	1925	hsa-miR-576-5p	2946	3967
hsa-miR-3680-3p	905	1926	hsa-miR-577	2947	3968
hsa-miR-3680-5p	906	1927	hsa-miR-578	2948	3969
hsa-miR-3681-3p	907	1928	hsa-miR-5787	2949	3970
hsa-miR-3681-5p	908	1929	hsa-miR-579	2950	3971
hsa-miR-3682-3p	909	1930	hsa-miR-580	2951	3972
hsa-miR-3682-5p	910	1931	hsa-miR-581	2952	3973
hsa-miR-3683	911	1932	hsa-miR-582-3p	2953	3974
hsa-miR-3684	912	1933	hsa-miR-582-5p	2954	3975
hsa-miR-3685	913	1934	hsa-miR-583	2955	3976
hsa-miR-3686	914	1935	hsa-miR-584-3p	2956	3977
hsa-miR-3687	915	1936	hsa-miR-584-5p	2957	3978
hsa-miR-3688-3p	916	1937	hsa-miR-585	2958	3979
hsa-miR-3688-5p	917	1938	hsa-miR-586	2959	3980
hsa-miR-3689a-3p	918	1939	hsa-miR-587	2960	3981
hsa-miR-3689a-5p	919	1940	hsa-miR-588	2961	3982
hsa-miR-3689b-3p	920	1941	hsa-miR-589-3p	2962	3983
hsa-miR-3689b-5p	921	1942	hsa-miR-589-5p	2963	3984
hsa-miR-3689c	922	1943	hsa-miR-590-3p	2964	3985
hsa-miR-3689d	923	1944	hsa-miR-590-5p	2965	3986
hsa-miR-3689e	924	1945	hsa-miR-591	2966	3987
hsa-miR-3689f	925	1946	hsa-miR-592	2967	3988
hsa-miR-3690	926	1947	hsa-miR-593-3p	2968	3989
hsa-miR-3691-3p	927	1948	hsa-miR-593-5p	2969	3990
hsa-miR-3691-5p	928	1949	hsa-miR-595	2970	3991
hsa-miR-3692-3p	929	1950	hsa-miR-596	2971	3992
hsa-miR-3692-5p	930	1951	hsa-miR-597	2972	3993

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hsa-miR-369-5p	932	1953	hsa-miR-599	2974	3995
hsa-miR-370	933	1954	hsa-miR-600	2975	3996
hsa-miR-3713	934	1955	hsa-miR-601	2976	3997
hsa-miR-3714	935	1956	hsa-miR-602	2977	3998
hsa-miR-371a-3p	936	1957	hsa-miR-603	2978	3999
hsa-miR-371a-5p	937	1958	hsa-miR-604	2979	4000
hsa-miR-371b-3p	938	1959	hsa-miR-605	2980	4001
hsa-miR-371b-5p	939	1960	hsa-miR-606	2981	4002
hsa-miR-372	940	1961	hsa-miR-6068	2982	4003
hsa-miR-373-3p	941	1962	hsa-miR-6069	2983	4004
hsa-miR-373-5p	942	1963	hsa-miR-607	2984	4005
hsa-miR-374a-3p	943	1964	hsa-miR-6070	2985	4006
hsa-miR-374a-5p	944	1965	hsa-miR-607 1	2986	4007
hsa-miR-374b-3p	945	1966	hsa-miR-6072	2987	4008
hsa-miR-374b-5p	946	1967	hsa-miR-6073	2988	4009
hsa-miR-374c-3p	947	1968	hsa-miR-6074	2989	4010
hsa-miR-374c-5p	948	1969	hsa-miR-6075	2990	401 1
hsa-miR-375	949	1970	hsa-miR-6076	2991	4012
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hsa-miR-376a-3p	951	1972	hsa-miR-6078	2993	4014
hsa-miR-376a-5p	952	1973	hsa-miR-6079	2994	4015
hsa-miR-376b-3p	953	1974	hsa-miR-608	2995	4016
hsa-miR-376b-5p	954	1975	hsa-miR-6080	2996	4017
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hsa-miR-378a-3p	959	1980	hsa-miR-6085	3001	4022
hsa-miR-378a-5p	960	1981	hsa-miR-6086	3002	4023
hsa-miR-378b	961	1982	hsa-miR-6087	3003	4024
hsa-miR-378c	962	1983	hsa-miR-6088	3004	4025
hsa-miR-378d	963	1984	hsa-miR-6089	3005	4026

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hsa-miR-378f	965	1986	hsa-miR-6090	3007	4028
hsa-miR-378g	966	1987	hsa-miR-610	3008	4029
hsa-miR-378h	967	1988	hsa-miR-6 11	3009	4030
hsa-miR-378i	968	1989	hsa-miR-612	3010	4031
hsa-miR-378j	969	1990	hsa-miR-6 124	301 1	4032
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hsa-miR-379-5p	971	1992	hsa-miR-6 126	3013	4034
hsa-miR-380-3p	972	1993	hsa-miR-6 127	3014	4035
hsa-miR-380-5p	973	1994	hsa-miR-6 128	3015	4036
hsa-miR-381-3p	974	1995	hsa-miR-6 129	3016	4037
hsa-miR-381-5p	975	1996	hsa-miR-6 13	3017	4038
hsa-miR-382-3p	976	1997	hsa-miR-6 130	3018	4039
hsa-miR-382-5p	977	1998	hsa-miR-6 131	3019	4040
hsa-miR-383	978	1999	hsa-miR-6 132	3020	4041
hsa-miR-384	979	2000	hsa-miR-6 133	3021	4042
hsa-miR-3907	980	2001	hsa-miR-6 134	3022	4043
hsa-miR-3908	981	2002	hsa-miR-6 14	3023	4044
hsa-miR-3909	982	2003	hsa-miR-6 15-3p	3024	4045
hsa-miR-3910	983	2004	hsa-miR-6 15-5p	3025	4046
hsa-miR-391 1	984	2005	hsa-miR-6 16-3p	3026	4047
hsa-miR-3912	985	2006	hsa-miR-6 165	3027	4048
hsa-miR-3913-3p	986	2007	hsa-miR-6 16-5p	3028	4049
hsa-miR-3913-5p	987	2008	hsa-miR-6 17	3029	4050
hsa-miR-3914	988	2009	hsa-miR-6 18	3030	4051
hsa-miR-3915	989	2010	hsa-miR-6 19	3031	4052
hsa-miR-3916	990	201 1	hsa-miR-620	3032	4053
hsa-miR-3917	991	2012	hsa-miR-62 1	3033	4054
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hsa-miR-3919	993	2014	hsa-miR-623	3035	4056
hsa-miR-3920	994	2015	hsa-miR-624-3p	3036	4057
hsa-miR-3921	995	2016	hsa-miR-624-5p	3037	4058
hsa-miR-3922-3p	996	2017	hsa-miR-625-3p	3038	4059

hsa-miR-3922-5p	997	2018	hsa-miR-625-5p	3039	4060
hsa-miR-3923	998	2019	hsa-miR-626	3040	4061
hsa-miR-3924	999	2020	hsa-miR-627	3041	4062
hsa-miR-3925-3p	1000	2021	hsa-miR-628-3p	3042	4063
hsa-miR-3925-5p	1001	2022	hsa-miR-628-5p	3043	4064
hsa-miR-3926	1002	2023	hsa-miR-629-3p	3044	4065
hsa-miR-3927-3p	1003	2024	hsa-miR-629-5p	3045	4066
hsa-miR-3927-5p	1004	2025	hsa-miR-630	3046	4067
hsa-miR-3928	1005	2026	hsa-miR-63 1	3047	4068
hsa-miR-3929	1006	2027	hsa-miR-632	3048	4069
hsa-miR-3934-3p	1007	2028	hsa-miR-63 3	3049	4070
hsa-miR-3934-5p	1008	2029	hsa-miR-634	3050	4071
hsa-miR-3935	1009	2030	hsa-miR-63 5	3051	4072
hsa-miR-3936	1010	2031	hsa-miR-63 6	3052	4073
hsa-miR-3937	101 1	2032	hsa-miR-63 7	3053	4074
hsa-miR-3938	1012	2033	hsa-miR-63 8	3054	4075
hsa-miR-3939	1013	2034	hsa-miR-63 9	3055	4076
hsa-miR-3940-3p	1014	2035	hsa-miR-640	3056	4077
hsa-miR-3940-5p	1015	2036	hsa-miR-641	3057	4078
hsa-miR-3941	1016	2037	hsa-miR-642a-3p	3058	4079
hsa-miR-3942-3p	1017	2038	hsa-miR-642a-5p	3059	4080
hsa-miR-3942-5p	1018	2039	hsa-miR-642b-3p	3060	4081
hsa-miR-3943	1019	2040	hsa-miR-642b-5p	3061	4082
hsa-miR-3944-3p	1020	2041	hsa-miR-643	3062	4083
hsa-miR-3944-5p	1021	2042	hsa-miR-644a	3063	4084
hsa-miR-3945	1022	2043	hsa-miR-645	3064	4085
hsa-miR-3960	1023	2044	hsa-miR-646	3065	4086
hsa-miR-3972	1024	2045	hsa-miR-647	3066	4087
hsa-miR-3973	1025	2046	hsa-miR-648	3067	4088
hsa-miR-3974	1026	2047	hsa-miR-649	3068	4089
hsa-miR-3975	1027	2048	hsa-miR-6499-3p	3069	4090
hsa-miR-3976	1028	2049	hsa-miR-6499-5p	3070	4091
hsa-miR-3977	1029	2050	hsa-miR-650	3071	4092

hsa-miR-3978	1030	2051	hsa-miR-6500-3p	3072	4093
hsa-miR-409-3p	1031	2052	hsa-miR-6500-5p	3073	4094
hsa-miR-409-5p	1032	2053	hsa-miR-6501-3p	3074	4095
hsa-miR-410	1033	2054	hsa-miR-6501-5p	3075	4096
hsa-miR-41 1-3p	1034	2055	hsa-miR-6502-3p	3076	4097
hsa-miR-41 1-5p	1035	2056	hsa-miR-6502-5p	3077	4098
hsa-miR-412	1036	2057	hsa-miR-6503-3p	3078	4099
hsa-miR-421	1037	2058	hsa-miR-6503-5p	3079	4100
hsa-miR-422a	1038	2059	hsa-miR-6504-3p	3080	4101
hsa-miR-423-3p	1039	2060	hsa-miR-6504-5p	3081	4102
hsa-miR-423-5p	1040	2061	hsa-miR-6505-3p	3082	4103
hsa-miR-424-3p	1041	2062	hsa-miR-6505-5p	3083	4104
hsa-miR-424-5p	1042	2063	hsa-miR-6506-3p	3084	4105
hsa-miR-4251	1043	2064	hsa-miR-6506-5p	3085	4106
hsa-miR-4252	1044	2065	hsa-miR-6507-3p	3086	4107
hsa-miR-4253	1045	2066	hsa-miR-6507-5p	3087	4108
hsa-miR-425-3p	1046	2067	hsa-miR-6508-3p	3088	4109
hsa-miR-4254	1047	2068	hsa-miR-6508-5p	3089	4 1 10
hsa-miR-4255	1048	2069	hsa-miR-6509-3p	3090	4 1 11
hsa-miR-425-5p	1049	2070	hsa-miR-6509-5p	3091	4 1 12
hsa-miR-4256	1050	2071	hsa-miR-65 1	3092	4 1 13
hsa-miR-4257	1051	2072	hsa-miR-65 10-3p	3093	4 1 14
hsa-miR-4258	1052	2073	hsa-miR-65 10-5p	3094	4 1 15
hsa-miR-4259	1053	2074	hsa-miR-65 11a-3p	3095	4 1 16
hsa-miR-4260	1054	2075	hsa-miR-65 11a-5p	3096	4 1 17
hsa-miR-4261	1055	2076	hsa-miR-65 11b-3p	3097	4 1 18
hsa-miR-4262	1056	2077	hsa-miR-65 11b-5p	3098	4 1 19
hsa-miR-4263	1057	2078	hsa-miR-65 12-3p	3099	4120
hsa-miR-4264	1058	2079	hsa-miR-65 12-5p	3100	4121
hsa-miR-4265	1059	2080	hsa-miR-65 13-3p	3101	4122
hsa-miR-4266	1060	2081	hsa-miR-65 13-5p	3102	4123
hsa-miR-4267	1061	2082	hsa-miR-65 14-3p	3103	4124
hsa-miR-4268	1062	2083	hsa-miR-65 14-5p	3104	4125

hsa-miR-4269	1063	2084	hsa-miR-6515-3p	3105	4126
hsa-miR-4270	1064	2085	hsa-miR-6515-5p	3106	4127
hsa-miR-4271	1065	2086	hsa-miR-652-3p	3107	4128
hsa-miR-4272	1066	2087	hsa-miR-652-5p	3108	4129
hsa-miR-4273	1067	2088	hsa-miR-653	3109	4130
hsa-miR-4274	1068	2089	hsa-miR-654-3p	3 110	4131
hsa-miR-4275	1069	2090	hsa-miR-654-5p	3 111	4132
hsa-miR-4276	1070	2091	hsa-miR-655	3 112	4133
hsa-miR-4277	1071	2092	hsa-miR-656	3 113	4134
hsa-miR-4278	1072	2093	hsa-miR-657	3 114	4135
hsa-miR-4279	1073	2094	hsa-miR-658	3 115	4136
hsa-miR-4280	1074	2095	hsa-miR-659-3p	3 116	4137
hsa-miR-4281	1075	2096	hsa-miR-659-5p	3 117	4138
hsa-miR-4282	1076	2097	hsa-miR-660-3p	3 118	4139
hsa-miR-4283	1077	2098	hsa-miR-660-5p	3 119	4140
hsa-miR-4284	1078	2099	hsa-miR-66 1	3120	4141
hsa-miR-4285	1079	2100	hsa-miR-662	3121	4142
hsa-miR-4286	1080	2101	hsa-miR-663a	3122	4143
hsa-miR-4287	1081	2102	hsa-miR-663b	3123	4144
hsa-miR-4288	1082	2103	hsa-miR-664a-3p	3124	4145
hsa-miR-4289	1083	2104	hsa-miR-664a-5p	3125	4146
hsa-miR-429	1084	2105	hsa-miR-664b-3p	3126	4147
hsa-miR-4290	1085	2106	hsa-miR-664b-5p	3127	4148
hsa-miR-429 1	1086	2107	hsa-miR-665	3128	4149
hsa-miR-4292	1087	2108	hsa-miR-668	3129	4150
hsa-miR-4293	1088	2109	hsa-miR-670	3130	4151
hsa-miR-4294	1089	2 110	hsa-miR-67 1-3p	3131	4152
hsa-miR-4295	1090	2 111	hsa-miR-67 15a-3p	3132	4153
hsa-miR-4296	1091	2 112	hsa-miR-67 15b-3p	3133	4154
hsa-miR-4297	1092	2 113	hsa-miR-67 15b-5p	3134	4155
hsa-miR-4298	1093	2 114	hsa-miR-67 l-5p	3135	4156
hsa-miR-4299	1094	2 115	hsa-miR-67 16-3p	3136	4157
hsa-miR-4300	1095	2 116	hsa-miR-67 16-5p	3137	4158

hsa-miR-4301	1096	2 1 17	hsa-miR-6717-5p	3138	4159
hsa-miR-4302	1097	2 1 18	hsa-miR-6718-5p	3139	4160
hsa-miR-4303	1098	2 1 19	hsa-miR-6719-3p	3140	4161
hsa-miR-4304	1099	2120	hsa-miR-6720-3p	3141	4162
hsa-miR-4305	1100	2121	hsa-miR-6721-5p	3142	4163
hsa-miR-4306	1101	2122	hsa-miR-6722-3p	3143	4164
hsa-miR-4307	1102	2123	hsa-miR-6722-5p	3144	4165
hsa-miR-4308	1103	2124	hsa-miR-6723-5p	3145	4166
hsa-miR-4309	1104	2125	hsa-miR-6724-5p	3146	4167
hsa-miR-4310	1105	2126	hsa-miR-675-3p	3147	4168
hsa-miR-43 11	1106	2127	hsa-miR-675-5p	3148	4169
hsa-miR-4312	1107	2128	hsa-miR-676-3p	3149	4170
hsa-miR-43 13	1108	2129	hsa-miR-676-5p	3150	4171
hsa-miR-43 l-3p	1109	2130	hsa-miR-708-3p	3151	4172
hsa-miR-43 14	1110	2131	hsa-miR-708-5p	3152	4173
hsa-miR-43 15	1111	2132	hsa-miR-7 11	3153	4174
hsa-miR-43 l-5p	1112	2133	hsa-miR-7-l-3p	3154	4175
hsa-miR-43 16	1113	2134	hsa-miR-7 18	3155	4176
hsa-miR-43 17	1114	2135	hsa-miR-7-2-3p	3156	4177
hsa-miR-43 18	1115	2136	hsa-miR-744-3p	3157	4178
hsa-miR-43 19	1116	2137	hsa-miR-744-5p	3158	4179
hsa-miR-4320	1117	2138	hsa-miR-758-3p	3159	4180
hsa-miR-4321	1118	2139	hsa-miR-758-5p	3160	4181
hsa-miR-4322	1119	2140	hsa-miR-759	3161	4182
hsa-miR-4323	1120	2141	hsa-miR-7-5p	3162	4183
hsa-miR-432-3p	1121	2142	hsa-miR-760	3163	4184
hsa-miR-4324	1122	2143	hsa-miR-76 1	3164	4185
hsa-miR-4325	1123	2144	hsa-miR-762	3165	4186
hsa-miR-432-5p	1124	2145	hsa-miR-764	3166	4187
hsa-miR-4326	1125	2146	hsa-miR-765	3167	4188
hsa-miR-4327	1126	2147	hsa-miR-766-3p	3168	4189
hsa-miR-4328	1127	2148	hsa-miR-766-5p	3169	4190
hsa-miR-4329	1128	2149	hsa-miR-767-3p	3170	4191

hsa-miR-433	1129	2150	hsa-miR-767-5p	3171	4192
hsa-miR-4330	1130	2151	hsa-miR-769-3p	3172	4193
hsa-miR-4417	1131	2152	hsa-miR-769-5p	3173	4194
hsa-miR-4418	1132	2153	hsa-miR-770-5p	3174	4195
hsa-miR-4419a	1133	2154	hsa-miR-802	3175	4196
hsa-miR-4419b	1134	2155	hsa-miR-873-3p	3176	4197
hsa-miR-4420	1135	2156	hsa-miR-873-5p	3177	4198
hsa-miR-4421	1136	2157	hsa-miR-874	3178	4199
hsa-miR-4422	1137	2158	hsa-miR-875-3p	3179	4200
hsa-miR-4423-3p	1138	2159	hsa-miR-875-5p	3180	4201
hsa-miR-4423-5p	1139	2160	hsa-miR-876-3p	3181	4202
hsa-miR-4424	1140	2161	hsa-miR-876-5p	3182	4203
hsa-miR-4425	1141	2162	hsa-miR-877-3p	3183	4204
hsa-miR-4426	1142	2163	hsa-miR-877-5p	3184	4205
hsa-miR-4427	1143	2164	hsa-miR-885-3p	3185	4206
hsa-miR-4428	1144	2165	hsa-miR-885-5p	3186	4207
hsa-miR-4429	1145	2166	hsa-miR-887	3187	4208
hsa-miR-4430	1146	2167	hsa-miR-888-3p	3188	4209
hsa-miR-443 1	1147	2168	hsa-miR-888-5p	3189	4210
hsa-miR-4432	1148	2169	hsa-miR-889	3190	421 1
hsa-miR-4433-3p	1149	2170	hsa-miR-890	3191	4212
hsa-miR-4433-5p	1150	2171	hsa-miR-891a	3192	4213
hsa-miR-4434	1151	2172	hsa-miR-891b	3193	4214
hsa-miR-443 5	1152	2173	hsa-miR-892a	3194	4215
hsa-miR-443 6a	1153	2174	hsa-miR-892b	3195	4216
hsa-miR-4436b-3p	1154	2175	hsa-miR-892c-3p	3196	4217
hsa-miR-4436b-5p	1155	2176	hsa-miR-892c-5p	3197	4218
hsa-miR-443 7	1156	2177	hsa-miR-920	3198	4219
hsa-miR-443 8	1157	2178	hsa-miR-921	3199	4220
hsa-miR-443 9	1158	2179	hsa-miR-922	3200	4221
hsa-miR-4440	1159	2180	hsa-miR-924	3201	4222
hsa-miR-4441	1160	2181	hsa-miR-92a- 1-5p	3202	4223
hsa-miR-4442	1161	2182	hsa-miR-92a-2-5p	3203	4224

hsa-miR-4443	1162	2183	hsa-miR-92a-3p	3204	4225
hsa-miR-4444	1163	2184	hsa-miR-92b-3p	3205	4226
hsa-miR-4445-3p	1164	2185	hsa-miR-92b-5p	3206	4227
hsa-miR-4445-5p	1165	2186	hsa-miR-933	3207	4228
hsa-miR-4446-3p	1166	2187	hsa-miR-93-3p	3208	4229
hsa-miR-4446-5p	1167	2188	hsa-miR-934	3209	4230
hsa-miR-4447	1168	2189	hsa-miR-935	3210	4231
hsa-miR-4448	1169	2190	hsa-miR-93-5p	321 1	4232
hsa-miR-4449	1170	2191	hsa-miR-936	3212	4233
hsa-miR-4450	1171	2192	hsa-miR-937-3p	3213	4234
hsa-miR-445 1	1172	2193	hsa-miR-937-5p	3214	4235
hsa-miR-4452	1173	2194	hsa-miR-938	3215	4236
hsa-miR-4453	1174	2195	hsa-miR-939-3p	3216	4237
hsa-miR-4454	1175	2196	hsa-miR-939-5p	3217	4238
hsa-miR-4455	1176	2197	hsa-miR-9-3p	3218	4239
hsa-miR-4456	1177	2198	hsa-miR-940	3219	4240
hsa-miR-4457	1178	2199	hsa-miR-941	3220	4241
hsa-miR-445 8	1179	2200	hsa-miR-942	3221	4242
hsa-miR-4459	1180	2201	hsa-miR-943	3222	4243
hsa-miR-4460	1181	2202	hsa-miR-944	3223	4244
hsa-miR-4461	1182	2203	hsa-miR-95	3224	4245
hsa-miR-4462	1183	2204	hsa-miR-9-5p	3225	4246
hsa-miR-4463	1184	2205	hsa-miR-96-3p	3226	4247
hsa-miR-4464	1185	2206	hsa-miR-96-5p	3227	4248
hsa-miR-4465	1186	2207	hsa-miR-98-3p	3228	4249
hsa-miR-4466	1187	2208	hsa-miR-98-5p	3229	4250
hsa-miR-4467	1188	2209	hsa-miR-99a-3p	3230	4251
hsa-miR-4468	1189	2210	hsa-miR-99a-5p	3231	4252
hsa-miR-4469	1190	221 1	hsa-miR-99b-3p	3232	4253
hsa-miR-4470	1191	2212	hsa-miR-99b-5p	3233	4254

[00350] As shown in Table 12, microRNAs are differentially expressed in different tissues and cells, and often associated with different types of diseases (e.g. cancer cells).

The decision of removal or insertion of microRNA binding sites, or any combination, is dependent on microRNA expression patterns and their profilings in cancer cells. In Table 12, "HCC" represents hepatocellular carcinoma, "ALL" stands for acute lymphoblastic leukemia, "RCC" stands for renal cell carcinoma, "CLL" stands for chronic lymphocytic leukemia and "MALT" stands for mucosa-associated lymphoid tissue.

Table 12 mirs, tissues/cell expression and diseases

microRNA	mir SEQ ID	BS SEQ ID	Tissues/cells	Associated disease	Biological function
hsa-let-7a-2-3p	171	1192	Embryonic stem cells, lung, myeloid cells	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor,
hsa-let-7a-3p	172	1193	Embryonic stem cells, lung	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor,
hsa-let-7a-5p	173	1194	Embryonic stem cells, lung	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor,
hsa-let-7b-3p	174	1195	epithelial cells, endothelial cells (vascular)	lung cancer, colorectal cancer, cervical cancer, inflammation and immune response after infection	tumor angiogenesis
hsa-let-7b-5p	175	1196	epithelial cells, endothelial cells (vascular)	cervical cancer, inflammation and immune response after infection	tumor angiogenesis
hsa-let-7c	176	1197	dendritic cells	various cancers (cervical, pancreatic, lung, esophageal, etc)	tumor suppressor, apoptosis
hsa-let-7d-3p	177	1198	embryonic stem cells	associated with various cancer cells	tumor suppressor
hsa-let-7d-5p	178	1199	embryonic stem cells	associated with various cancer cells	tumor suppressor
hsa-let-7e-3p	179	1200	immune cells	various cancer cells,	tumor suppressor

				autoimmunity, endotoxin tolerance	
hsa-let-7e-5p	180	1201	immune cells	various cancer cells	tumor suppressor
hsa-let-7f-1-3p	181	1202	immune cells (T cells)	various cancer cells	tumor suppressor
hsa-let-7f-2-3p	182	1203	immune cells (T cells)	various cancer cells	tumor suppressor
hsa-let-7f-5p	183	1204	immune cells (T cells)	Various cancer cells	tumor suppressor
hsa-let-7g-3p	184	1205	hematopoietic cells, adipose, smooth muscle cells	various cancer cells (lung, breast, etc)	tumor suppressor
hsa-let-7g-5p	185	1206	hematopoietic cells, adipose, smooth muscle cells	various cancer cells (lung, breast, etc)	tumor suppressor
hsa-let-7i-3p	186	1207	immune cells	chronic lymphocyte leukimia	tumor suppressor
hsa-let-7i-5p	187	1208	immune cells	chronic lymphocyte leukimia	tumor suppressor
hsa-miR-1	188	1209	muscle, heart		angiogenesis, cell proliferation(my ogenesis)
hsa-miR-100-3p	189	1210	hematopoietic cells, endothelial cells	gastric cancer, pancreatic cancer	tumor angiogenesis
hsa-miR-100-5p	190	1211	hematopoietic cells, endothelial cells	gastric cancer, pancreatic cancer	tumor angiogenesis
hsa-miR-101-3p	191	1212	endothelial cells	various cancers (breast,non-small cell lung, colon, gastric, pancreatic, bladder, etc); lupus erythematosus	angiogenesis
hsa-miR-101-5p	192	1213	endothelial cells	various cancers (breast,non-small cell lung, colon, gastric, pancreatic, bladder, etc); lupus erythematosus	angiogenesis
hsa-miR-103a-2-5p	193	1214	embryonic stem	various cancers	oncogene, cell

			cells, many tissues/cells	(endometrial, neuroblastoma, colorectal, breast, liver, etc)	growth
hsa-miR-103a-3p	194	1215	embryonic stem cells, many tissues/cells	various cancers (endometrial, neuroblastoma, colorectal, breast, liver, etc)	oncogene, cell growth
hsa-miR-103b	195	1216	Many tissues/cells	various cancers (endometrial, neuroblastoma, colorectal, breast, liver, etc)	oncogene, cell growth
hsa-miR-105-3p	196	1217	pancreatic cells		
hsa-miR-105-5p	197	1218	pancreatic cells		
hsa-miR-106a-3p	198	1219	osteogenic cells	osteocarcinoma, other cancers	cell differentiation
hsa-miR-106a-5p	199	1220	osteogenic cells	osteocarcinoma, other cancers	cell differentiation
hsa-miR-106b-3p	200	1221	embryonic stem cells	various cancers (non-small lung cancer, gastric cancer, HCC, gliomas, etc)	oncogene
hsa-miR-106b-5p	201	1222	embryonic stem cells	various cancers (non-small lung cancer, gastric cancer, HCC, gliomas, etc)	oncogene
hsa-miR-107	202	1223	many tissues, brain hepatocytes/liver	breast cancer, pituitary adenoma, obesity/diabetes	
hsa-miR-10a-3p	203	1224	hematopoietic cells	acute myeloid leukemia	oncogene, cell growth
hsa-miR-10a-5p	204	1225	hematopoietic cells	acute myeloid leukemia	oncogene, cell growth
hsa-miR-10b-3p	205	1226	multiple tissues and cells	various cancers (breast, ovarian, glioblastoma, pancreatic ductal adenocarcinoma, gastric, etc)	oncogene
hsa-miR-10b-5p	206	1227	multiple tissues and cells	various cancers (breast, ovarian, glioblastoma, pancreatic ductal	oncogene

				adenocarcinoma, gastric, etc)	
hsa-miR-1178-3p	207	1228		osteocarcoma	
hsa-miR-1178-5p	208	1229		osteocarcoma	
hsa-miR-1179	209	1230		osteocarcoma	
hsa-miR-1180	210	1231	discovered in sarcoma, no expression data		
hsa-miR-1181	211	1232		downregulated in ovarian cancer cells, associated with HCV infection in hepatocytes	
hsa-miR-1182	212	1233	placenta		
hsa-miR-1183	213	1234		associated with rectal cancer	
hsa-miR-1184	214	1235	Hematopoietic cells	downregulated in oral leukoplakia (OLK)	
hsa-miR-1185-1-3p	215	1236	placenta		
hsa-miR-1185-2-3p	216	1237	placenta		
hsa-miR-1185-5p	217	1238	placenta		
hsa-miR-1193	218	1239		melanoma	
hsa-miR-1197	219	1240		neublastoma	
hsa-miR-1200	220	1241		chronic lymphocytic leukemia	
hsa-miR-1202	221	1242		chronic lymphocytic leukemia, downregulated in ovarian cancer cells	
hsa-miR-1203	222	1243		in the chromosome 8q24 region, cancer cells	
hsa-miR-1204	223	1244		in the chromosome 8q24 region, cancer cells	
hsa-miR-1205	224	1245		in the chromosome 8q24 region, cancer cells	
hsa-miR-1206	225	1246		in the chromosome 8q24 region,	

				cancer cells	
hsa-miR-1207-3p	226	1247		in the chromosome 8q24 region, cancer cells	
hsa-miR-1207-5p	227	1248		in the chromosome 8q24 region, cancer cells	
hsa-miR-1208	228	1249		in the chromosome 8q24 region, cancer cells	
hsa-miR-122-3p	229	1250	kidney, liver/hepatocytes	Renal Cell Carcinoma (RCC), cancer cells	lipid metabolism
hsa-miR-1224-3p	230	1251		Lupus nephritis	
hsa-miR-1224-5p	231	1252		rectal cancer	
hsa-miR-1225-3p	232	1253		adrenal pheochromocytomas; upregulated in MITF KnockDown melanocytes	
hsa-miR-1225-5p	233	1254		prostate cancer	
hsa-miR-122-5p	234	1255	liver/hepatocytes	cancer cells	lipid metabolism
hsa-miR-1226-3p	235	1256	discovered in a mirtron screening		
hsa-miR-1226-5p	236	1257	discovered in a mirtron screening		
hsa-miR-1227-3p	237	1258	cartilage/chondrocytes		
hsa-miR-1227-5p	238	1259	cartilage/chondrocytes		
hsa-miR-1228-3p	239	1260	liver/hepatocytes	Hepatocellular carcinoma(HCC)	anti-apoptosis
hsa-miR-1228-5p	240	1261	liver/hepatocytes	Hepatocellular carcinoma(HCC)	anti-apoptosis
hsa-miR-1229-3p	241	1262	discovered in a mirtron screening		
hsa-miR-1229-5p	242	1263	discovered in a mirtron screening		
hsa-miR-1231	243	1264		HCC	
hsa-miR-1233-1-5p	244	1265	serum		
hsa-miR-1233-3p	245	1266	serum		
hsa-miR-1234-3p	246	1267	discovered in embryonic stem cell		

hsa-miR-1234-5p	247	1268	discovered in embryonic stem cell		
hsa-miR-1236-3p	248	1269	lymphatic endothelial cells		target to VEGFR-3
hsa-miR-1236-5p	249	1270	lymphatic endothelial cells		target to VEGFR-3
hsa-miR-1237-3p	250	1271	esophageal cell line KYSE-150R		
hsa-miR-1237-5p	251	1272	esophageal cell line KYSE-150R		
hsa-miR-1238-3p	252	1273		colorectal cancer	
hsa-miR-1238-5p	253	1274		colorectal cancer	
hsa-miR-1243	254	1275	discovered in embryonic stem cells		
hsa-miR-124-3p	255	1276	brain, plasma (exosomal)	glioma	cell differentiation
hsa-miR-1244	256	1277	discovered in embryonic stem cells		
hsa-miR-1245a	257	1278	discovered in embryonic stem cells		
hsa-miR-1245b-3p	258	1279	discovered in embryonic stem cells		
hsa-miR-1245b-5p	259	1280	discovered in embryonic stem cells		
hsa-miR-124-5p	260	1281	brain, Plasma (circulating)	upregulated in heart dysfunction, glioma	cell differentiation
hsa-miR-1246	261	1282	embryonic stem cells, epithelial cells		
hsa-miR-1247-3p	262	1283	embryoid body cells		
hsa-miR-1247-5p	263	1284	embryoid body cells		
hsa-miR-1248	264	1285			component of SnoRNAs
hsa-miR-1249	265	1286	liver(hepatocytes)		
hsa-miR-1250	266	1287	oligodendrocytes		
hsa-miR-1251	267	1288	discovered in embryonic stem cells		

hsa-miR-1252	268	1289	discovered in embryonic stem cells		
hsa-miR-1253	269	1290	discovered in embryonic stem cells		
hsa-miR-1254	270	1291	embryonic stem cells		
hsa-miR-1255a	271	1292	discovered in embryonic stem cells		
hsa-miR-1255b-2-3p	272	1293	discovered in embryonic stem cells		
hsa-miR-1255b-5p	273	1294	discovered in embryonic stem cells		
hsa-miR-1256	274	1295	discovered in embryonic stem cells	prostate cancer	
hsa-miR-1257	275	1296	discovered in embryonic stem cells	liposarcoma (soft tissue sarcoma)	
hsa-miR-1258	276	1297	discovered in embryonic stem cells	breast cancer and lung cancer	
hsa-miR-125a-3p	277	1298	brain, hematopoietic cells	various cancer (prostate, HCC, etc)	cell proliferation and differentiation
hsa-miR-125a-5p	278	1299	brain, hematopoietic cells	various cancer (prostate, HCC, etc)	cell proliferation and differentiation
hsa-miR-125b-1-3p	279	1300	hematopoietic cells (monocytes), brain(neuron)	various cancer (prostate, HCC, etc)	oncogene, cell differentiation
hsa-miR-125b-2-3p	280	1301	hematopoietic cells (monocytes), brain(neuron)	various cancer (prostate, HCC, etc)	oncogene, cell differentiation
hsa-miR-125b-5p	281	1302	hematopoietic cells, brain (neuron)	various cancer (cutaneous T cell lymphoma, prostate, HCC, etc)	oncogene, cell differentiation
hsa-miR-1260a	282	1303	periodontal tissue		
hsa-miR-1260b	283	1304	periodontal tissue		
hsa-miR-1261	284	1305	embryonic stem cells		
hsa-miR-1262	285	1306	embryoid body cells		

hsa-miR-1263	286	1307	discovered in embryonic stem cells		
hsa-miR-126-3p	287	1308	endothelial cells, lung	B-leage ALL	angiogenesis
hsa-miR-1264	288	1309	discovered in embryonic stem cells		
hsa-miR-1265	289	1310	discovered in embryonic stem cells		
hsa-miR-126-5p	290	131 1	endothelial cells, lung	breast cancer, B-leage ALL	angiogenesis
hsa-miR-1266	291	1312	embryonic stem cells		
hsa-miR-1267	292	1313	discovered in embryonic stem cells		
hsa-miR- 1268a	293	1314	embryonic stem cells		
hsa-miR- 1268b	294	1315	embryonic stem cells		
hsa-miR- 1269a	295	1316	embryoid body cells		
hsa-miR- 1269b	296	1317	embryoid body cells		
hsa-miR- 1270	297	1318	discovered in embryonic stem cells		
hsa-miR- 1271-3p	298	1319	brain	Hepatocellular carcinoma(HCC)	Suppress GPC-3 in HCC
hsa-miR- 1271-5p	299	1320	brain	Hepatocellular carcinoma(HCC)	Suppress GPC-3 in HCC
hsa-miR- 1272	300	1321	embryonic stem cells		
hsa-miR- 1273 a	301	1322	discovered in embryonic stem cells		
hsa-miR- 1273c	302	1323		colorectal cancer	
hsa-miR- 1273 d	303	1324	discovered in embryonic stem cells		
hsa-miR- 1273 e	304	1325		solid tumor cells	
hsa-miR- 1273 f	305	1326		cervical cancer	
hsa-miR- 1273g-3p	306	1327		cervical cancer	
hsa-miR- 1273g-5p	307	1328		cervical cancer	
hsa-miR- 127-3p	308	1329	lung, placenta		

hsa-miR-1275	309	1330	embryonic stem cells	gastric carcinoma	
hsa-miR-127-5p	310	1331	lung, placenta(islet)		
hsa-miR-1276	311	1332	discovered in embryonic stem cells		
hsa-miR-1277-3p	312	1333	embryoid body cells		
hsa-miR-1277-5p	313	1334	embryoid body cells		
hsa-miR-1278	314	1335	discovered in embryonic stem cells		
hsa-miR-1279	315	1336	monocytes		
hsa-miR-128	316	1337	glioblast, brain	B-leage ALL	target to neurofibrominlin neuron
hsa-miR-1281	317	1338		muscle invasive bladder cancer	
hsa-miR-1282	318	1339	discovered in embryonic stem cells		
hsa-miR-1283	319	1340	placenta		
hsa-miR-1284	320	1341		lung cancer	
hsa-miR-1285-3p	321	1342		various cancer cells	inhibit P53 expression
hsa-miR-1285-5p	322	1343		various cancer cells	inhibit P53 expression
hsa-miR-1286	323	1344	smooth muscle	esophageal cancer	
hsa-miR-1287	324	1345	embryoid body cells	breast cancer	
hsa-miR-1288	325	1346	discovered in embryonic stem cells		
hsa-miR-1289	326	1347	multiple cell types		
hsa-miR-1290	327	1348	embryoid body cells	gastric carcinoma	
hsa-miR-1291	328	1349	hepatocytes		component of SnoRNAs
hsa-miR-129-l-3p	329	1350	multiple cell types	HCC cancer cells	
hsa-miR-1292-3p	330	1351			
hsa-miR-129-2-3p	331	1352	multiple cell types	various cancer cells	
hsa-miR-1292-5p	332	1353			

hsa-miR-1293	333	1354	discovered in embryonic stem cells		
hsa-miR-1294	334	1355	discovered in embryonic stem cells		
hsa-miR-1295a	335	1356		tumor cells (follicular lymphoma)	
hsa-miR-1295b-3p	336	1357		tumor cells (follicular lymphoma)	
hsa-miR-1295b-5p	337	1358		tumor cells (follicular lymphoma)	
hsa-miR-129-5p	338	1359	liver(hepatocytes)	HCC, thyroid cancer	cell death in cancer cell
hsa-miR-1296	339	1360		breast cancer	
hsa-miR-1297	340	1361	discovered in embryonic stem cells		
hsa-miR-1298	341	1362			
hsa-miR-1299	342	1363	discovered in embryonic stem cells		
hsa-miR-1301	343	1364		breast cancer	
hsa-miR-1302	344	1365			
hsa-miR-1303	345	1366	hepatocyte	colorectal cancer, liver cancer	
hsa-miR-1304-3p	346	1367			dental development
hsa-miR-1304-5p	347	1368			dental development
hsa-miR-1305	348	1369	discovered in embryonic stem cells		
hsa-miR-1306-3p	349	1370	discovered in embryonic stem cells		
hsa-miR-1306-5p	350	1371	discovered in embryonic stem cells		
hsa-miR-1307-3p	351	1372	discovered in embryonic stem cells		
hsa-miR-1307-5p	352	1373	discovered in embryonic stem cells		

hsa-miR-130a-3p	353	1374	lung, monocytes, vascular endothelial cells	various cancers (basal cell carcinoma, HCC, ovarian, etc), drug resistance	pro-angiogenic
hsa-miR-130a-5p	354	1375	lung, monocytes, vascular endothelial cells	various cancers (basal cell carcinoma, HCC, ovarian, etc), drug resistance	pro-angiogenic
hsa-miR-130b-3p	355	1376	Lung, epidermal cells(keratinocytes)	various cancers (gastric, renal cell carcinoma)	cell proliferation/senescence
hsa-miR-130b-5p	356	1377	Lung, epidermal cells(keratinocytes)	various cancers (gastric, renal cell carcinoma)	cell proliferation/senescence
hsa-miR-1321	357	1378		neuroblastoma	
hsa-miR-1322	358	1379		neuroblastoma	
hsa-miR-1323	359	1380	placenta	neuroblastoma	
hsa-miR-132-3p	360	1381	Brain(neuron), immune cells		
hsa-miR-1324	361	1382		neuroblastoma	
hsa-miR-132-5p	362	1383	brain(neuron), immune cells		
hsa-miR-133a	363	1384	muscle, heart, epithelial cells (lung)	heart failure, esophageal cancer	myogenesis
hsa-miR-133b	364	1385	muscle, heart, epithelial cells (lung)	heart failure, esophageal cancer	myogenesis
hsa-miR-134	365	1386	lung (epithelial)	non-small cell lung cancer, pulmonary embolism	
hsa-miR-1343	366	1387		breast cancer cells	
hsa-miR-135a-3p	367	1388	brain, other tissues	various cancer cells (lung, breast, colorectal, HCC, etc)	tumor suppressor
hsa-miR-135a-5p	368	1389	brain, other tissues	various cancer cells (lung, breast, colorectal, HCC, etc)	tumor suppressor
hsa-miR-135b-3p	369	1390	brain, placenta, other tissues	various cancers (gastric, mammary, neuroblastomas, pancreatic, etc)	

hsa-miR-135b-5p	370	1391	brain, placenta, other tissues	various cancers (gastric, mammary,neurobl astomas, pancreatic, etc)	
hsa-miR-136-3p	371	1392	stem cells, placenta	glioma	tumor suppressor
hsa-miR-136-5p	372	1393	stem cells, placenta	glioma	tumor suppressor
hsa-miR-137	373	1394	brain	various cancers (glioblastoma, breast, gastric etc), Alzheimer's disease	inhibiting cancer cell proliferation and migration
hsa-miR-138-1-3p	374	1395	stem cells, epidermal cells(keratinocytes)	arious cancer cells, downregulated in HCC	cell proliferation/sen escence
hsa-miR-138-2-3p	375	1396	stem cells	arious cancer cells, downregulated in HCC	
hsa-miR-138-5p	376	1397	stem cells	arious cancer cells, downregulated in HCC	
hsa-miR-139-3p	377	1398	hematocytes, brain	various cancer cells (colorectal, gastric, ovarian)	repress cancer metastasis
hsa-miR-139-5p	378	1399	hematocytes, brain	various cancer cells (colorectal, gastric, ovarian)	repress cancer metastasis
hsa-miR-140-3p	379	1400	airway smooth muscle	Virus infection, cancers	
hsa-miR-140-5p	380	1401	cartilage (chondrocytes)	csncers	
hsa-miR-141-3p	381	1402	Many tissues/cells	various cancer cells (HCC, prostate, kidney, etc)	cell differentiation
hsa-miR-141-5p	382	1403	Many tissues/cells	various cancer cells (HCC, prostate, kidney, etc)	cell differentiation
hsa-miR-142-3p	383	1404	meyloid cells, hematopoiesis, APC cells		immune response
hsa-miR-142-5p	384	1405	meyloid cells, hematopoiesis, APC cells		immune response
hsa-miR-143-3p	385	1406	vascular smooth muscle	pre-B-cell acute lymphocytic	

				leukemia , virus infection	
hsa-miR-143-5p	386	1407	vascular smooth muscle, T-cells	virus infection	
hsa-miR-144-3p	387	1408	erythroid	various cancers (lung, colorectal, etc)	cell differentiation
hsa-miR-144-5p	388	1409	erythroid	various cancers (lung, colorectal, etc)	cell differentiation
hsa-miR-145-3p	389	1410	kidney, cartilage, vascular smooth muscle	T-cell lupus	tumor suppressor
hsa-miR-145-5p	390	1411	kidney, cartilage, vascular smooth muscle	T-cell lupus	tumor suppressor
hsa-miR-1468	391	1412		lung cancer	
hsa-miR-1469	392	1413		tumor cell(follicular lymphoma), rectal cancer	
hsa-miR-146a-3p	393	1414	immune cells, hematopoiesis	various cancers, endotoxin tolerance	
hsa-miR-146a-5p	394	1415	immune cells, hematopoiesis	various cancers, endotoxin tolerance	
hsa-miR-146b-3p	395	1416	immune cells	various cancers	
hsa-miR-146b-5p	396	1417	Embryonic stem cells	various cancers (glioma)	tumor invasion, migration
hsa-miR-1470	397	1418			
hsa-miR-1471	398	1419		tumor cell(follicular lymphoma), rectal cancer	
hsa-miR-147a	399	1420	Macrophage	inflammatory response	
hsa-miR-147b	400	1421	Macrophage	inflammatory response	
hsa-miR-148a-3p	401	1422	hematopoietic cells	CLL, T-lineage ALL	
hsa-miR-148a-5p	402	1423	hematopoietic cells	CLL, T-lineage ALL	
hsa-miR-148b-3p	403	1424	neuron		
hsa-miR-148b-5p	404	1425	neuron		
hsa-miR-149-3p	405	1426	heart, brain	various cancers (glioma,	

				colorectal, gastric, etc)	
hsa-miR-149-5p	406	1427	heart, brain	various cancers (glioma, colorectal, gastric, etc)	
hsa-miR-150-3p	407	1428	hematopoietic cells (lymphoid)	circulating plasma (acute myeloid leukemia)	
hsa-miR-150-5p	408	1429	hematopoietic cells (lymphoid)	circulating plasma (acute myeloid leukemia)	
hsa-miR-151a-3p	409	1430	neuron, fetal liver		
hsa-miR-151a-5p	410	1431	neuron, fetal liver		
hsa-miR-151b	411	1432	immune cells (B-cells)		
hsa-miR-152	412	1433	liver		
hsa-miR-153	413	1434	brain		
hsa-miR-1537	414	1435			
hsa-miR-1538	415	1436	blood	Cancer cells	
hsa-miR-1539	416	1437	esophageal cell line KYSE-150R		
hsa-miR-154-3p	417	1438	embryonic stem cells		
hsa-miR-154-5p	418	1439	embryonic stem cells		
hsa-miR-155-3p	419	1440	T/B cells, monocytes, breast	various cancers (CLL, B cell lymphoma, breast, lung, ovarian, cervical, colorectal, prostate)	
hsa-miR-155-5p	420	1441	T/B cells, monocytes, breast	various cancers (CLL, B cell lymphoma, breast, lung, ovarian, cervical, colorectal, prostate)	
hsa-miR-1587	421	1442	identified in B-cells		
hsa-miR-15a-3p	422	1443	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation

hsa-miR- 15a-5p	423	1444	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR- 15b-3p	424	1445	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR- 15b-5p	425	1446	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR- 16-l-3p	426	1447	embryonic stem cells, blood, hematopoietic tissues (spleen)		
hsa-miR- 16-2-3p	427	1448	blood, lymphocyte, hematopoietic tissues (spleen)		
hsa-miR- 16-5p	428	1449	Many tissues, blood		
hsa-miR- 17-3p	429	1450	embryonic stem cells, endothelial cells,		tumor angiogenesis
hsa-miR- 17-5p	430	1451	endothelial cells, kidney, breast;		tumor angiogenesis
hsa-miR- 181 a-2-3p	431	1452	glioblast, stem cells		
hsa-miR-181a-3p	432	1453	glioblast, myeloid cells, Embryonic stem cells		
hsa-miR-181a-5p	433	1454	glioblast, myeloid cells, Embryonic stem cells		
hsa-miR- 181b-3p	434	1455	glioblast, Embryonic stem cells , epidermal (keratinocytes)		cell proiferation/sene scence
hsa-miR- 181b-5p	435	1456	glioblast, Embryonic stem cells , epidermal (keratinocytes)		cell proiferation/sene scence
hsa-miR- 181c-3p	436	1457	brain, stem cells/progenitor	variou cance cells (gliobasloma, basal cell carcinoma, prostate)	cell differentiation
hsa-miR- 181c-5p	437	1458	brain, stem cells/progenitor	variou cance cells (gliobasloma,	cell differentiation

				basal cell carcinoma, prostate)	
hsa-miR-181d	438	1459	glia cells		
hsa-miR-182-3p	439	1460	immune cells	autoimmune	immune response
hsa-miR-1825	440	1461	discovered in a MiRDeep screening		
hsa-miR-182-5p	441	1462	lung, immune cells	autoimmune	immune response
hsa-miR-1827	442	1463		small cell lung cancer	
hsa-miR-183-3p	443	1464	brain		
hsa-miR-183-5p	444	1465	brain		
hsa-miR-184	445	1466	blood, tongue, pancreas (islet)		
hsa-miR-185-3p	446	1467			
hsa-miR-185-5p	447	1468			
hsa-miR-186-3p	448	1469	osteoblasts, heart	various cancer cells	
hsa-miR-186-5p	449	1470	osteoblasts, heart	various cancer cells	
hsa-miR-187-3p	450	1471		thyroid tumor	
hsa-miR-187-5p	451	1472		thyroid tumor	
hsa-miR-188-3p	452	1473	irway smooth muscle, central nervous system		
hsa-miR-188-5p	453	1474	irway smooth muscle, central nervous system		
hsa-miR-18a-3p	454	1475	endothelial cells, lung		
hsa-miR-18a-5p	455	1476	endothelial cells, lung		
hsa-miR-18b-3p	456	1477	lung		
hsa-miR-18b-5p	457	1478	lung		
hsa-miR-1908	458	1479		breast cancer	
hsa-miR-1909-3p	459	1480		rectal cancer	
hsa-miR-1909-5p	460	1481		rectal cancer	
hsa-miR-190a	461	1482	brain		
hsa-miR-190b	462	1483	brain		

hsa-miR-1910	463	1484	embryonic stem cells		
hsa-miR-1911-3p	464	1485	embryonic stem cells, neural precursor		
hsa-miR-1911-5p	465	1486	embryonic stem cells, neural precursor		
hsa-miR-1912	466	1487	embryonic stem cells, neural precursor		
hsa-miR-1913	467	1488	embryonic stem cells		
hsa-miR-191-3p	468	1489		chronic lymphocyte leukemia, B-lineage ALL	
hsa-miR-1914-3p	469	1490	embryonic stem cells		
hsa-miR-1914-5p	470	1491	embryonic stem cells		
hsa-miR-1915-3p	471	1492	embryonic stem cells		
hsa-miR-1915-5p	472	1493	embryonic stem cells		
hsa-miR-191-5p	473	1494		chronic lymphocyte leukemia, B-lineage ALL	
hsa-miR-192-3p	474	1495	kidney		
hsa-miR-192-5p	475	1496	kidney		
hsa-miR-193a-3p	476	1497	many tissues/cells	various cancer cells (lung, osteoblastoma, ALL, follicular lymphoma, etc)	tumor suppressor, proliferation
hsa-miR-193a-5p	477	1498	many tissues/cells	various cancer cells (lung, osteoblastoma, ALL, follicular lymphoma, etc)	tumor suppressor, proliferation
hsa-miR-193b-3p	478	1499	many tissues/cells, semen	various cancer cells (prostate, breast, melanoma, myeloma, non small cell lung, etc)follicular lymphoma)	tumor suppressor
hsa-miR-193b-5p	479	1500	many tissues/cells,	various cancer cells	tumor

			semen	(prostate, breast, melanoma, myeloma, non small cell lung, etc)follicular lymphoma)	suppressor
hsa-miR-194-3p	480	1501	kidney,liver	various cancers	
hsa-miR-194-5p	481	1502	kidney,liver	various cancers	
hsa-miR-195-3p	482	1503	breast, pancreas (islet)		
hsa-miR-195-5p	483	1504	breast, pancreas (islet)		
hsa-miR-196a-3p	484	1505	pancreatic cells,endometrial tissues, mesenchymal stem cells	various cancer cells (pancreatic, osteosarcoma, endometrial, AML etc)	oncogenic, tumor suppressor
hsa-miR-196a-5p	485	1506	pancreatic cells,endometrial tissues, mesenchymal stem cells	various cancer cells (pancreatic, osteosarcoma, endometrial, AML etc)	oncogenic, tumor suppressor
hsa-miR-196b-3p	486	1507	endometrial tissues	glioblastoma	apoptosis
hsa-miR-196b-5p	487	1508	endometrial tissues	glioblastoma	apoptosis
hsa-miR-1972	488	1509		acute lymphoblastic leukemia	
hsa-miR-1973	489	1510		acute lymphoblastic leukemia	
hsa-miR-197-3p	490	151 1	blood (myeloid), other tissues/cells	various cancers (thyroid tumor, leukemia, etc)	
hsa-miR-197-5p	491	1512	blood (myeloid), other tissues/cells	various cancers (thyroid tumor, leukemia, etc)	
hsa-miR-1976	492	1513		acute lymphoblastic leukemia	
hsa-miR-198	493	1514	central nevous system(CNS)		
hsa-miR-199a-3p	494	1515	liver, embryoid body cells, cardiomyocytes		
hsa-miR-199a-5p	495	1516	liver, cardiomyocytes		
hsa-miR-199b-3p	496	1517	liver, osteoblast	various cancers	osteogenesis

hsa-miR-199b-5p	497	1518	liver, osteoblast	various cancers	osteogenesis
hsa-miR-19a-3p	498	1519	endothelial cells		tumor angiogenesis
hsa-miR-19a-5p	499	1520	endothelial cells		tumor angiogenesis
hsa-miR-19b-1-5p	500	1521	endothelial cells		tumor angiogenesis
hsa-miR-19b-2-5p	501	1522	endothelial cells		tumor angiogenesis
hsa-miR-19b-3p	502	1523	endothelial cells		tumor angiogenesis
hsa-miR-200a-3p	503	1524	epithelial cells, many other tissues	various cancers (breast, cervical, bladder, etc)	tumor progression and metastasis
hsa-miR-200a-5p	504	1525	epithelial cells, many other tissues	various cancers (breast, cervical, bladder, etc)	tumor progression and metastasis
hsa-miR-200b-3p	505	1526	epithelial cells, many other tissues		tumor progression and metastasis
hsa-miR-200b-5p	506	1527	epithelial cells, many other tissues		tumor progression and metastasis
hsa-miR-200c-3p	507	1528	epithelial cells, many other tissues, embryonic stem cells		tumor progression and metastasis
hsa-miR-200c-5p	508	1529	epithelial cells, many other tissues, embryonic stem cells		tumor progression and metastasis
hsa-miR-202-3p	509	1530	blood	lymphomagenesis, other cancers	
hsa-miR-202-5p	510	1531	blood	lymphomagenesis, other cancers	
hsa-miR-203a	511	1532	skin (epithelium)	psoriasis, autoimmune	
hsa-miR-203b-3p	512	1533	skin specific (epithelium)	psoriasis, autoimmune	
hsa-miR-203b-5p	513	1534	skin specific (epithelium)	psoriasis, autoimmune	
hsa-miR-204-3p	514	1535	adipose, other tissues/cells. kidney	various cancers	tumor metastasis
hsa-miR-204-5p	515	1536	adipose, other tissues/cells, kidney	various cancers	tumor metastasis
hsa-miR-2052	516	1537			

hsa-miR-2053	517	1538			
hsa-miR-205-3p	518	1539	blood(plasma)	various cancer cells (breast, glioma, melanoma, endometrial, etc)	
hsa-miR-2054	519	1540			
hsa-miR-205-5p	520	1541	blood(plasma)	various cancer cells (breast, glioma, melanoma, endometrial, etc)	
hsa-miR-206	521	1542	muscle (cardiac and skeletal)		myogenesis
hsa-miR-208a	522	1543	heart(cardiomyocyte), muscle	cardiac defects	
hsa-miR-208b	523	1544	heart(cardiomyocyte), muscle	cardiac defects	
hsa-miR-20a-3p	524	1545	endothelial cells, kidney, osteogenic cells		
hsa-miR-20a-5p	525	1546	endothelial cells, kidney, osteogenic cells		
hsa-miR-20b-3p	526	1547	osteogenic cells		
hsa-miR-20b-5p	527	1548	osteogenic cells		
hsa-miR-210	528	1549	kidney, heart, vascular endothelial cells	RCC, B-cell lymphocytes	angiogenesis
hsa-miR-2110	529	1550		rectal cancer	
hsa-miR-2113	530	1551	embryonic stem cells		
hsa-miR-211-3p	531	1552	melanocytes	melanoma and other cancers	
hsa-miR-2114-3p	532	1553	ovary, female reproductive tract		
hsa-miR-2114-5p	533	1554	ovary, female reproductive tract		
hsa-miR-2115-3p	534	1555	female reproductive tract	ovarian cancer	
hsa-miR-2115-5p	535	1556	female reproductive tract	ovarian cancer	
hsa-miR-211-5p	536	1557	melanocytes	melanoma and other cancers	
hsa-miR-2116-3p	537	1558		live cancer(hepatocytes) and ovarian cancer	
hsa-miR-2116-5p	538	1559		live	

				cancer(hepatocytes) and ovarian cancer	
hsa-miR-21 17	539	1560		ovarian cancer	
hsa-miR-212-3p	540	1561	brain(neuron), spleen	lymphoma	
hsa-miR-212-5p	541	1562	brain(neuron), spleen	lymphoma	
hsa-miR-2 1-3p	542	1563	glioblast, Blood (meyloid cells), liver, vascular endothelial cells	autoimmune, heart diseases, cancers	
hsa-miR-214-3p	543	1564	immune cerlls, pancreas	varioua cancers (melanoma, pancreatic, ovarian)	immune response
hsa-miR-2 14-5p	544	1565	immune cells, pancreas	varioua cancers (melanoma, pancreatic, ovarian)	immune response
hsa-miR-2 15	545	1566	many tissues/cells	various cancers (renal, colon, osteosarcoma)	cell cycle arrest/p53 inducible
hsa-miR-2 1-5p	546	1567	blood (myeloid cells), liver, endothelial cells	autoimmune, heart diseases, cancers	
hsa-miR-2 16a-3p	547	1568	kidney, pancreas		
hsa-miR-2 16a-5p	548	1569	kidney, pancreas		
hsa-miR-2 16b	549	1570		cancers	senescence
hsa-miR-2 17	550	1571	endothelial cells	various cancer cells (pancreas, kidney, breast)	
hsa-miR-2 18-1-3p	551	1572	endothelial cells	various cancer cells (gastric tumor, bladder, cervical, etc)	
hsa-miR-2 18-2-3p	552	1573		various cancer cells (gastric tumor, bladder, cervical, etc)	
hsa-miR-2 18-5p	553	1574		various cancer cells (gastric tumor, bladder, cervical, etc)	
hsa-miR-2 19-1-3p	554	1575	brain, oligodendrocytes		
hsa-miR-2 19-2-3p	555	1576	brain, oligodendrocytes		

hsa-miR-219-5p	556	1577	brain, oligodendrocytes		
hsa-miR-22-1-3p	557	1578	endothelial cells, immune cells	leukemia and other cancers	angiogenesis/vas culogenesis
hsa-miR-22-1-5p	558	1579	endothelial cells, immune cells	leukemia and other cancers	angiogenesis/vas culogenesis
hsa-miR-222-3p	559	1580	endothelial cells	various cancers	angiogenesis
hsa-miR-222-5p	560	1581	endothelial cells	various cancers	angiogenesis
hsa-miR-223-3p	561	1582	myeloid cells	leukemia	
hsa-miR-223-5p	562	1583	myeloid cells	leukemia	
hsa-miR-22-3p	563	1584	many tissues/cells	various cancers	tumorigenesis
hsa-miR-224-3p	564	1585	blood(plasma), ovary	cancers and inflammation	
hsa-miR-224-5p	565	1586	blood(plasma), ovary	cancers and inflammation	
hsa-miR-22-5p	566	1587	many tissues/cells	Various cancers	tumorigenesis
hsa-miR-2276	567	1588		breast cancer	
hsa-miR-2277-3p	568	1589	female reproductive tract		
hsa-miR-2277-5p	569	1590	female reproductive tract		
hsa-miR-2278	570	1591		breast cancer	
hsa-miR-2355-3p	571	1592	embryonic stem cells		
hsa-miR-2355-5p	572	1593	embryonic stem cells		
hsa-miR-2392	573	1594	identified in B- cells		
hsa-miR-23a-3p	574	1595	brain(astrocyte), endothelial cells, blood(erythroid)	Cancers	
hsa-miR-23a-5p	575	1596	brain(astrocyte), endothelial cells, blood(erythroid)	cancers	
hsa-miR-23b-3p	576	1597	blood, myeloid cells	cancers (renal cancer, glioblastoma, prostate, etc) and autoimmune	
hsa-miR-23b-5p	577	1598	blood, myeloid cells	cancers(glioblasto ma, prostate, etc) and autoimmune	
hsa-miR-23c	578	1599		cervical cancer	
hsa-miR-24-1-5p	579	1600	lung, myeloid cells		

hsa-miR-24-2-5p	580	1601	lung, meylold cells		
hsa-miR-24-3p	581	1602	lung, meylold cells		
hsa-miR-2467-3p	582	1603		breast cancer	
hsa-miR-2467-5p	583	1604		breast cancer	
hsa-miR-25-3p	584	1605	embryonic stem cells, airway smooth muscle		
hsa-miR-25-5p	585	1606	embryonic stem cells, airway smooth muscle		
hsa-miR-2681-3p	586	1607		breast cancer	
hsa-miR-2681-5p	587	1608		breast cancer	
hsa-miR-2682-3p	588	1609			
hsa-miR-2682-5p	589	1610			
hsa-miR-26a- 1-3p	590	161 1	embryonic stem cells, blood , other tissues	CLL and other cancers	cell cycle and differentiation
hsa-miR-26a-2-3p	591	1612	blood , other tissues	CLL and other cancers	cell cycle and differentiation
hsa-miR-26a-5p	592	1613	blood , other tissues	CLL and other cancers	cell cycle and differentiation
hsa-miR-26b-3p	593	1614	hematopoietic cells		
hsa-miR-26b-5p	594	1615	hematopoietic cells		
hsa-miR-27a-3p	595	1616	meyloid cells	various cancer cells	
hsa-miR-27a-5p	596	1617	meyloid cells	various cancer cells	
hsa-miR-27b-3p	597	1618	meyloid cells, vascular endothelial cells	various cancer cells	pro-angiogenic
hsa-miR-27b-5p	598	1619	meyloid cells, vascular endothelial cells	various cancer cells	pro-angiogenic
hsa-miR-28-3p	599	1620	blood(immune cells)	B/T cell lymphoma	
hsa-miR-28-5p	600	1621	blood(immune cells)	B/T cell lymphoma	
hsa-miR-2861	601	1622	osteoblasts	basal cell carcinoma	
hsa-miR-2909	602	1623	T-Lymphocytes		
hsa-miR-296-3p	603	1624	kidney, heart, lung, entothelial cells		angiogenesis
hsa-miR-2964a-3p	604	1625			

hsa-miR-2964a-5p	605	1626			
hsa-miR-296-5p	606	1627	lung, liver, endothelial cells		angiogenesis
hsa-miR-297	607	1628	oocyte and prostate		
hsa-miR-298	608	1629		breast cancer	
hsa-miR-299-3p	609	1630		myeloid leukaemia, hepatoma, breast cancer	
hsa-miR-299-5p	610	1631		myeloid leukaemia, hepatoma, breast cancer	
hsa-miR-29a-3p	611	1632	immuno system	CLL, other cancers, neurodegenerative disease	tumor suppression, immune modulation
hsa-miR-29a-5p	612	1633	immuno system	CLL, other cancers, neurodegenerative disease	tumor suppression, immune modulation
hsa-miR-29b-1-5p	613	1634	immuno system	CLL, other cancers, neurodegenerative disease	tumor suppression, immune modulation
hsa-miR-29b-2-5p	614	1635	immuno system	CLL, other cancers	tumor suppression, immune modulation
hsa-miR-29b-3p	615	1636	immuno system	CLL, other cancers	tumor suppression, immune modulation
hsa-miR-29c-3p	616	1637	immuno system	CLL, other cancers	tumor suppression, immune modulation
hsa-miR-29c-5p	617	1638	immuno system	CLL, other cancers	tumor suppression, immune modulation
hsa-miR-300	618	1639	osteoblast	Bladder cancer	
hsa-miR-301a-3p	619	1640	embryonic stem cells		
hsa-miR-301a-5p	620	1641	embryonic stem cells		
hsa-miR-301b	621	1642		esophageal adenocarcinoma,	

				colonic cancer	
hsa-miR-302a-3p	622	1643	embryonic stem cells, lipid metabolism		lipid metabolism
hsa-miR-302a-5p	623	1644	embryonic stem cells, lipid metabolism		lipid metabolism
hsa-miR-302b-3p	624	1645	embryonic stem cells		
hsa-miR-302b-5p	625	1646	embryonic stem cells		
hsa-miR-302c-3p	626	1647	embryonic stem cells		
hsa-miR-302c-5p	627	1648	embryonic stem cells		
hsa-miR-302d-3p	628	1649	embryonic stem cells		
hsa-miR-302d-5p	629	1650	embryonic stem cells		
hsa-miR-302e	630	1651	embryoid body cells		
hsa-miR-302f	631	1652		gastric cancer	
hsa-miR-3064-3p	632	1653			
hsa-miR-3064-5p	633	1654			
hsa-miR-3065-3p	634	1655	oligodendrocytes	anti-virus response	
hsa-miR-3065-5p	635	1656	oligodendrocytes	solid tumors	
hsa-miR-3074-3p	636	1657		various cancer(melanoma, breast)	
hsa-miR-3074-5p	637	1658		various cancer(melanoma, breast)	
hsa-miR-30a-3p	638	1659	kidney, pancreatic cells	various cancers	autophagy
hsa-miR-30a-5p	639	1660	CNS(prefrontal cortex), other tissues	glioma, colon carcinoma	autophagy
hsa-miR-30b-3p	640	1661	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30b-5p	641	1662	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30c-l-3p	642	1663	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30c-2-3p	643	1664	kidney, adipose,		

			CNS(prefrontal cortex)		
hsa-miR-30c-5p	644	1665	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30d-3p	645	1666	CNS (prefrontal cortex)		
hsa-miR-30d-5p	646	1667	CNS (prefrontal cortex, embryoid body cells)		
hsa-miR-30e-3p	647	1668	myeloid cells, glia cells		
hsa-miR-30e-5p	648	1669	myeloid cells, glia cells		
hsa-miR-3 115	649	1670		various cancer (melanoma, breast tumor)	
hsa-miR-3 116	650	1671	discovered in the melanoma miRNAome		
hsa-miR-3 117-3p	651	1672	discovered in the melanoma miRNAome		
hsa-miR-3 117-5p	652	1673	discovered in the melanoma miRNAome		
hsa-miR-3 118	653	1674	discovered in the melanoma miRNAome		
hsa-miR-3 119	654	1675	discovered in the melanoma miRNAome		
hsa-miR-3 120-3p	655	1676	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 120-5p	656	1677	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 121-3p	657	1678	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 121-5p	658	1679	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 122	659	1680	discovered in the melanoma miRNAome		
hsa-miR-3 123	660	1681	discovered in the melanoma miRNAome		

hsa-miR-3124-3p	661	1682	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3124-5p	662	1683	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3125	663	1684	discovered in the melanoma miRNAome		
hsa-miR-3 126-3p	664	1685	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 126-5p	665	1686	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 127-3p	666	1687	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 127-5p	667	1688	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 128	668	1689	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 129-3p	669	1690	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 129-5p	670	1691	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 130-3p	671	1692	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 130-5p	672	1693	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 131	673	1694	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 132	674	1695	discovered in the melanoma miRNAome		
hsa-miR-3 133	675	1696	discovered in the melanoma miRNAome		
hsa-miR-3 134	676	1697	discovered in the melanoma miRNAome		
hsa-miR-3 135a	677	1698	discovered in the melanoma		

			miRNAome		
hsa-miR-3135b	678	1699	discovered in B cells		
hsa-miR-3136-3p	679	1700	discovered in the melanoma miRNAome	lymphoblastic leukaemia and breast tumor	
hsa-miR-3136-5p	680	1701	discovered in the melanoma miRNAome	lymphoblastic leukaemia and breast tumor	
hsa-miR-3137	681	1702	discovered in the melanoma miRNAome		
hsa-miR-3138	682	1703	discovered in the melanoma miRNAome, ovary		
hsa-miR-3139	683	1704	discovered in the melanoma miRNAome		
hsa-miR-31-3p	684	1705			
hsa-miR-3140-3p	685	1706	discovered in the melanoma miRNAome, ovary	lymphoblastic leukaemia and breast tumor	
hsa-miR-3140-5p	686	1707	discovered in the melanoma miRNAome, ovary	lymphoblastic leukaemia and breast tumor	
hsa-miR-3141	687	1708	discovered in the melanoma miRNAome		
hsa-miR-3142	688	1709	discovered in the melanoma miRNAome; immune cells		
hsa-miR-3143	689	1710	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3144-3p	690	1711	discovered in the melanoma miRNAome, ovary		
hsa-miR-3144-5p	691	1712	discovered in the melanoma miRNAome, ovary		
hsa-miR-3145-3p	692	1713	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3145-5p	693	1714	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3146	694	1715	discovered in the melanoma	breast tumor	

			miRNAome		
hsa-miR-3147	695	1716	discovered in the melanoma miRNAome		
hsa-miR-3148	696	1717	discovered in the melanoma miRNAome		
hsa-miR-3149	697	1718	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 150a-3p	698	1719	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 150a-5p	699	1720	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 150b-3p	700	1721	discovered in the melanoma miRNAome	breast tumor and lymphoblastic leukaemia	
hsa-miR-3 150b-5p	701	1722	discovered in the melanoma miRNAome	breast tumor and lymphoblastic leukaemia	
hsa-miR-3 151	702	1723	discovered in the melanoma miRNAome	lymphoblastic leukaemia	
hsa-miR-3 152-3p	703	1724	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 152-5p	704	1725	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 153	705	1726	discovered in the melanoma miRNAome		
hsa-miR-3 154	706	1727	discovered in the melanoma miRNAome	lymphoblastic leukaemia	
hsa-miR-3 155a	707	1728	discovered in the melanoma miRNAome		
hsa-miR-3 155b	708	1729	discovered in B cells		
hsa-miR-3 156-3p	709	1730	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 156-5p	710	1731	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 157-3p	711	1732	discovered in the melanoma	breast tumor	

			miRNAome		
hsa-miR-3157-5p	712	1733	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3158-3p	713	1734	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3158-5p	714	1735	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3159	715	1736	discovered in the melanoma miRNAome		
hsa-miR-3 1-5p	716	1737		various cancer cells (breast, lung, prostate)	
hsa-miR-3160-3p	717	1738	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 160-5p	718	1739	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 161	719	1740	discovered in the melanoma miRNAome		
hsa-miR-3 162-3p	720	1741	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 162-5p	721	1742	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 163	722	1743	discovered in the melanoma miRNAome		
hsa-miR-3 164	723	1744	discovered in the melanoma miRNAome		
hsa-miR-3 165	724	1745	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 166	725	1746	discovered in the melanoma miRNAome		
hsa-miR-3 167	726	1747	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 168	727	1748	discovered in the melanoma miRNAome		

hsa-miR-3169	728	1749	discovered in the melanoma miRNAome		
hsa-miR-3170	729	1750	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3171	730	1751	discovered in the melanoma miRNAome, ovary		
hsa-miR-3173-3p	731	1752	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3173-5p	732	1753	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3174	733	1754	discovered in the melanoma miRNAome		
hsa-miR-3 175	734	1755	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 176	735	1756	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 177-3p	736	1757	discovered in the melanoma miRNAome	breast tumor and lymphoblastic leukaemia	
hsa-miR-3 177-5p	737	1758	discovered in the melanoma miRNAome	breast tumor and lymphoblastic leukaemia	
hsa-miR-3 178	738	1759	discovered in the melanoma miRNAome		
hsa-miR-3 179	739	1760	discovered in the melanoma miRNAome		
hsa-miR-3 180	740	1761	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 180-3p	741	1762	discovered in breast tumor		
hsa-miR-3 180-5p	742	1763	discovered in breast tumor		
hsa-miR-3 181	743	1764	discovered in the melanoma miRNAome		
hsa-miR-3 182	744	1765	discovered in the melanoma miRNAome		

hsa-miR-3183	745	1766	discovered in the melanoma miRNAome		
hsa-miR-3184-3p	746	1767	discovered in the melanoma miRNAome		
hsa-miR-3184-5p	747	1768	discovered in the melanoma miRNAome		
hsa-miR-3185	748	1769	discovered in the melanoma miRNAome		
hsa-miR-3186-3p	749	1770	discovered in the melanoma miRNAome, ovary		
hsa-miR-3186-5p	750	1771	discovered in the melanoma miRNAome, ovary		
hsa-miR-3187-3p	751	1772	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3187-5p	752	1773	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3188	753	1774	discovered in the melanoma miRNAome		
hsa-miR-3189-3p	754	1775	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3189-5p	755	1776	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3190-3p	756	1777	discovered in the melanoma miRNAome	lymphoblastic leukaemia	
hsa-miR-3190-5p	757	1778	discovered in the melanoma miRNAome	lymphoblastic leukaemia	
hsa-miR-3191-3p	758	1779	discovered in the melanoma miRNAome		
hsa-miR-3191-5p	759	1780	discovered in the melanoma miRNAome		
hsa-miR-3192	760	1781	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3193	761	1782	discovered in the melanoma		

			miRNAome		
hsa-miR-3194-3p	762	1783	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3194-5p	763	1784	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3195	764	1785	discovered in the melanoma miRNAome		
hsa-miR-3196	765	1786		basal cell carcinoma	
hsa-miR-3197	766	1787	discovered in the melanoma miRNAome		
hsa-miR-3198	767	1788	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3199	768	1789	discovered in the melanoma miRNAome		
hsa-miR-3200-3p	769	1790	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3200-5p	770	1791	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3201	771	1792	discovered in the melanoma miRNAome,		
hsa-miR-3202	772	1793	discovered in the melanoma miRNAome, epithelial cell BEAS2B		
hsa-miR-320a	773	1794	blood, heart(myocardiac)	colon cancer cells, heart disease	
hsa-miR-320b	774	1795	central nervous system		
hsa-miR-320c	775	1796	chondrocyte		cartilage metabolism
hsa-miR-320d	776	1797		cancer stem cells	
hsa-miR-320e	777	1798	neural cells		
hsa-miR-323a-3p	778	1799	neurons	myeloid leukaemia, mudulla thyroid carcinoma	
hsa-miR-323a-5p	779	1800	neurons	myeloid leukaemia, mudulla thyroid	

				carcinoma	
hsa-miR-323b-3p	780	1801		myeloid leukaemia	
hsa-miR-323b-5p	781	1802		myeloid leukaemia	
hsa-miR-32-3p	782	1803	blood, glia	various cancers (lung, kidney, prostate, etc), virus infection	
hsa-miR-324-3p	783	1804	kidney		
hsa-miR-324-5p	784	1805	neurons	tumor cells	
hsa-miR-325	785	1806	neurons, placenta		
hsa-miR-32-5p	786	1807	blood, glia	various cancers (lung, kidney, prostate, etc), virus infection	
hsa-miR-326	787	1808	neurons	tumor cells	
hsa-miR-328	788	1809	neuron, blood	tumor cells	
hsa-miR-329	789	1810	brain and platelet		
hsa-miR-330-3p	790	1811		various cancers(prostate, glioblastoma, colorectal)	
hsa-miR-330-5p	791	1812		various cancers(prostate, glioblastoma, colorectal)	
hsa-miR-331-3p	792	1813		gastric cancer	
hsa-miR-331-5p	793	1814	lymphocytes		
hsa-miR-335-3p	794	1815	kidney, breast	RCC, multiple myeloma	
hsa-miR-335-5p	795	1816	kidney, breast	RCC, multiple myeloma	
hsa-miR-337-3p	796	1817	lung	gastric cancer	
hsa-miR-337-5p	797	1818	lung		
hsa-miR-338-3p	798	1819	epithelial cells, oligodendrocytes	gastric, rectal cancer cells, osteosarcoma	
hsa-miR-338-5p	799	1820	oligodendrocytes	gastric cancer	
hsa-miR-339-3p	800	1821	immune cell		
hsa-miR-339-5p	801	1822	immune cell		
hsa-miR-33a-3p	802	1823	pancreatic islet, lipid metabolism		lipid metabolism
hsa-miR-33a-5p	803	1824	pancreatic islet,		lipid metabolism

			lipid metabolism		
hsa-miR-33b-3p	804	1825	lipid metabolism		lipid metabolism
hsa-miR-33b-5p	805	1826	lipid metabolism		lipid metabolism
hsa-miR-340-3p	806	1827		various cancers	
hsa-miR-340-5p	807	1828	embryoid body cells		
hsa-miR-342-3p	808	1829	brain, circulating plasma	multiple myeloma, other cancers	
hsa-miR-342-5p	809	1830	circulating plasma	multiple myeloma, other cancers	
hsa-miR-345-3p	810	1831	hematopoietic cells	follicular lymphoma, other cancers	
hsa-miR-345-5p	811	1832	hematopoietic cells	follicular lymphoma, other cancers	
hsa-miR-346	812	1833	immune cells	cancers and autoimmune	
hsa-miR-34a-3p	813	1834	breast, myeloid cells, ciliated epithelial cells	gastric cancer, CLL, other	tumor suppressor, p53 inducible
hsa-miR-34a-5p	814	1835	breast, myeloid cells, ciliated epithelial cells	gastric cancer, CLL, other	tumor suppressor, p53 inducible
hsa-miR-34b-3p	815	1836	ciliated epithelial cells	various cancers	tumor suppressor, p53 inducible
hsa-miR-34b-5p	816	1837	ciliated epithelial cells	various cancers	tumor suppressor, p53 inducible
hsa-miR-34c-3p	817	1838	ciliated epithelial cells, placenta	various cancers	tumor suppressor, p53 inducible
hsa-miR-34c-5p	818	1839	ciliated epithelial cells, placenta	various cancers	tumor suppressor, p53 inducible
hsa-miR-3529-3p	819	1840	discovered in breast tumor		
hsa-miR-3529-5p	820	1841	discovered in breast tumor		
hsa-miR-3591-3p	821	1842	discovered in breast tumor		
hsa-miR-3591-5p	822	1843	discovered in breast tumor		
hsa-miR-3605-3p	823	1844	discovered in reproductive tracts		
hsa-miR-3605-5p	824	1845	discovered in reproductive tracts		

hsa-miR-3606-3p	825	1846	discovered in cervical tumors		
hsa-miR-3606-5p	826	1847	discovered in cervical tumors		
hsa-miR-3607-3p	827	1848	discovered in cervical tumors		
hsa-miR-3607-5p	828	1849	discovered in cervical tumors		
hsa-miR-3609	829	1850	discovered in cervical tumors		
hsa-miR-3610	830	1851	discovered in cervical tumors		
hsa-miR-361 1	831	1852	discovered in cervical tumors		
hsa-miR-3612	832	1853	discovered in cervical tumors		
hsa-miR-361 3-3p	833	1854	discovered in cervical tumors		
hsa-miR-361 3-5p	834	1855	discovered in cervical tumors		
hsa-miR-361 -3p	835	1856	blood, endothelial cells		
hsa-miR-3614-3p	836	1857	discovered in cervical and breast tumors		
hsa-miR-3614-5p	837	1858	discovered in cervical and breast tumors		
hsa-miR-361 5	838	1859	discovered in cervical tumors		
hsa-miR-361 -5p	839	1860	endothelial cells		
hsa-miR-361 6-3p	840	1861	discovered in cervical tumors		
hsa-miR-361 6-5p	841	1862	discovered in cervical tumors		
hsa-miR-361 7-3p	842	1863	discovered in cervical tumors and psoriasis		
hsa-miR-361 7-5p	843	1864	discovered in cervical tumors and psoriasis		
hsa-miR-361 8	844	1865	discovered in cervical tumors		
hsa-miR-361 9-3p	845	1866	discovered in breast tumors		
hsa-miR-361 9-5p	846	1867	discovered in breast tumors		
hsa-miR-3620-3p	847	1868	discovered in cervical tumors		

hsa-miR-3620-5p	848	1869	discovered in cervical tumors		
hsa-miR-3621	849	1870	discovered in cervical tumors		
hsa-miR-3622a-3p	850	1871	discovered in breast tumors		
hsa-miR-3622a-5p	851	1872	discovered in breast tumors		
hsa-miR-3622b-3p	852	1873	discovered in cervical tumors		
hsa-miR-3 622b-5p	853	1874	discovered in cervical tumors		
hsa-miR-362-3p	854	1875		melanoma	
hsa-miR-3 62-5p	855	1876		melanoma	
hsa-miR-3 63-3p	856	1877	kidney stem cell, blood cells		
hsa-miR-3 63-5p	857	1878	kidney stem cell, blood cells		
hsa-miR-3 646	858	1879	discovered in solid tumor		
hsa-miR-3648	859	1880	discovered in solid tumor		
hsa-miR-3 649	860	1881	discovered in solid tumor		
hsa-miR-3650	861	1882	discovered in solid tumor		
hsa-miR-3 651	862	1883	discovered in solid tumor		
hsa-miR-3 652	863	1884	discovered in solid tumor		
hsa-miR-3653	864	1885	discovered in solid tumor		
hsa-miR-3 654	865	1886	discovered in solid tumor		
hsa-miR-3655	866	1887	discovered in solid tumor		
hsa-miR-3656	867	1888	discovered in solid tumor		
hsa-miR-3657	868	1889	discovered in solid tumor		
hsa-miR-3 658	869	1890	discovered in solid tumor		
hsa-miR-3659	870	1891	discovered in breast tumors		
hsa-miR-365a-3p	871	1892		various cancer cells (Immune cells, lung, colon, endometriotic)	apoptosis

hsa-miR-365a-5p	872	1893		various cancer cells (Immune cells, lung, colon, endometriotic))	apoptosis
hsa-miR-365b-3p	873	1894		various cancers (retinoblastoma, colon, endometriotic)	apoptosis
hsa-miR-365b-5p	874	1895		various cancers (colon, endometriotic)	apoptosis
hsa-miR-3660	875	1896	discovered in breast tumors		
hsa-miR-3661	876	1897	discovered in breast tumors		
hsa-miR-3662	877	1898	-		
hsa-miR-3663-3p	878	1899	-		
hsa-miR-3663-5p	879	1900	-		
hsa-miR-3664-3p	880	1901	discovered in breast tumors		
hsa-miR-3664-5p	881	1902	discovered in breast tumors		
hsa-miR-3665	882	1903	brain		
hsa-miR-3666	883	1904	brain		
hsa-miR-3667-3p	884	1905	discovered in peripheral blood		
hsa-miR-3667-5p	885	1906	discovered in peripheral blood		
hsa-miR-3668	886	1907	discovered in peripheral blood		
hsa-miR-3669	887	1908	discovered in peripheral blood		
hsa-miR-3670	888	1909	discovered in peripheral blood		
hsa-miR-3671	889	1910	discovered in peripheral blood		
hsa-miR-3672	890	1911	discovered in peripheral blood		
hsa-miR-3673	891	1912	discovered in peripheral blood		
hsa-miR-367-3p	892	1913	embryonic stem cells		reprogramming
hsa-miR-3674	893	1914	discovered in peripheral blood		
hsa-miR-3675-3p	894	1915	discovered in peripheral blood		
hsa-miR-3675-5p	895	1916	discovered in peripheral blood		

hsa-miR-367-5p	896	1917	embryonic stem cells		reprogramming
hsa-miR-3676-3p	897	1918	discovered in peripheral blood		
hsa-miR-3676-5p	898	1919	discovered in peripheral blood		
hsa-miR-3677-3p	899	1920	discovered in peripheral blood		
hsa-miR-3677-5p	900	1921	discovered in peripheral blood		
hsa-miR-3678-3p	901	1922	discovered in peripheral blood		
hsa-miR-3678-5p	902	1923	discovered in peripheral blood		
hsa-miR-3679-3p	903	1924	discovered in peripheral blood		
hsa-miR-3679-5p	904	1925	discovered in peripheral blood		
hsa-miR-3680-3p	905	1926	discovered in peripheral blood		
hsa-miR-3680-5p	906	1927	discovered in peripheral blood		
hsa-miR-3681-3p	907	1928	discovered in peripheral blood		
hsa-miR-3681-5p	908	1929	discovered in peripheral blood		
hsa-miR-3682-3p	909	1930	discovered in peripheral blood		
hsa-miR-3682-5p	910	1931	discovered in peripheral blood		
hsa-miR-3683	911	1932	discovered in peripheral blood		
hsa-miR-3684	912	1933	discovered in peripheral blood		
hsa-miR-3685	913	1934	discovered in peripheral blood		
hsa-miR-3686	914	1935	discovered in peripheral blood		
hsa-miR-3687	915	1936	discovered in peripheral blood		
hsa-miR-3688-3p	916	1937	discovered in breast tumor		
hsa-miR-3688-5p	917	1938	discovered in breast tumor		
hsa-miR-3689a-3p	918	1939	discovered in female reproductive tract		
hsa-miR-3689a-5p	919	1940	discovered in female reproductive tract		

			and peripheral blood		
hsa-miR-3689b-3p	920	1941	discovered in female reproductive tract and peripheral blood		
hsa-miR-3689b-5p	921	1942	discovered in female reproductive tract		
hsa-miR-3689c	922	1943	discovered in B cells		
hsa-miR-3689d	923	1944	discovered in B cells		
hsa-miR-3689e	924	1945	discovered in B cells		
hsa-miR-3689f	925	1946	discovered in B cells		
hsa-miR-3690	926	1947	discovered in peripheral blood		
hsa-miR-3691-3p	927	1948	discovered in peripheral blood		
hsa-miR-3691-5p	928	1949	discovered in peripheral blood		
hsa-miR-3692-3p	929	1950	discovered in peripheral blood		
hsa-miR-3692-5p	930	1951	discovered in peripheral blood		
hsa-miR-369-3p	931	1952	stem cells		reprogramming
hsa-miR-369-5p	932	1953	stem cells		reprogramming
hsa-miR-370	933	1954		acute myeloid leukaemia and other cancers	tumor suppressor, lipid metabolism
hsa-miR-3713	934	1955	discovered in neuroblastoma		
hsa-miR-3714	935	1956	discovered in neuroblastoma		
hsa-miR-371a-3p	936	1957	serum		
hsa-miR-371a-5p	937	1958	serum		
hsa-miR-371b-3p	938	1959	serum		
hsa-miR-371b-5p	939	1960	serum		
hsa-miR-372	940	1961	hematopoietic cells, lung, placental (blood)		
hsa-miR-373-3p	941	1962		breast cancer	
hsa-miR-373-5p	942	1963		breast cancer	

hsa-miR-374a-3p	943	1964	muscle (myoblasts)	breast and lung cancer	myogenic differentiation
hsa-miR-374a-5p	944	1965	muscle (myoblasts)	breast and lung cancer	myogenic differentiation
hsa-miR-374b-3p	945	1966	muscle (myoblasts)		myogenic differentiation
hsa-miR-374b-5p	946	1967	muscle (myoblasts)		myogenic differentiation
hsa-miR-374c-3p	947	1968	muscle (myoblasts)		myogenic differentiation
hsa-miR-374c-5p	948	1969	muscle (myoblasts)		myogenic differentiation
hsa-miR-375	949	1970	pancreas (islet)		
hsa-miR-376a-2-5p	950	1971	regulatory miRs for hematopoietic cells (erythroid,platelet, lympho)		
hsa-miR-376a-3p	951	1972	regulatory miRs for hematopoietic cells (erythroid,platelet, lympho)		
hsa-miR-376a-5p	952	1973	regulatory miRs for hematopoietic cells (erythroid,platelet, lympho)		
hsa-miR-376b-3p	953	1974	blood	various cancer cells	autophagy
hsa-miR-376b-5p	954	1975	blood	various cancer cells	autophagy
hsa-miR-376c-3p	955	1976	trophoblast	various cancer cells	cell proliferatio
hsa-miR-376c-5p	956	1977	trophoblast	various cancer cells	cell proliferatio
hsa-miR-377-3p	957	1978	hematopoietic cells		
hsa-miR-377-5p	958	1979	hematopoietic cells		
hsa-miR-378a-3p	959	1980	ovary, lipid metabolism		
hsa-miR-378a-5p	960	1981	ovary, placenta/trophoblast, lipid metabolism		
hsa-miR-378b	961	1982	lipid metabolism		
hsa-miR-378c	962	1983	lipid metabolism		

hsa-miR-378d	963	1984	lipid metabolism		
hsa-miR-378e	964	1985	lipid metabolism		
hsa-miR-378f	965	1986	lipid metabolism		
hsa-miR-378g	966	1987	lipid metabolism		
hsa-miR-378h	967	1988	lipid metabolism		
hsa-miR-378i	968	1989	lipid metabolism		
hsa-miR-378j	969	1990	lipid metabolism		
hsa-miR-379-3p	970	1991		various cancers (breast, hepatocytes, colon)	
hsa-miR-379-5p	971	1992		various cancers (breast, hepatocytes, colon)	
hsa-miR-380-3p	972	1993	brain	neuroblastoma	
hsa-miR-380-5p	973	1994	brain, embryonic stem cells	neuroblastoma	
hsa-miR-381-3p	974	1995	chondrogenesis, lung, brain		
hsa-miR-381-5p	975	1996	chondrogenesis, lung, brain		
hsa-miR-382-3p	976	1997	renal epithelial cells		
hsa-miR-382-5p	977	1998	renal epithelial cells		
hsa-miR-383	978	1999	testes, brain (medulla)		
hsa-miR-384	979	2000	epithelial cells		
hsa-miR-3907	980	2001	discovered in female reproductive tract		
hsa-miR-3908	981	2002	discovered in female reproductive tract		
hsa-miR-3909	982	2003	discovered in female reproductive tract		
hsa-miR-3910	983	2004	discovered in female reproductive tract		
hsa-miR-391 1	984	2005	discovered in breast tumor and female reproductive tract		

hsa-miR-3912	985	2006	discovered in female reproductive tract		
hsa-miR-3913-3p	986	2007	discovered in breast tumor and female reproductive tract		
hsa-miR-3913-5p	987	2008	discovered in breast tumor and female reproductive tract		
hsa-miR-3914	988	2009	discovered in breast tumor and female reproductive tract		
hsa-miR-3915	989	2010	discovered in female reproductive tract		
hsa-miR-3916	990	2011	discovered in female reproductive tract		
hsa-miR-3917	991	2012	discovered in female reproductive tract		
hsa-miR-3918	992	2013	discovered in female reproductive tract		
hsa-miR-3919	993	2014	discovered in female reproductive tract		
hsa-miR-3920	994	2015	discovered in female reproductive tract		
hsa-miR-3921	995	2016	discovered in female reproductive tract		
hsa-miR-3922-3p	996	2017	discovered in breast tumor and female reproductive tract		
hsa-miR-3922-5p	997	2018	discovered in breast tumor and female reproductive tract		
hsa-miR-3923	998	2019	discovered in female reproductive tract		
hsa-miR-3924	999	2020	discovered in female reproductive tract		

hsa-miR-3925-3p	1000	2021	discovered in breast tumor and female reproductive tract		
hsa-miR-3925-5p	1001	2022	discovered in breast tumor and female reproductive tract		
hsa-miR-3926	1002	2023	discovered in female reproductive tract		
hsa-miR-3927-3p	1003	2024	discovered in female reproductive tract and psoriasis		
hsa-miR-3927-5p	1004	2025	discovered in female reproductive tract and psoriasis		
hsa-miR-3928	1005	2026	discovered in female reproductive tract		
hsa-miR-3929	1006	2027	discovered in female reproductive tract		
hsa-miR-3934-3p	1007	2028	discovered in abnormal skin (psoriasis)		
hsa-miR-3934-5p	1008	2029	discovered in abnormal skin (psoriasis)		
hsa-miR-3935	1009	2030			
hsa-miR-3936	1010	2031	discovered in breast tumor and lymphoblastic leukaemia		
hsa-miR-3937	1011	2032			
hsa-miR-3938	1012	2033			
hsa-miR-3939	1013	2034			
hsa-miR-3940-3p	1014	2035	discovered in breast tumor		
hsa-miR-3940-5p	1015	2036	discovered in breast tumor		
hsa-miR-3941	1016	2037			
hsa-miR-3942-3p	1017	2038	discovered in breast tumor and lymphoblastic		

			leukaemia		
hsa-miR-3942-5p	1018	2039	discovered in breast tumor and lymphoblastic leukaemia		
hsa-miR-3943	1019	2040			
hsa-miR-3944-3p	1020	2041	discovered in breast tumor		
hsa-miR-3944-5p	1021	2042	discovered in breast tumor		
hsa-miR-3945	1022	2043			
hsa-miR-3960	1023	2044	osteoblast		
hsa-miR-3972	1024	2045	discovered in Acute Myeloid Leukaemia		
hsa-miR-3973	1025	2046	discovered in Acute Myeloid Leukaemia		
hsa-miR-3974	1026	2047	discovered in Acute Myeloid Leukaemia		
hsa-miR-3975	1027	2048	discovered in Acute Myeloid Leukaemia		
hsa-miR-3976	1028	2049	discovered in Acute Myeloid Leukaemia		
hsa-miR-3977	1029	2050	discovered in Acute Myeloid Leukaemia		
hsa-miR-3978	1030	2051	discovered in Acute Myeloid Leukaemia		
hsa-miR-409-3p	1031	2052		gastric cancer	
hsa-miR-409-5p	1032	2053		gastric cancer	
hsa-miR-410	1033	2054	brain	glioma	
hsa-miR-411-3p	1034	2055		Glioblastoma others	
hsa-miR-411-5p	1035	2056		Glioblastoma others	
hsa-miR-412	1036	2057		upregulated in lung cancer	
hsa-miR-421	1037	2058	endothelial cells	gastric cancer, HCC	
hsa-miR-422a	1038	2059	circulating microRNA (in plasma)		

hsa-miR-423-3p	1039	2060	embryonic stem cells		
hsa-miR-423-5p	1040	2061	heart, embryonic stem cells		
hsa-miR-424-3p	1041	2062	endothelial cells	various cancers(e.g B-lieage ALL), cardiac diseases	pro-angiogenic
hsa-miR-424-5p	1042	2063	endothelial cells	various cancers(e.g B-lieage ALL), cardiac diseases	pro-angiogenic
hsa-miR-4251	1043	2064	discovered in embryonic stem cells and neural precursors		
hsa-miR-4252	1044	2065	discovered in embryonic stem cells and neural precursors		
hsa-miR-4253	1045	2066	discovered in embryonic stem cells and neural precursors		
hsa-miR-425-3p	1046	2067	brain	ovarian cancer, brain tumor	
hsa-miR-4254	1047	2068	discovered in embryonic stem cells and neural precursors		
hsa-miR-4255	1048	2069	discovered in embryonic stem cells and neural precursors		
hsa-miR-425-5p	1049	2070	brain	B-lieage ALL, brain tumor	
hsa-miR-4256	1050	2071	discovered in embryonic stem cells and neural precursors		
hsa-miR-4257	1051	2072	discovered in embryonic stem cells and neural precursors		
hsa-miR-4258	1052	2073	discovered in embryonic stem cells and neural precursors		
hsa-miR-4259	1053	2074	discovered in embryonic stem		

			cells and neural precursors		
hsa-miR-4260	1054	2075	discovered in embryonic stem cells and neural precursors		
hsa-miR-4261	1055	2076	discovered in embryonic stem cells and neural precursors		
hsa-miR-4262	1056	2077	discovered in embryonic stem cells and neural precursors		
hsa-miR-4263	1057	2078	discovered in embryonic stem cells and neural precursors		
hsa-miR-4264	1058	2079	discovered in embryonic stem cells and neural precursors		
hsa-miR-4265	1059	2080	discovered in embryonic stem cells and neural precursors		
hsa-miR-4266	1060	2081	discovered in embryonic stem cells and neural precursors		
hsa-miR-4267	1061	2082	discovered in embryonic stem cells and neural precursors		
hsa-miR-4268	1062	2083	discovered in embryonic stem cells and neural precursors		
hsa-miR-4269	1063	2084	discovered in embryonic stem cells and neural precursors		
hsa-miR-4270	1064	2085	discovered in embryonic stem cells and neural precursors		
hsa-miR-4271	1065	2086	discovered in embryonic stem cells and neural precursors		

hsa-miR-4272	1066	2087	discovered in embryonic stem cells and neural precursors		
hsa-miR-4273	1067	2088			
hsa-miR-4274	1068	2089	discovered in embryonic stem cells and neural precursors		
hsa-miR-4275	1069	2090	discovered in embryonic stem cells and neural precursors		
hsa-miR-4276	1070	2091	discovered in embryonic stem cells and neural precursors		
hsa-miR-4277	1071	2092	discovered in embryonic stem cells and neural precursors		
hsa-miR-4278	1072	2093	discovered in embryonic stem cells and neural precursors		
hsa-miR-4279	1073	2094	discovered in embryonic stem cells and neural precursors		
hsa-miR-4280	1074	2095	discovered in embryonic stem cells and neural precursors		
hsa-miR-4281	1075	2096	discovered in embryonic stem cells and neural precursors		
hsa-miR-4282	1076	2097	discovered in embryonic stem cells and neural precursors		
hsa-miR-4283	1077	2098	discovered in embryonic stem cells and neural precursors		
hsa-miR-4284	1078	2099	discovered in embryonic stem cells and neural precursors		

hsa-miR-4285	1079	2100	discovered in embryonic stem cells and neural precursors		
hsa-miR-4286	1080	2101	discovered in embryonic stem cells and neural precursors		
hsa-miR-4287	1081	2102	discovered in embryonic stem cells and neural precursors		
hsa-miR-4288	1082	2103	discovered in embryonic stem cells and neural precursors		
hsa-miR-4289	1083	2104	discovered in embryonic stem cells and neural precursors		
hsa-miR-429	1084	2105	Epithelial cells	various cancers (colorectal, endometrial, gastric, ovarian etc)	
hsa-miR-4290	1085	2106	discovered in embryonic stem cells and neural precursors		
hsa-miR-4291	1086	2107	discovered in embryonic stem cells and neural precursors		
hsa-miR-4292	1087	2108	discovered in embryonic stem cells and neural precursors		
hsa-miR-4293	1088	2109	discovered in embryonic stem cells and neural precursors		
hsa-miR-4294	1089	2110	discovered in embryonic stem cells and neural precursors		
hsa-miR-4295	1090	2111	discovered in embryonic stem cells and neural precursors		
hsa-miR-4296	1091	2112	discovered in		

			embryonic stem cells and neural precursors		
hsa-miR-4297	1092	2 113	discovered in embryonic stem cells and neural precursors		
hsa-miR-4298	1093	2 114	discovered in embryonic stem cells and neural precursors		
hsa-miR-4299	1094	2 115	discovered in embryonic stem cells and neural precursors		
hsa-miR-4300	1095	2 116	discovered in embryonic stem cells and neural precursors		
hsa-miR-4301	1096	2 117	discovered in embryonic stem cells and neural precursors		
hsa-miR-4302	1097	2 118	discovered in embryonic stem cells and neural precursors		
hsa-miR-4303	1098	2 119	discovered in embryonic stem cells and neural precursors		
hsa-miR-4304	1099	2120	discovered in embryonic stem cells and neural precursors		
hsa-miR-4305	1100	2121	discovered in embryonic stem cells and neural precursors		
hsa-miR-4306	1101	2122	discovered in embryonic stem cells and neural precursors		
hsa-miR-4307	1102	2123	discovered in embryonic stem cells and neural precursors		
hsa-miR-4308	1103	2124	discovered in embryonic stem cells and neural		

			precursors		
hsa-miR-4309	1104	2125	discovered in embryonic stem cells and neural precursors		
hsa-miR-4310	1105	2126	discovered in embryonic stem cells and neural precursors		
hsa-miR-43 11	1106	2127	discovered in embryonic stem cells and neural precursors		
hsa-miR-4312	1107	2128	discovered in embryonic stem cells and neural precursors		
hsa-miR-43 13	1108	2129	discovered in embryonic stem cells and neural precursors		
hsa-miR-43 1-3p	1109	2130		Cancers (follicular lymphoma)	
hsa-miR-43 14	1110	2131	discovered in embryonic stem cells and neural precursors		
hsa-miR-43 15	1111	2132	discovered in embryonic stem cells and neural precursors		
hsa-miR-43 1-5p	1112	2133		Cancers (follicular lymphoma)	
hsa-miR-43 16	1113	2134	discovered in embryonic stem cells and neural precursors		
hsa-miR-43 17	1114	2135	discovered in embryonic stem cells and neural precursors		
hsa-miR-43 18	1115	2136	discovered in embryonic stem cells and neural precursors		
hsa-miR-43 19	1116	2137	discovered in embryonic stem cells and neural precursors		
hsa-miR-4320	1117	2138	discovered in		

			embryonic stem cells and neural precursors		
hsa-miR-4321	1118	2139	discovered in embryonic stem cells and neural precursors		
hsa-miR-4322	1119	2140	discovered in embryonic stem cells and neural precursors		
hsa-miR-4323	1120	2141	discovered in embryonic stem cells and neural precursors		
hsa-miR-432-3p	1121	2142	myoblast		myogenic differentiation
hsa-miR-4324	1122	2143	discovered in embryonic stem cells and neural precursors		
hsa-miR-4325	1123	2144	discovered in embryonic stem cells and neural precursors		
hsa-miR-432-5p	1124	2145	myoblast		myogenic differentiation
hsa-miR-4326	1125	2146	discovered in embryonic stem cells and neural precursors		
hsa-miR-4327	1126	2147	discovered in embryonic stem cells and neural precursors		
hsa-miR-4328	1127	2148	discovered in embryonic stem cells and neural precursors		
hsa-miR-4329	1128	2149	discovered in embryonic stem cells and neural precursors		
hsa-miR-433	1129	2150		various diseases (cancer, Parkinson's, Chondrodysplasia)	
hsa-miR-4330	1130	2151	discovered in embryonic stem cells and neural		

			precursors		
hsa-miR-4417	1131	2152	discovered in B cells		
hsa-miR-4418	1132	2153	discovered in B cells		
hsa-miR-4419a	1133	2154	discovered in B cells		
hsa-miR-4419b	1134	2155	discovered in B cells		
hsa-miR-4420	1135	2156	discovered in B cells		
hsa-miR-4421	1136	2157	discovered in B cells		
hsa-miR-4422	1137	2158	discovered in breast tumor and B cells		
hsa-miR-4423-3p	1138	2159	discovered in breast tumor, B cells and skin(psoriasis)		
hsa-miR-4423-5p	1139	2160	discovered in breast tumor B cells and skin(psoriasis)		
hsa-miR-4424	1140	2161	discovered in B cells		
hsa-miR-4425	1141	2162	discovered in B cells		
hsa-miR-4426	1142	2163	discovered in B cells		
hsa-miR-4427	1143	2164	discovered in B cells		
hsa-miR-4428	1144	2165	discovered in B cells		
hsa-miR-4429	1145	2166	discovered in B cells		
hsa-miR-4430	1146	2167	discovered in B cells		
hsa-miR-443 1	1147	2168	discovered in B cells		
hsa-miR-4432	1148	2169	discovered in B cells		
hsa-miR-443 3-3p	1149	2170	discovered in B cells		
hsa-miR-443 3-5p	1150	2171	discovered in B cells		
hsa-miR-4434	1151	2172	discovered in B cells		
hsa-miR-443 5	1152	2173	discovered in B cells		

hsa-miR-4436a	1153	2174	discovered in breast tumor and B cells		
hsa-miR-4436b-3p	1154	2175	discovered in breast tumor		
hsa-miR-4436b-5p	1155	2176	discovered in breast tumor		
hsa-miR-4437	1156	2177	discovered in B cells		
hsa-miR-4438	1157	2178	discovered in B cells		
hsa-miR-4439	1158	2179	discovered in B cells		
hsa-miR-4440	1159	2180	discovered in B cells		
hsa-miR-4441	1160	2181	discovered in B cells		
hsa-miR-4442	1161	2182	discovered in B cells		
hsa-miR-4443	1162	2183	discovered in B cells		
hsa-miR-4444	1163	2184	discovered in B cells		
hsa-miR-4445-3p	1164	2185	discovered in B cells		
hsa-miR-4445-5p	1165	2186	discovered in B cells		
hsa-miR-4446-3p	1166	2187	discovered in breast tumor and B cells		
hsa-miR-4446-5p	1167	2188	discovered in breast tumor and B cells		
hsa-miR-4447	1168	2189	discovered in B cells		
hsa-miR-4448	1169	2190	discovered in B cells		
hsa-miR-4449	1170	2191	discovered in B cells		
hsa-miR-4450	1171	2192	discovered in B cells		
hsa-miR-445 1	1172	2193	discovered in B cells		
hsa-miR-4452	1173	2194	discovered in B cells		
hsa-miR-4453	1174	2195	discovered in B cells		
hsa-miR-4454	1175	2196	discovered in B cells		

hsa-miR-4455	1176	2197	discovered in B cells		
hsa-miR-4456	1177	2198	discovered in B cells		
hsa-miR-4457	1178	2199	discovered in B cells		
hsa-miR-4458	1179	2200	discovered in B cells		
hsa-miR-4459	1180	2201	discovered in B cells		
hsa-miR-4460	1181	2202	discovered in B cells		
hsa-miR-4461	1182	2203	discovered in B cells		
hsa-miR-4462	1183	2204	discovered in B cells		
hsa-miR-4463	1184	2205	discovered in B cells		
hsa-miR-4464	1185	2206	discovered in B cells		
hsa-miR-4465	1186	2207	discovered in B cells		
hsa-miR-4466	1187	2208	discovered in B cells		
hsa-miR-4467	1188	2209	discovered in breast tumor and B cells		
hsa-miR-4468	1189	2210	discovered in B cells		
hsa-miR-4469	1190	221 1	discovered in breast tumor and B cells		
hsa-miR-4470	1191	2212	discovered in B cells		
hsa-miR-4471	2213	3234	discovered in breast tumor and B cells		
hsa-miR-4472	2214	3235	discovered in B cells		
hsa-miR-4473	2215	3236	discovered in B cells		
hsa-miR-4474-3p	2216	3237	discovered in breast tumor, lymphoblastic leukaemia and B cells		
hsa-miR-4474-5p	2217	3238	discovered in breast tumor, lymphoblastic leukaemia and B		

			cells		
hsa-miR-4475	2218	3239	discovered in B cells		
hsa-miR-4476	2219	3240	discovered in B cells		
hsa-miR-4477a	2220	3241	discovered in B cells		
hsa-miR-4477b	2221	3242	discovered in B cells		
hsa-miR-4478	2222	3243	discovered in B cells		
hsa-miR-4479	2223	3244	discovered in B cells		
hsa-miR-448	2224	3245	liver(hepatocytes)	HCC	
hsa-miR-4480	2225	3246	discovered in B cells		
hsa-miR-448 1	2226	3247	discovered in B cells		
hsa-miR-4482-3p	2227	3248	discovered in B cells		
hsa-miR-4482-5p	2228	3249	discovered in B cells		
hsa-miR-4483	2229	3250	discovered in B cells		
hsa-miR-4484	2230	3251	discovered in B cells		
hsa-miR-4485	2231	3252	discovered in B cells		
hsa-miR-4486	2232	3253	discovered in B cells		
hsa-miR-4487	2233	3254	discovered in B cells		
hsa-miR-4488	2234	3255	discovered in B cells		
hsa-miR-4489	2235	3256	discovered in breast tumor and B cells		
hsa-miR-4490	2236	3257	discovered in B cells		
hsa-miR-4491	2237	3258	discovered in B cells		
hsa-miR-4492	2238	3259	discovered in B cells		
hsa-miR-4493	2239	3260	discovered in B cells		
hsa-miR-4494	2240	3261	discovered in B cells		
hsa-miR-4495	2241	3262	discovered in B cells		

hsa-miR-4496	2242	3263	discovered in B cells		
hsa-miR-4497	2243	3264	discovered in B cells		
hsa-miR-4498	2244	3265	discovered in B cells		
hsa-miR-4499	2245	3266	discovered in B cells		
hsa-miR-449a	2246	3267	chondrocytes, ciliated epithelial cells	lung, colonic, ovarian cancer	cell cycle progression and proliferation
hsa-miR-449b-3p	2247	3268	ciliated epithelial cells, other tissues	various cancer cells	cell cycle progression and proliferation
hsa-miR-449b-5p	2248	3269	ciliated epithelial cells, other tissues	various cancer cells	cell cycle progression and proliferation
hsa-miR-449c-3p	2249	3270		epithelial ovarian cancer cells	
hsa-miR-449c-5p	2250	3271		epithelial ovarian cancer cells	
hsa-miR-4500	2251	3272	discovered in B cells		
hsa-miR-4501	2252	3273	discovered in B cells		
hsa-miR-4502	2253	3274	discovered in B cells		
hsa-miR-4503	2254	3275	discovered in B cells		
hsa-miR-4504	2255	3276	discovered in B cells		
hsa-miR-4505	2256	3277	discovered in B cells		
hsa-miR-4506	2257	3278	discovered in B cells		
hsa-miR-4507	2258	3279	discovered in B cells		
hsa-miR-4508	2259	3280	discovered in B cells		
hsa-miR-4509	2260	3281	discovered in B cells		
hsa-miR-450a-3p	2261	3282			
hsa-miR-450a-5p	2262	3283			
hsa-miR-450b-3p	2263	3284			
hsa-miR-450b-5p	2264	3285			
hsa-miR-4510	2265	3286	discovered in B cells		

hsa-miR-451 1	2266	3287	discovered in B cells		
hsa-miR-4512	2267	3288	discovered in B cells		
hsa-miR-4513	2268	3289	discovered in B cells		
hsa-miR-4514	2269	3290	discovered in B cells		
hsa-miR-4515	2270	3291	discovered in B cells		
hsa-miR-4516	2271	3292	discovered in B cells		
hsa-miR-4517	2272	3293	discovered in B cells		
hsa-miR-4518	2273	3294	discovered in B cells		
hsa-miR-4519	2274	3295	discovered in B cells		
hsa-miR-45 1a	2275	3296	heart, central nervous system, epithelial cells		
hsa-miR-451b	2276	3297	heart, central nervous system, epithelial cells		
hsa-miR-4520a-3p	2277	3298	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4520a-5p	2278	3299	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4520b-3p	2279	3300	discovered in breast tumor		
hsa-miR-4520b-5p	2280	3301	discovered in breast tumor		
hsa-miR-4521	2281	3302	discovered in B cells		
hsa-miR-4522	2282	3303	discovered in B cells		
hsa-miR-4523	2283	3304	discovered in B cells		
hsa-miR-452-3p	2284	3305	myoblast	bladder cancer and others	
hsa-miR-4524a-3p	2285	3306	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4524a-5p	2286	3307	discovered in breast tumor and		

			B cells, skin(psoriasis)		
hsa-miR-4524b-3p	2287	3308	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4524b-5p	2288	3309	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4525	2289	3310	discovered in B cells		
hsa-miR-452-5p	2290	331 1	myoblast	bladder cancer and others	
hsa-miR-4526	2291	3312	discovered in breast tumor and B cells		
hsa-miR-4527	2292	3313	discovered in B cells		
hsa-miR-4528	2293	3314	discovered in B cells		
hsa-miR-4529-3p	2294	3315	discovered in breast tumor and B cells		
hsa-miR-4529-5p	2295	3316	discovered in breast tumor and B cells		
hsa-miR-4530	2296	3317	discovered in B cells		
hsa-miR-453 1	2297	3318	discovered in B cells		
hsa-miR-4532	2298	3319	discovered in B cells		
hsa-miR-4533	2299	3320	discovered in B cells		
hsa-miR-4534	2300	3321	discovered in B cells		
hsa-miR-4535	2301	3322	discovered in B cells		
hsa-miR-4536-3p	2302	3323	discovered in B cells		
hsa-miR-4536-5p	2303	3324	discovered in B cells		
hsa-miR-4537	2304	3325	discovered in B cells		
hsa-miR-453 8	2305	3326	discovered in B cells		
hsa-miR-4539	2306	3327	discovered in B cells		

hsa-miR-4540	2307	3328	discovered in B cells		
hsa-miR-454-3p	2308	3329	embryoid body cells, central nervous system, monocytes		
hsa-miR-454-5p	2309	3330	embryoid body cells, central nervous system, monocytes		
hsa-miR-455-3p	2310	3331		basal cell carcinoma, other cancers	
hsa-miR-455-5p	2311	3332		basal cell carcinoma, other cancers	
hsa-miR-4632-3p	2312	3333	discovered in breast tumor		
hsa-miR-4632-5p	2313	3334	discovered in breast tumor		
hsa-miR-4633-3p	2314	3335	discovered in breast tumor		
hsa-miR-4633-5p	2315	3336	discovered in breast tumor		
hsa-miR-4634	2316	3337	discovered in breast tumor		
hsa-miR-4635	2317	3338	discovered in breast tumor		
hsa-miR-4636	2318	3339	discovered in breast tumor		
hsa-miR-4637	2319	3340	discovered in breast tumor and lymphoblastic leukaemia		
hsa-miR-4638-3p	2320	3341	discovered in breast tumor		
hsa-miR-4638-5p	2321	3342	discovered in breast tumor		
hsa-miR-4639-3p	2322	3343	discovered in breast tumor		
hsa-miR-4639-5p	2323	3344	discovered in breast tumor		
hsa-miR-4640-3p	2324	3345	discovered in breast tumor		
hsa-miR-4640-5p	2325	3346	discovered in breast tumor		
hsa-miR-4641	2326	3347	discovered in breast tumor		
hsa-miR-4642	2327	3348	discovered in breast tumor		

hsa-miR-4643	2328	3349	discovered in breast tumor		
hsa-miR-4644	2329	3350	discovered in breast tumor		
hsa-miR-4645-3p	2330	3351	discovered in breast tumor		
hsa-miR-4645-5p	2331	3352	discovered in breast tumor		
hsa-miR-4646-3p	2332	3353	discovered in breast tumor		
hsa-miR-4646-5p	2333	3354	discovered in breast tumor		
hsa-miR-4647	2334	3355	discovered in breast tumor		
hsa-miR-4648	2335	3356	discovered in breast tumor		
hsa-miR-4649-3p	2336	3357	discovered in breast tumor		
hsa-miR-4649-5p	2337	3358	discovered in breast tumor		
hsa-miR-4650-3p	2338	3359	discovered in breast tumor		
hsa-miR-4650-5p	2339	3360	discovered in breast tumor		
hsa-miR-465 1	2340	3361	discovered in breast tumor		
hsa-miR-4652-3p	2341	3362	discovered in breast tumor		
hsa-miR-4652-5p	2342	3363	discovered in breast tumor		
hsa-miR-4653-3p	2343	3364	discovered in breast tumor		
hsa-miR-4653-5p	2344	3365	discovered in breast tumor		
hsa-miR-4654	2345	3366	discovered in breast tumor		
hsa-miR-4655-3p	2346	3367	discovered in breast tumor		
hsa-miR-4655-5p	2347	3368	discovered in breast tumor		
hsa-miR-4656	2348	3369	discovered in breast tumor		
hsa-miR-4657	2349	3370	discovered in breast tumor		
hsa-miR-465 8	2350	3371	discovered in breast tumor		
hsa-miR-4659a-3p	2351	3372	discovered in breast tumor		
hsa-miR-4659a-5p	2352	3373	discovered in breast tumor		

hsa-miR-4659b-3p	2353	3374	discovered in breast tumor		
hsa-miR-4659b-5p	2354	3375	discovered in breast tumor		
hsa-miR-466	2355	3376			
hsa-miR-4660	2356	3377	discovered in breast tumor		
hsa-miR-4661-3p	2357	3378	discovered in breast tumor		
hsa-miR-4661-5p	2358	3379	discovered in breast tumor		
hsa-miR-4662a-3p	2359	3380	discovered in breast tumor, psoriasis		
hsa-miR-4662a-5p	2360	3381	discovered in breast tumor, psoriasis		
hsa-miR-4662b	2361	3382	discovered in breast tumor		
hsa-miR-4663	2362	3383	discovered in breast tumor		
hsa-miR-4664-3p	2363	3384	discovered in breast tumor		
hsa-miR-4664-5p	2364	3385	discovered in breast tumor		
hsa-miR-4665-3p	2365	3386	discovered in breast tumor		
hsa-miR-4665-5p	2366	3387	discovered in breast tumor		
hsa-miR-4666a-3p	2367	3388	discovered in breast tumor		
hsa-miR-4666a-5p	2368	3389	discovered in breast tumor		
hsa-miR-4666b	2369	3390			
hsa-miR-4667-3p	2370	3391	discovered in breast tumor		
hsa-miR-4667-5p	2371	3392	discovered in breast tumor		
hsa-miR-4668-3p	2372	3393	discovered in breast tumor		
hsa-miR-4668-5p	2373	3394	discovered in breast tumor		
hsa-miR-4669	2374	3395	discovered in breast tumor		
hsa-miR-4670-3p	2375	3396	discovered in breast tumor		
hsa-miR-4670-5p	2376	3397	discovered in breast tumor		

hsa-miR-4671-3p	2377	3398	discovered in breast tumor		
hsa-miR-4671-5p	2378	3399	discovered in breast tumor		
hsa-miR-4672	2379	3400	discovered in breast tumor		
hsa-miR-4673	2380	3401	discovered in breast tumor		
hsa-miR-4674	2381	3402	discovered in breast tumor		
hsa-miR-4675	2382	3403	discovered in breast tumor		
hsa-miR-4676-3p	2383	3404	discovered in breast tumor		
hsa-miR-4676-5p	2384	3405	discovered in breast tumor		
hsa-miR-4677-3p	2385	3406	discovered in breast tumor, psoriasis		
hsa-miR-4677-5p	2386	3407	discovered in breast tumor, psoriasis		
hsa-miR-4678	2387	3408	discovered in breast tumor		
hsa-miR-4679	2388	3409	discovered in breast tumor		
hsa-miR-4680-3p	2389	3410	discovered in breast tumor		
hsa-miR-4680-5p	2390	341 1	discovered in breast tumor		
hsa-miR-4681	2391	3412	discovered in breast tumor		
hsa-miR-4682	2392	3413	discovered in breast tumor		
hsa-miR-4683	2393	3414	discovered in breast tumor		
hsa-miR-4684-3p	2394	3415	discovered in breast tumor		
hsa-miR-4684-5p	2395	3416	discovered in breast tumor		
hsa-miR-4685-3p	2396	3417	discovered in breast tumor		
hsa-miR-4685-5p	2397	3418	discovered in breast tumor		
hsa-miR-4686	2398	3419	discovered in breast tumor		
hsa-miR-4687-3p	2399	3420	discovered in breast tumor		
hsa-miR-4687-5p	2400	3421	discovered in breast tumor		

hsa-miR-4688	2401	3422	discovered in breast tumor		
hsa-miR-4689	2402	3423	discovered in breast tumor		
hsa-miR-4690-3p	2403	3424	discovered in breast tumor		
hsa-miR-4690-5p	2404	3425	discovered in breast tumor		
hsa-miR-4691-3p	2405	3426	discovered in breast tumor		
hsa-miR-4691-5p	2406	3427	discovered in breast tumor		
hsa-miR-4692	2407	3428	discovered in breast tumor		
hsa-miR-4693-3p	2408	3429	discovered in breast tumor		
hsa-miR-4693-5p	2409	3430	discovered in breast tumor		
hsa-miR-4694-3p	2410	3431	discovered in breast tumor		
hsa-miR-4694-5p	241 1	3432	discovered in breast tumor		
hsa-miR-4695-3p	2412	3433	discovered in breast tumor		
hsa-miR-4695-5p	2413	3434	discovered in breast tumor		
hsa-miR-4696	2414	3435	discovered in breast tumor		
hsa-miR-4697-3p	2415	3436	discovered in breast tumor		
hsa-miR-4697-5p	2416	3437	discovered in breast tumor		
hsa-miR-4698	2417	3438	discovered in breast tumor		
hsa-miR-4699-3p	2418	3439	discovered in breast tumor		
hsa-miR-4699-5p	2419	3440	discovered in breast tumor		
hsa-miR-4700-3p	2420	3441	discovered in breast tumor		
hsa-miR-4700-5p	2421	3442	discovered in breast tumor		
hsa-miR-4701-3p	2422	3443	discovered in breast tumor		
hsa-miR-4701-5p	2423	3444	discovered in breast tumor		
hsa-miR-4703-3p	2424	3445	discovered in breast tumor		
hsa-miR-4703-5p	2425	3446	discovered in breast tumor		

hsa-miR-4704-3p	2426	3447	discovered in breast tumor		
hsa-miR-4704-5p	2427	3448	discovered in breast tumor		
hsa-miR-4705	2428	3449	discovered in breast tumor		
hsa-miR-4706	2429	3450	discovered in breast tumor		
hsa-miR-4707-3p	2430	3451	discovered in breast tumor		
hsa-miR-4707-5p	2431	3452	discovered in breast tumor		
hsa-miR-4708-3p	2432	3453	discovered in breast tumor		
hsa-miR-4708-5p	2433	3454	discovered in breast tumor		
hsa-miR-4709-3p	2434	3455	discovered in breast tumor		
hsa-miR-4709-5p	2435	3456	discovered in breast tumor		
hsa-miR-4710	2436	3457	discovered in breast tumor		
hsa-miR-471 1-3p	2437	3458	discovered in breast tumor		
hsa-miR-471 1-5p	2438	3459	discovered in breast tumor		
hsa-miR-4712-3p	2439	3460	discovered in breast tumor		
hsa-miR-4712-5p	2440	3461	discovered in breast tumor		
hsa-miR-4713-3p	2441	3462	discovered in breast tumor		
hsa-miR-4713-5p	2442	3463	discovered in breast tumor		
hsa-miR-4714-3p	2443	3464	discovered in breast tumor		
hsa-miR-4714-5p	2444	3465	discovered in breast tumor		
hsa-miR-4715-3p	2445	3466	discovered in breast tumor		
hsa-miR-4715-5p	2446	3467	discovered in breast tumor		
hsa-miR-4716-3p	2447	3468	discovered in breast tumor		
hsa-miR-4716-5p	2448	3469	discovered in breast tumor		
hsa-miR-4717-3p	2449	3470	discovered in breast tumor		
hsa-miR-4717-5p	2450	3471	discovered in breast tumor		

hsa-miR-4718	2451	3472	discovered in breast tumor		
hsa-miR-4719	2452	3473	discovered in breast tumor		
hsa-miR-4720-3p	2453	3474	discovered in breast tumor		
hsa-miR-4720-5p	2454	3475	discovered in breast tumor		
hsa-miR-4721	2455	3476	discovered in breast tumor		
hsa-miR-4722-3p	2456	3477	discovered in breast tumor		
hsa-miR-4722-5p	2457	3478	discovered in breast tumor		
hsa-miR-4723-3p	2458	3479	discovered in breast tumor		
hsa-miR-4723-5p	2459	3480	discovered in breast tumor		
hsa-miR-4724-3p	2460	3481	discovered in breast tumor		
hsa-miR-4724-5p	2461	3482	discovered in breast tumor		
hsa-miR-4725-3p	2462	3483	discovered in breast tumor		
hsa-miR-4725-5p	2463	3484	discovered in breast tumor		
hsa-miR-4726-3p	2464	3485	discovered in breast tumor		
hsa-miR-4726-5p	2465	3486	discovered in breast tumor		
hsa-miR-4727-3p	2466	3487	discovered in breast tumor		
hsa-miR-4727-5p	2467	3488	discovered in breast tumor		
hsa-miR-4728-3p	2468	3489	discovered in breast tumor		
hsa-miR-4728-5p	2469	3490	discovered in breast tumor		
hsa-miR-4729	2470	3491	discovered in breast tumor		
hsa-miR-4730	2471	3492	discovered in breast tumor		
hsa-miR-4731-3p	2472	3493	discovered in breast tumor		
hsa-miR-4731-5p	2473	3494	discovered in breast tumor		
hsa-miR-4732-3p	2474	3495	discovered in breast tumor		
hsa-miR-4732-5p	2475	3496	discovered in breast tumor		

hsa-miR-4733-3p	2476	3497	discovered in breast tumor		
hsa-miR-4733-5p	2477	3498	discovered in breast tumor		
hsa-miR-4734	2478	3499	discovered in breast tumor		
hsa-miR-4735-3p	2479	3500	discovered in breast tumor		
hsa-miR-4735-5p	2480	3501	discovered in breast tumor		
hsa-miR-4736	2481	3502	discovered in breast tumor		
hsa-miR-4737	2482	3503	discovered in breast tumor		
hsa-miR-4738-3p	2483	3504	discovered in breast tumor		
hsa-miR-4738-5p	2484	3505	discovered in breast tumor		
hsa-miR-4739	2485	3506	discovered in breast tumor		
hsa-miR-4740-3p	2486	3507	discovered in breast tumor		
hsa-miR-4740-5p	2487	3508	discovered in breast tumor		
hsa-miR-4741	2488	3509	discovered in breast tumor, psoriasis		
hsa-miR-4742-3p	2489	3510	discovered in breast tumor, psoriasis		
hsa-miR-4742-5p	2490	351 1	discovered in breast tumor		
hsa-miR-4743-3p	2491	3512	discovered in breast tumor		
hsa-miR-4743-5p	2492	3513	discovered in breast tumor		
hsa-miR-4744	2493	3514	discovered in breast tumor		
hsa-miR-4745-3p	2494	3515	discovered in breast tumor		
hsa-miR-4745-5p	2495	3516	discovered in breast tumor		
hsa-miR-4746-3p	2496	3517	discovered in breast tumor		
hsa-miR-4746-5p	2497	3518	discovered in breast tumor		
hsa-miR-4747-3p	2498	3519	discovered in breast tumor		
hsa-miR-4747-5p	2499	3520	discovered in breast tumor		

hsa-miR-4748	2500	3521	discovered in breast tumor		
hsa-miR-4749-3p	2501	3522	discovered in breast tumor		
hsa-miR-4749-5p	2502	3523	discovered in breast tumor		
hsa-miR-4750-3p	2503	3524	discovered in breast tumor		
hsa-miR-4750-5p	2504	3525	discovered in breast tumor		
hsa-miR-4751	2505	3526	discovered in breast tumor		
hsa-miR-4752	2506	3527	discovered in breast tumor		
hsa-miR-4753-3p	2507	3528	discovered in breast tumor		
hsa-miR-4753-5p	2508	3529	discovered in breast tumor		
hsa-miR-4754	2509	3530	discovered in breast tumor		
hsa-miR-4755-3p	2510	3531	discovered in breast tumor		
hsa-miR-4755-5p	251 1	3532	discovered in breast tumor		
hsa-miR-4756-3p	2512	3533	discovered in breast tumor		
hsa-miR-4756-5p	2513	3534	discovered in breast tumor		
hsa-miR-4757-3p	2514	3535	discovered in breast tumor		
hsa-miR-4757-5p	2515	3536	discovered in breast tumor		
hsa-miR-4758-3p	2516	3537	discovered in breast tumor		
hsa-miR-4758-5p	2517	3538	discovered in breast tumor		
hsa-miR-4759	2518	3539	discovered in breast tumor		
hsa-miR-4760-3p	2519	3540	discovered in breast tumor		
hsa-miR-4760-5p	2520	3541	discovered in breast tumor		
hsa-miR-4761-3p	2521	3542	discovered in breast tumor		
hsa-miR-4761-5p	2522	3543	discovered in breast tumor		
hsa-miR-4762-3p	2523	3544	discovered in breast tumor		
hsa-miR-4762-5p	2524	3545	discovered in breast tumor		

hsa-miR-4763-3p	2525	3546	discovered in breast tumor		
hsa-miR-4763-5p	2526	3547	discovered in breast tumor		
hsa-miR-4764-3p	2527	3548	discovered in breast tumor		
hsa-miR-4764-5p	2528	3549	discovered in breast tumor		
hsa-miR-4765	2529	3550	discovered in breast tumor		
hsa-miR-4766-3p	2530	3551	discovered in breast tumor		
hsa-miR-4766-5p	2531	3552	discovered in breast tumor		
hsa-miR-4767	2532	3553	discovered in breast tumor		
hsa-miR-4768-3p	2533	3554	discovered in breast tumor		
hsa-miR-4768-5p	2534	3555	discovered in breast tumor		
hsa-miR-4769-3p	2535	3556	discovered in breast tumor		
hsa-miR-4769-5p	2536	3557	discovered in breast tumor		
hsa-miR-4770	2537	3558	discovered in breast tumor		
hsa-miR-4771	2538	3559	discovered in breast tumor		
hsa-miR-4772-3p	2539	3560	discovered in breast tumor, blood monoclear cells	energy metabolism/obesity	
hsa-miR-4772-5p	2540	3561	discovered in breast tumor, blood monoclear cells	energy metabolism/obesity	
hsa-miR-4773	2541	3562	discovered in breast tumor		
hsa-miR-4774-3p	2542	3563	discovered in breast tumor and Lymphoblastic leukemia		
hsa-miR-4774-5p	2543	3564	discovered in breast tumor and Lymphoblastic leukemia		
hsa-miR-4775	2544	3565	discovered in breast tumor		
hsa-miR-4776-3p	2545	3566	discovered in breast tumor		

hsa-miR-4776-5p	2546	3567	discovered in breast tumor		
hsa-miR-4777-3p	2547	3568	discovered in breast tumor		
hsa-miR-4777-5p	2548	3569	discovered in breast tumor		
hsa-miR-4778-3p	2549	3570	discovered in breast tumor		
hsa-miR-4778-5p	2550	3571	discovered in breast tumor		
hsa-miR-4779	2551	3572	discovered in breast tumor		
hsa-miR-4780	2552	3573	discovered in breast tumor		
hsa-miR-4781-3p	2553	3574	discovered in breast tumor		
hsa-miR-4781-5p	2554	3575	discovered in breast tumor		
hsa-miR-4782-3p	2555	3576	discovered in breast tumor		
hsa-miR-4782-5p	2556	3577	discovered in breast tumor		
hsa-miR-4783-3p	2557	3578	discovered in breast tumor		
hsa-miR-4783-5p	2558	3579	discovered in breast tumor		
hsa-miR-4784	2559	3580	discovered in breast tumor		
hsa-miR-4785	2560	3581	discovered in breast tumor		
hsa-miR-4786-3p	2561	3582	discovered in breast tumor		
hsa-miR-4786-5p	2562	3583	discovered in breast tumor		
hsa-miR-4787-3p	2563	3584	discovered in breast tumor		
hsa-miR-4787-5p	2564	3585	discovered in breast tumor		
hsa-miR-4788	2565	3586	discovered in breast tumor		
hsa-miR-4789-3p	2566	3587	discovered in breast tumor		
hsa-miR-4789-5p	2567	3588	discovered in breast tumor		
hsa-miR-4790-3p	2568	3589	discovered in breast tumor		
hsa-miR-4790-5p	2569	3590	discovered in breast tumor		
hsa-miR-4791	2570	3591	discovered in breast tumor		

hsa-miR-4792	2571	3592	discovered in breast tumor		
hsa-miR-4793-3p	2572	3593	discovered in breast tumor		
hsa-miR-4793-5p	2573	3594	discovered in breast tumor		
hsa-miR-4794	2574	3595	discovered in breast tumor		
hsa-miR-4795-3p	2575	3596	discovered in breast tumor		
hsa-miR-4795-5p	2576	3597	discovered in breast tumor		
hsa-miR-4796-3p	2577	3598	discovered in breast tumor		
hsa-miR-4796-5p	2578	3599	discovered in breast tumor		
hsa-miR-4797-3p	2579	3600	discovered in breast tumor		
hsa-miR-4797-5p	2580	3601	discovered in breast tumor		
hsa-miR-4798-3p	2581	3602	discovered in breast tumor		
hsa-miR-4798-5p	2582	3603	discovered in breast tumor		
hsa-miR-4799-3p	2583	3604	discovered in breast tumor		
hsa-miR-4799-5p	2584	3605	discovered in breast tumor		
hsa-miR-4800-3p	2585	3606	discovered in breast tumor		
hsa-miR-4800-5p	2586	3607	discovered in breast tumor		
hsa-miR-4801	2587	3608	discovered in breast tumor		
hsa-miR-4802-3p	2588	3609	discovered in breast tumor, psoriasis		
hsa-miR-4802-5p	2589	3610	discovered in breast tumor, psoriasis		
hsa-miR-4803	2590	361 1	discovered in breast tumor		
hsa-miR-4804-3p	2591	3612	discovered in breast tumor		
hsa-miR-4804-5p	2592	3613	discovered in breast tumor		
hsa-miR-483-3p	2593	3614		aderonocortical carcinoma, rectal/pancreatic cancer,	oncogenic

				proliferation of wounded epithelial cells	
hsa-miR-483-5p	2594	3615	cartilage (chondrocyte), fetal brain	adrenocortical carcinoma	angiogenesis
hsa-miR-484	2595	3616			mitochondrial network
hsa-miR-485-3p	2596	3617			
hsa-miR-485-5p	2597	3618		ovarian epithelial tumor	
hsa-miR-486-3p	2598	3619	erythroid cells	various cancers	
hsa-miR-486-5p	2599	3620	stem cells (adipose)	various cancers	
hsa-miR-487a	2600	3621		laryngeal carcinoma	
hsa-miR-487b	2601	3622		neuroblastoma, pulmonary carcinogenesis	
hsa-miR-488-3p	2602	3623		prostate cancer, others	
hsa-miR-488-5p	2603	3624		prostate cancer, others	
hsa-miR-489	2604	3625	mesenchymal stem cells	osteogenesis	
hsa-miR-490-3p	2605	3626		neuroblastoma, uterine leiomyoma (ULM)/muscle	
hsa-miR-490-5p	2606	3627		neuroblastoma, uterine leiomyoma (ULM)/muscle	
hsa-miR-491-3p	2607	3628		various cancers, brain disease	pro-apoptosis
hsa-miR-491-5p	2608	3629		various cancers, brain disease	pro-apoptosis
hsa-miR-492	2609	3630			
hsa-miR-493-3p	2610	3631	myeloid cells, pancreas (islet)		
hsa-miR-493-5p	2611	3632	myeloid cells, pancreas (islet)		
hsa-miR-494	2612	3633	epithelial cells	various cancers	cell cycle
hsa-miR-495-3p	2613	3634	platelet	various cancers (gastric, MLL leukemia, pancreatic etc) and inflammation	
hsa-miR-495-5p	2614	3635	platelet	various cancers (gastric, MLL	

				leukemia, pancreatic etc) and inflammation	
hsa-miR-496	2615	3636	Blood		
hsa-miR-497-3p	2616	3637		various cancers (breast, colorectal, etc)	tumor suppressor/pro-apoptosis
hsa-miR-497-5p	2617	3638		various cancers (breast, colorectal, etc)	tumor suppressor/pro-apoptosis
hsa-miR-498	2618	3639		autoimmuno (e.g. rheumatoid arthritis)	
hsa-miR-4999-3p	2619	3640			
hsa-miR-4999-5p	2620	3641			
hsa-miR-499a-3p	2621	3642	heart, cardiac stem cells	cardiovascular disease	cardiomyocyte differentiation
hsa-miR-499a-5p	2622	3643	heart, cardiac stem cells	cardiovascular disease	cardiomyocyte differentiation
hsa-miR-499b-3p	2623	3644	heart, cardiac stem cells	cardiovascular disease	cardiomyocyte differentiation
hsa-miR-499b-5p	2624	3645	heart, cardiac stem cells	cardiovascular disease	cardiomyocyte differentiation
hsa-miR-5000-3p	2625	3646	discovered in lymphoblastic leukaemia		
hsa-miR-5000-5p	2626	3647	discovered in lymphoblastic leukaemia		
hsa-miR-5001-3p	2627	3648			
hsa-miR-5001-5p	2628	3649			
hsa-miR-5002-3p	2629	3650			
hsa-miR-5002-5p	2630	3651			
hsa-miR-5003-3p	2631	3652			
hsa-miR-5003-5p	2632	3653			
hsa-miR-5004-3p	2633	3654			
hsa-miR-5004-5p	2634	3655			
hsa-miR-5006-3p	2635	3656	discovered in lymphoblastic leukaemia		
hsa-miR-5006-5p	2636	3657	discovered in lymphoblastic leukaemia		
hsa-miR-5007-3p	2637	3658			

hsa-miR-5007-5p	2638	3659			
hsa-miR-5008-3p	2639	3660			
hsa-miR-5008-5p	2640	3661			
hsa-miR-5009-3p	2641	3662			
hsa-miR-5009-5p	2642	3663			
hsa-miR-500a-3p	2643	3664			
hsa-miR-500a-5p	2644	3665			
hsa-miR-500b	2645	3666	Blood (plasma)		
hsa-miR-5010-3p	2646	3667		abnormal skin (psoriasis)	
hsa-miR-5010-5p	2647	3668		abnormal skin (psoriasis)	
hsa-miR-5011-3p	2648	3669			
hsa-miR-5011-5p	2649	3670			
hsa-miR-501-3p	2650	3671			
hsa-miR-501-5p	2651	3672			
hsa-miR-502-3p	2652	3673		various cancers (hepatocellular, ovarian, breast)	
hsa-miR-502-5p	2653	3674		various cancers (hepatocellular, ovarian, breast)	
hsa-miR-503-3p	2654	3675	ovary		
hsa-miR-503-5p	2655	3676	ovary		
hsa-miR-504	2656	3677		glioblastoma	
hsa-miR-5047	2657	3678			
hsa-miR-505-3p	2658	3679		breast cancer	
hsa-miR-505-5p	2659	3680		breast cancer	
hsa-miR-506-3p	2660	3681		various cancers	
hsa-miR-506-5p	2661	3682		various cancers	
hsa-miR-507	2662	3683			
hsa-miR-508-3p	2663	3684		renal cell carcinoma	
hsa-miR-508-5p	2664	3685	endothelial progenitor cells (EPCs)		
hsa-miR-5087	2665	3686			
hsa-miR-5088	2666	3687			

hsa-miR-5089-3p	2667	3688			
hsa-miR-5089-5p	2668	3689			
hsa-miR-5090	2669	3690			
hsa-miR-5091	2670	3691			
hsa-miR-5092	2671	3692			
hsa-miR-5093	2672	3693			
hsa-miR-509-3-5p	2673	3694	testis		
hsa-miR-509-3p	2674	3695		renal cell carcinoma, brain disease	
hsa-miR-5094	2675	3696			
hsa-miR-5095	2676	3697		cervical cancer	
hsa-miR-509-5p	2677	3698		metabolic syndrome, brain disease	
hsa-miR-5096	2678	3699		cervical cance	
hsa-miR-510	2679	3700	brain		
hsa-miR-5100	2680	3701	discoverd in Salivary gland		
hsa-miR-511	2681	3702	dendritic cells and macrophages		
hsa-miR-512-3p	2682	3703	embryonic stem cells, placenta		
hsa-miR-512-5p	2683	3704	embryonic stem cells, placenta,		
hsa-miR-513a-3p	2684	3705		lung carcinoma	
hsa-miR-513a-5p	2685	3706	endothelial cells		
hsa-miR-513b	2686	3707		follicular lymphoma	
hsa-miR-513c-3p	2687	3708			
hsa-miR-513c-5p	2688	3709			
hsa-miR-514a-3p	2689	3710			
hsa-miR-514a-5p	2690	3711			
hsa-miR-514b-3p	2691	3712		various cancer cells	
hsa-miR-514b-5p	2692	3713		various cancer cells	
hsa-miR-515-3p	2693	3714			
hsa-miR-515-5p	2694	3715	placenta		
hsa-miR-516a-3p	2695	3716	frontal cortex		

hsa-miR-5 16a-5p	2696	3717	placenta		
hsa-miR-5 16b-3p	2697	3718			
hsa-miR-5 16b-5p	2698	3719			
hsa-miR-5 17-5p	2699	3720	placenta		
hsa-miR-5 17a-3p	2700	3721	placenta		
hsa-miR-5 17b-3p	2701	3722	placenta		
hsa-miR-5 17c-3p	2702	3723	placenta		
hsa-miR-5 186	2703	3724	discovered in lymphoblastic leukaemia		
hsa-miR-5 187-3p	2704	3725	discovered in lymphoblastic leukaemia, skin (psoriasis)		
hsa-miR-5 187-5p	2705	3726	discovered in lymphoblastic leukaemia, skin (psoriasis)		
hsa-miR-5 188	2706	3727	discovered in lymphoblastic leukaemia		
hsa-miR-5 189	2707	3728	discovered in lymphoblastic leukaemia		
hsa-miR-5 18a-3p	2708	3729		HCC	
hsa-miR-5 18a-5p	2709	3730		various cancer cells	
hsa-miR-5 18b	2710	3731	placenta	HCC	cell cycle progression
hsa-miR-5 18c-3p	2711	3732	placenta		
hsa-miR-5 18c-5p	2712	3733	placenta		
hsa-miR-5 18d-3p	2713	3734			
hsa-miR-5 18d-5p	2714	3735			
hsa-miR-5 18e-3p	2715	3736		HCC	cell cycle progression
hsa-miR-5 18e-5p	2716	3737		HCC	cell cycle progression
hsa-miR-5 18f-3p	2717	3738	placenta		
hsa-miR-5 18f-5p	2718	3739	placenta		
hsa-miR-5 190	2719	3740	discovered in lymphoblastic leukaemia		

hsa-miR-5191	2720	3741	discovered in lymphoblastic leukaemia		
hsa-miR-5192	2721	3742	discovered in lymphoblastic leukaemia		
hsa-miR-5193	2722	3743	discovered in lymphoblastic leukaemia		
hsa-miR-5194	2723	3744	discovered in lymphoblastic leukaemia		
hsa-miR-5195-3p	2724	3745	discovered in lymphoblastic leukaemia		
hsa-miR-5195-5p	2725	3746	discovered in lymphoblastic leukaemia		
hsa-miR-5196-3p	2726	3747	discovered in lymphoblastic leukaemia		
hsa-miR-5196-5p	2727	3748	discovered in lymphoblastic leukaemia		
hsa-miR-5197-3p	2728	3749	discovered in lymphoblastic leukaemia		
hsa-miR-5197-5p	2729	3750	discovered in lymphoblastic leukaemia		
hsa-miR-519a-3p	2730	3751	placenta	HCC	
hsa-miR-519a-5p	2731	3752	placenta	HCC	
hsa-miR-519b-3p	2732	3753		breast cancer	
hsa-miR-519b-5p	2733	3754		breast cancer	
hsa-miR-519c-3p	2734	3755			
hsa-miR-519c-5p	2735	3756			
hsa-miR-519d	2736	3757	placenta		
hsa-miR-519e-3p	2737	3758	placenta		
hsa-miR-519e-5p	2738	3759	placenta		
hsa-miR-520a-3p	2739	3760	placenta		
hsa-miR-520a-5p	2740	3761	placenta		
hsa-miR-520b	2741	3762		breast cancer	
hsa-miR-520c-3p	2742	3763		gastric cancer, breast tumor	

hsa-miR-520c-5p	2743	3764		breast tumor	
hsa-miR-520d-3p	2744	3765		various cancer cells	
hsa-miR-520d-5p	2745	3766		various cancer cells	
hsa-miR-520e	2746	3767		hepatoma	tumor suppressor
hsa-miR-520f	2747	3768		breast cancer	
hsa-miR-520g	2748	3769		HCC, bladder cancer, breast cancer	
hsa-miR-520h	2749	3770	placental specific		
hsa-miR-521	2750	3771		prostate cancer	
hsa-miR-522-3p	2751	3772		HCC	
hsa-miR-522-5p	2752	3773		HCC	
hsa-miR-523-3p	2753	3774			
hsa-miR-523-5p	2754	3775			
hsa-miR-524-3p	2755	3776		colon cancer stem cells	
hsa-miR-524-5p	2756	3777	placental specific	gliomas	
hsa-miR-525-3p	2757	3778	placental specific	HCC	
hsa-miR-525-5p	2758	3779	placental specific		
hsa-miR-526a	2759	3780	placental specific		
hsa-miR-526b-3p	2760	3781	placental specific		
hsa-miR-526b-5p	2761	3782	placental specific		
hsa-miR-527	2762	3783			
hsa-miR-532-3p	2763	3784		ALL	
hsa-miR-532-5p	2764	3785		ALL	
hsa-miR-539-3p	2765	3786			
hsa-miR-539-5p	2766	3787			
hsa-miR-541-3p	2767	3788			
hsa-miR-541-5p	2768	3789			
hsa-miR-542-3p	2769	3790	monocytes		
hsa-miR-542-5p	2770	3791		basal cell carcinoma, neuroblastoma	
hsa-miR-543	2771	3792			
hsa-miR-544a	2772	3793		osteosarcoma	

hsa-miR-544b	2773	3794		osteocarcinoma	
hsa-miR-545-3p	2774	3795			
hsa-miR-545-5p	2775	3796		rectal cancer	
hsa-miR-548	2776	3797			
hsa-miR-548-3p	2777	3798			
hsa-miR-548-5p	2778	3799			
hsa-miR-548a	2779	3800	identified in colorectal microRNAome		
hsa-miR-548a-3p	2780	3801	identified in colorectal microRNAome		
hsa-miR-548a-5p	2781	3802	identified in colorectal microRNAome		
hsa-miR-548aa	2782	3803	identified in cervical tumor		
hsa-miR-548ab	2783	3804	discovered in B-cells		
hsa-miR-548ac	2784	3805	discovered in B-cells		
hsa-miR-548ad	2785	3806	discovered in B-cells		
hsa-miR-548ae	2786	3807	discovered in B-cells		
hsa-miR-548ag	2787	3808	discovered in B-cells		
hsa-miR-548ah-3p	2788	3809	discovered in B-cells		
hsa-miR-548ah-5p	2789	3810	discovered in B-cells		
hsa-miR-548ai	2790	3811	discovered in B-cells		
hsa-miR-548aj-3p	2791	3812	discovered in B-cells		
hsa-miR-548aj-5p	2792	3813	discovered in B-cells		
hsa-miR-548ak	2793	3814	discovered in B-cells		
hsa-miR-548al	2794	3815	discovered in B-cells		
hsa-miR-548am-3p	2795	3816	discovered in B-cells		
hsa-miR-548am-5p	2796	3817	discovered in B-cells		
hsa-miR-548an	2797	3818	discovered in B-cells		

hsa-miR-548ao-3p	2798	3819			
hsa-miR-548ao-5p	2799	3820			
hsa-miR-548ap-3p	2800	3821			
hsa-miR-548ap-5p	2801	3822			
hsa-miR-548aq-3p	2802	3823			
hsa-miR-548aq-5p	2803	3824			
hsa-miR-548ar-3p	2804	3825			
hsa-miR-548ar-5p	2805	3826			
hsa-miR-548as-3p	2806	3827			
hsa-miR-548as-5p	2807	3828			
hsa-miR-548at-3p	2808	3829		prostate cancer	
hsa-miR-548at-5p	2809	3830		prostate cancer	
hsa-miR-548au-3p	2810	3831			
hsa-miR-548au-5p	281 1	3832			
hsa-miR-548av-3p	2812	3833			
hsa-miR-548av-5p	2813	3834			
hsa-miR-548aw	2814	3835		prostate cancer	
hsa-miR-548ay-3p	2815	3836	discovered in abnormal skin (psoriasis)		
hsa-miR-548ay-5p	2816	3837	discovered in abnormal skin (psoriasis)		
hsa-miR-548az-3p	2817	3838	discovered in abnormal skin (psoriasis)		
hsa-miR-548az-5p	2818	3839	discovered in abnormal skin (psoriasis)		
hsa-miR-548b-3p	2819	3840	identified in colorectal microRNAome		
hsa-miR-548b-5p	2820	3841	immune cells, frontal cortex		
hsa-miR-548c-3p	2821	3842	identified in colorectal microRNAome		
hsa-miR-548c-5p	2822	3843	immune cells, frontal cortex		
hsa-miR-548d-3p	2823	3844	identified in colorectal		

			microRNAome		
hsa-miR-548d-5p	2824	3845	identified in colorectal microRNAome		
hsa-miR-548e	2825	3846	embryonic stem cells		
hsa-miR-548f	2826	3847	embryonic stem cells		
hsa-miR-548g-3p	2827	3848	embryonic stem cells		
hsa-miR-548g-5p	2828	3849	embryonic stem cells		
hsa-miR-548h-3p	2829	3850	embryonic stem cells		
hsa-miR-548h-5p	2830	3851	embryonic stem cells		
hsa-miR-548i	2831	3852	embryonic stem cells, immune cells		
hsa-miR-548j	2832	3853	immune cells		
hsa-miR-548k	2833	3854	embryonic stem cells		
hsa-miR-548l	2834	3855	embryonic stem cells		
hsa-miR-548m	2835	3856	embryonic stem cells		
hsa-miR-548n	2836	3857	embryonic stem cells, immune cells		
hsa-miR-548o-3p	2837	3858	embryonic stem cells		
hsa-miR-548o-5p	2838	3859	embryonic stem cells		
hsa-miR-548p	2839	3860	embryonic stem cells		
hsa-miR-548q	2840	3861		ovarian cancer cells	
hsa-miR-548s	2841	3862	discovered in the melanoma MicroRNAome		
hsa-miR-548t-3p	2842	3863	discovered in the melanoma MicroRNAome		
hsa-miR-548t-5p	2843	3864	discovered in the melanoma MicroRNAome		
hsa-miR-548u	2844	3865	discovered in the melanoma MicroRNAome		
hsa-miR-548w	2845	3866	discovered in the melanoma		

			MicroRNAome		
hsa-miR-548y	2846	3867	/		
hsa-miR-548z	2847	3868	discovered in cervical tumor		
hsa-miR-549a	2848	3869	discovered in a colorectal MicroRNAome		
hsa-miR-550a-3-5p	2849	3870		Hepatocellular Carcinoma	
hsa-miR-550a-3p	2850	3871		Hepatocellular Carcinoma	
hsa-miR-550a-5p	2851	3872		Hepatocellular Carcinoma	
hsa-miR-550b-2-5p	2852	3873	discovered in cervical tumor		
hsa-miR-550b-3p	2853	3874	discovered in cervical tumor		
hsa-miR-551a	2854	3875		gastric cancer	
hsa-miR-551b-3p	2855	3876	hepatocytes		
hsa-miR-551b-5p	2856	3877	hepatocytes		
hsa-miR-552	2857	3878	discovered in a colorectal MicroRNAome		
hsa-miR-553	2858	3879	discovered in a colorectal MicroRNAome		
hsa-miR-554	2859	3880	discovered in a colorectal MicroRNAome		
hsa-miR-555	2860	3881	discovered in a colorectal MicroRNAome		
hsa-miR-556-3p	2861	3882	discovered in a colorectal MicroRNAome		
hsa-miR-556-5p	2862	3883	discovered in a colorectal MicroRNAome		
hsa-miR-557	2863	3884	liver(hepatocytes)		
hsa-miR-5571-3p	2864	3885	discovered in Salivary gland		
hsa-miR-557 l-5p	2865	3886	discovered in Salivary gland		
hsa-miR-5572	2866	3887	discovered in Salivary gland		
hsa-miR-5579-3p	2867	3888			

hsa-miR-5579-5p	2868	3889			
hsa-miR-558	2869	3890		neuroblastoma	
hsa-miR-5580-3p	2870	3891			
hsa-miR-5580-5p	2871	3892			
hsa-miR-5581-3p	2872	3893			
hsa-miR-5581-5p	2873	3894			
hsa-miR-5582-3p	2874	3895			
hsa-miR-5582-5p	2875	3896			
hsa-miR-5583-3p	2876	3897			
hsa-miR-5583-5p	2877	3898			
hsa-miR-5584-3p	2878	3899			
hsa-miR-5584-5p	2879	3900			
hsa-miR-5585-3p	2880	3901			
hsa-miR-5585-5p	2881	3902			
hsa-miR-5586-3p	2882	3903			
hsa-miR-5586-5p	2883	3904			
hsa-miR-5587-3p	2884	3905			
hsa-miR-5587-5p	2885	3906			
hsa-miR-5588-3p	2886	3907			
hsa-miR-5588-5p	2887	3908			
hsa-miR-5589-3p	2888	3909			
hsa-miR-5589-5p	2889	3910			
hsa-miR-559	2890	3911			
hsa-miR-5590-3p	2891	3912			
hsa-miR-5590-5p	2892	3913			
hsa-miR-5591-3p	2893	3914			
hsa-miR-5591-5p	2894	3915			
hsa-miR-561-3p	2895	3916		multiple myeloma	
hsa-miR-561-5p	2896	3917		multiple myeloma	
hsa-miR-562	2897	3918			
hsa-miR-563	2898	3919	discovered in a colorectal MicroRNAome		
hsa-miR-564	2899	3920		Chronic myeloid	

				leukemia	
hsa-miR-566	2900	3921		MALT lymphoma/lymphocyte	
hsa-miR-567	2901	3922		colorectal cancer	
hsa-miR-568	2902	3923	discovered in a colorectal MicroRNAome		
hsa-miR-5680	2903	3924		Associated with metastatic prostate cancer	
hsa-miR-5681a	2904	3925		Associated with metastatic prostate cancer	
hsa-miR-5681b	2905	3926		Associated with metastatic prostate cancer	
hsa-miR-5682	2906	3927		Associated with metastatic prostate cancer	
hsa-miR-5683	2907	3928		Associated with metastatic prostate cancer	
hsa-miR-5684	2908	3929		Associated with metastatic prostate cancer	
hsa-miR-5685	2909	3930		Associated with metastatic prostate cancer	
hsa-miR-5686	2910	3931		Associated with metastatic prostate cancer	
hsa-miR-5687	2911	3932		Associated with metastatic prostate cancer	
hsa-miR-5688	2912	3933		Associated with metastatic prostate cancer	
hsa-miR-5689	2913	3934		Associated with metastatic prostate cancer	
hsa-miR-569	2914	3935			
hsa-miR-5690	2915	3936		Associated with metastatic prostate cancer	
hsa-miR-5691	2916	3937		Associated with metastatic prostate cancer	

hsa-miR-5692a	2917	3938		Associated with metastatic prostate cancer	
hsa-miR-5692b	2918	3939		Associated with metastatic prostate cancer	
hsa-miR-5692c	2919	3940		Associated with metastatic prostate cancer	
hsa-miR-5693	2920	3941		Associated with metastatic prostate cancer	
hsa-miR-5694	2921	3942		Associated with metastatic prostate cancer	
hsa-miR-5695	2922	3943		Associated with metastatic prostate cancer	
hsa-miR-5696	2923	3944		Associated with metastatic prostate cancer	
hsa-miR-5697	2924	3945		Associated with metastatic prostate cancer	
hsa-miR-5698	2925	3946		Associated with metastatic prostate cancer	
hsa-miR-5699	2926	3947		Associated with metastatic prostate cancer	
hsa-miR-5700	2927	3948		Associated with metastatic prostate cancer	
hsa-miR-5701	2928	3949		Associated with metastatic prostate cancer	
hsa-miR-5702	2929	3950		Associated with metastatic prostate cancer	
hsa-miR-5703	2930	3951		Associated with metastatic prostate cancer	
hsa-miR-570-3p	2931	3952		follicular lymphoma	
hsa-miR-5704	2932	3953		Associated with metastatic prostate cancer	
hsa-miR-5705	2933	3954		Associated with metastatic prostate cancer	

hsa-miR-570-5p	2934	3955		follicular lymphoma	
hsa-miR-5706	2935	3956		Associated with metastatic prostate cancer	
hsa-miR-5707	2936	3957		Associated with metastatic prostate cancer	
hsa-miR-5708	2937	3958		Associated with metastatic prostate cancer	
hsa-miR-571	2938	3959	frontal cortex		
hsa-miR-572	2939	3960	circulating microRNA (in plasma)	basal cell carcinoma	
hsa-miR-573	2940	3961	discovered in the colorectal MicroRNAome		
hsa-miR-5739	2941	3962	endothelial cells		
hsa-miR-574-3p	2942	3963	blood (myeloid cells)	follicular lymphoma	
hsa-miR-574-5p	2943	3964	semen		
hsa-miR-575	2944	3965		gastric cancer	
hsa-miR-576-3p	2945	3966	discovered in a colorectal MicroRNAome		
hsa-miR-576-5p	2946	3967	cartilage/chondrocyte		
hsa-miR-577	2947	3968	discovered in a colorectal MicroRNAome		
hsa-miR-578	2948	3969	discovered in a colorectal MicroRNAome		
hsa-miR-5787	2949	3970	fibroblast		
hsa-miR-579	2950	3971			
hsa-miR-580	2951	3972		breast cancer	
hsa-miR-581	2952	3973	liver(hepatocytes)		
hsa-miR-582-3p	2953	3974	cartilage/chondrocyte	bladder cancer	
hsa-miR-582-5p	2954	3975		bladder cancer	
hsa-miR-583	2955	3976		rectal cancer cells	
hsa-miR-584-3p	2956	3977		tumor cells (follicular lymphoma, rectal	

				cancer cells)	
hsa-miR-584-5p	2957	3978		tumor cells (follicular lymphoma, rectal cancer cells)	
hsa-miR-585	2958	3979		oral squamous cell carcinoma	
hsa-miR-586	2959	3980	discovered in a colorectal MicroRNAome		
hsa-miR-587	2960	3981	discovered in a colorectal MicroRNAome		
hsa-miR-588	2961	3982	discovered in a colorectal MicroRNAome		
hsa-miR-589-3p	2962	3983	mesothelial cells		
hsa-miR-589-5p	2963	3984	mesothelial cells		
hsa-miR-590-3p	2964	3985	cardiomyocytes		Cell cycle progression
hsa-miR-590-5p	2965	3986	cardiomyocytes		Cell cycle progression
hsa-miR-591	2966	3987		neuroblastoma	
hsa-miR-592	2967	3988		hepatocellular carcinoma	
hsa-miR-593-3p	2968	3989		esophageal cancer	
hsa-miR-593-5p	2969	3990		esophageal cancer	
hsa-miR-595	2970	3991		heart failure	
hsa-miR-596	2971	3992		ependymoma, cancers	
hsa-miR-597	2972	3993	discovered in a colorectal MicroRNAome		
hsa-miR-598	2973	3994	Blood (lymphocytes)		
hsa-miR-599	2974	3995		Multiple sclerosis	
hsa-miR-600	2975	3996	discovered in a colorectal MicroRNAome		
hsa-miR-601	2976	3997		various cancers (colonrectal, gastric)	
hsa-miR-602	2977	3998	oocyte		
hsa-miR-603	2978	3999			
hsa-miR-604	2979	4000	discovered in a		

			colorectal MicroRNAome		
hsa-miR-605	2980	4001	discovered in a colorectal MicroRNAome		
hsa-miR-606	2981	4002	discovered in a colorectal MicroRNAome		
hsa-miR-6068	2982	4003	discovered in endothelial cells		
hsa-miR-6069	2983	4004	discovered in endothelial cells		
hsa-miR-607	2984	4005	discovered in a colorectal MicroRNAome		
hsa-miR-6070	2985	4006	discovered in a colorectal MicroRNAome		
hsa-miR-607 1	2986	4007	discovered in endothelial cells		
hsa-miR-6072	2987	4008	discovered in endothelial cells		
hsa-miR-6073	2988	4009	discovered in endothelial cells		
hsa-miR-6074	2989	4010	discovered in endothelial cells		
hsa-miR-6075	2990	401 1	discovered in endothelial cells		
hsa-miR-6076	2991	4012	discovered in endothelial cells		
hsa-miR-6077	2992	4013	discovered in endothelial cells		
hsa-miR-6078	2993	4014	discovered in endothelial cells		
hsa-miR-6079	2994	4015	discovered in endothelial cells		
hsa-miR-608	2995	4016		various cancers	
hsa-miR-6080	2996	4017	discovered in endothelial cells		
hsa-miR-608 1	2997	4018	discovered in endothelial cells		
hsa-miR-6082	2998	4019	discovered in endothelial cells		
hsa-miR-6083	2999	4020	discovered in endothelial cells		
hsa-miR-6084	3000	4021	discovered in endothelial cells		
hsa-miR-6085	3001	4022	discovered in endothelial cells		

hsa-miR-6086	3002	4023	embryonic stem cells		
hsa-miR-6087	3003	4024	embryonic stem cells		
hsa-miR-6088	3004	4025	embryonic stem cells		
hsa-miR-6089	3005	4026	embryonic stem cells		
hsa-miR-609	3006	4027	discovered in a colorectal MicroRNAome		
hsa-miR-6090	3007	4028	embryonic stem cells		
hsa-miR-610	3008	4029		gastric cancer	
hsa-miR-611	3009	4030		Renal cell carcinoma	
hsa-miR-612	3010	4031		AM leukemia	
hsa-miR-6124	3011	4032			
hsa-miR-6125	3012	4033			
hsa-miR-6126	3013	4034			
hsa-miR-6127	3014	4035			
hsa-miR-6128	3015	4036			
hsa-miR-6129	3016	4037			
hsa-miR-613	3017	4038	lipid metabolism		
hsa-miR-6130	3018	4039			
hsa-miR-6131	3019	4040			
hsa-miR-6132	3020	4041			
hsa-miR-6133	3021	4042			
hsa-miR-6134	3022	4043			
hsa-miR-614	3023	4044	circulating miRNAs (in Plasma)		
hsa-miR-615-3p	3024	4045			
hsa-miR-615-5p	3025	4046			
hsa-miR-616-3p	3026	4047		prostate cancer	
hsa-miR-6165	3027	4048			Pro-apoptotic factor
hsa-miR-616-5p	3028	4049		prostate cancer	
hsa-miR-617	3029	4050			
hsa-miR-618	3030	4051			

hsa-miR-619	3031	4052	discovered in a colorectal MicroRNAome		
hsa-miR-620	3032	4053	discovered in a colorectal MicroRNAome		
hsa-miR-621	3033	4054			
hsa-miR-622	3034	4055			
hsa-miR-623	3035	4056			
hsa-miR-624-3p	3036	4057	chondrocyte		
hsa-miR-624-5p	3037	4058	chondrocyte		
hsa-miR-625-3p	3038	4059	liver(hepatocytes), circulating (blood)	various cancers	
hsa-miR-625-5p	3039	4060	liver(hepatocytes), circulating (blood)	various cancers	
hsa-miR-626	3040	4061	discovered in the colorectal MicroRNAome		
hsa-miR-627	3041	4062		colorectal cancer	
hsa-miR-628-3p	3042	4063		neuroblastoma	
hsa-miR-628-5p	3043	4064		neuroblastoma	
hsa-miR-629-3p	3044	4065		B-lineage ALL, T cell lupus, RCC/kidney	
hsa-miR-629-5p	3045	4066		B-lineage ALL, T cell lupus, RCC/kidney	
hsa-miR-630	3046	4067	chondrocytes	rectal cancer	
hsa-miR-631	3047	4068	discovered in the colorectal MicroRNAom		
hsa-miR-632	3048	4069		myelodysplastic syndromes	
hsa-miR-633	3049	4070		multiple sclerosis	
hsa-miR-634	3050	4071	cartilage/chondrocyte		
hsa-miR-635	3051	4072	discovered in the colorectal MicroRNAome		
hsa-miR-636	3052	4073		myelodysplastic syndromes	
hsa-miR-637	3053	4074	discovered in the colorectal MicroRNAome		

hsa-miR-638	3054	4075		Lupus nephritis, basal cell carcinoma	
hsa-miR-639	3055	4076	discovered in the colorectal MicroRNAome		
hsa-miR-640	3056	4077		Chronic lymphocytic leukemia	
hsa-miR-641	3057	4078	cartilage/chondrocyte		
hsa-miR-642a-3p	3058	4079	adipocyte		
hsa-miR-642a-5p	3059	4080	discovered in the colorectal MicroRNAome		
hsa-miR-642b-3p	3060	4081	discovered in a cervical tumor		
hsa-miR-642b-5p	3061	4082	discovered in a cervical tumor		
hsa-miR-643	3062	4083	discovered in the colorectal MicroRNAome		
hsa-miR-644a	3063	4084			
hsa-miR-645	3064	4085		ovarian cancer	
hsa-miR-646	3065	4086			
hsa-miR-647	3066	4087		prostate and lung cancer	
hsa-miR-648	3067	4088	circulating microRNAs (in Plasma)		
hsa-miR-649	3068	4089	Serum		
hsa-miR-6499-3p	3069	4090	discovered in abnormal skin (psoriasis)		
hsa-miR-6499-5p	3070	4091	discovered in abnormal skin (psoriasis)		
hsa-miR-650	3071	4092		melanoma	
hsa-miR-6500-3p	3072	4093	discovered in abnormal skin (psoriasis)		
hsa-miR-6500-5p	3073	4094	discovered in abnormal skin (psoriasis)		
hsa-miR-6501-3p	3074	4095	discovered in abnormal skin (psoriasis)		

hsa-miR-6501-5p	3075	4096	discovered in abnormal skin (psoriasis)		
hsa-miR-6502-3p	3076	4097	discovered in abnormal skin (psoriasis)		
hsa-miR-6502-5p	3077	4098	discovered in abnormal skin (psoriasis)		
hsa-miR-6503-3p	3078	4099	discovered in abnormal skin (psoriasis)		
hsa-miR-6503-5p	3079	4100	discovered in abnormal skin (psoriasis)		
hsa-miR-6504-3p	3080	4101	discovered in abnormal skin (psoriasis)		
hsa-miR-6504-5p	3081	4102	discovered in abnormal skin (psoriasis)		
hsa-miR-6505-3p	3082	4103	discovered in abnormal skin (psoriasis)		
hsa-miR-6505-5p	3083	4104	discovered in abnormal skin (psoriasis)		
hsa-miR-6506-3p	3084	4105	discovered in abnormal skin (psoriasis)		
hsa-miR-6506-5p	3085	4106	discovered in abnormal skin (psoriasis)		
hsa-miR-6507-3p	3086	4107	discovered in abnormal skin (psoriasis)		
hsa-miR-6507-5p	3087	4108	discovered in abnormal skin (psoriasis)		
hsa-miR-6508-3p	3088	4109	discovered in abnormal skin (psoriasis)		
hsa-miR-6508-5p	3089	4 1 10	discovered in abnormal skin (psoriasis)		
hsa-miR-6509-3p	3090	4 1 11	discovered in abnormal skin (psoriasis)		
hsa-miR-6509-5p	3091	4 1 12	discovered in abnormal skin		

			(psoriasis)		
hsa-miR-65 1	3092	4 113	discovered in the colorectal MicroRNAome	lung cancer	
hsa-miR-65 10-3p	3093	4 114	discovered in abnormal skin (psoriasis)		
hsa-miR-65 10-5p	3094	4 115	discovered in abnormal skin (psoriasis)		
hsa-miR-65 11a-3p	3095	4 116	discovered in abnormal skin (psoriasis) and epididymis		
hsa-miR-65 11a-5p	3096	4 117	discovered in abnormal skin (psoriasis) and epididymis		
hsa-miR-65 11b-3p	3097	4 118	discovered in epididymis		
hsa-miR-65 11b-5p	3098	4 119	discovered in epididymis		
hsa-miR-65 12-3p	3099	4120	discovered in abnormal skin (psoriasis)		
hsa-miR-65 12-5p	3100	4121	discovered in abnormal skin (psoriasis)		
hsa-miR-65 13-3p	3101	4122	discovered in abnormal skin (psoriasis)		
hsa-miR-65 13-5p	3102	4123	discovered in abnormal skin (psoriasis)		
hsa-miR-65 14-3p	3103	4124	discovered in abnormal skin (psoriasis)		
hsa-miR-65 14-5p	3104	4125	discovered in abnormal skin (psoriasis)		
hsa-miR-65 15-3p	3105	4126	discovered in abnormal skin (psoriasis) and epididymis		
hsa-miR-65 15-5p	3106	4127	discovered in abnormal skin (psoriasis) and epididymis		
hsa-miR-652-3p	3107	4128		rectal cancer cells	

hsa-miR-652-5p	3108	4129		rectal cancer cells	
hsa-miR-653	3109	4130	Discovered in the colorectal MicroRNAome		
hsa-miR-654-3p	3110	4131	Discovered in the colorectal MicroRNAome		
hsa-miR-654-5p	3111	4132	bone marrow	prostate cancer	
hsa-miR-655	3112	4133			
hsa-miR-656	3113	4134		various cancers	
hsa-miR-657	3114	4135	oligodendrocytes	diabetes	
hsa-miR-658	3115	4136		gastric cancer	
hsa-miR-659-3p	3116	4137	myoblast		
hsa-miR-659-5p	3117	4138	myoblast		
hsa-miR-660-3p	3118	4139	myoblast		
hsa-miR-660-5p	3119	4140	myoblast		
hsa-miR-661	3120	4141		breast cancer	
hsa-miR-662	3121	4142	endothelial progenitor cells, oocytes		
hsa-miR-663a	3122	4143		follicular lymphoma, Lupus nephritis	
hsa-miR-663b	3123	4144		follicular lymphoma, Lupus nephritis	
hsa-miR-664a-3p	3124	4145	embryonic stem cells		component of SnoRNAs
hsa-miR-664a-5p	3125	4146	embryonic stem cells		component of SnoRNAs
hsa-miR-664b-3p	3126	4147	embryonic stem cells		component of SnoRNAs
hsa-miR-664b-5p	3127	4148	embryonic stem cells		component of SnoRNAs
hsa-miR-665	3128	4149		breast cancer	
hsa-miR-668	3129	4150	keratinocytes		senescence
hsa-miR-670	3130	4151			
hsa-miR-671-3p	3131	4152			
hsa-miR-6715a-3p	3132	4153	discovered in epididymis		
hsa-miR-6715b-3p	3133	4154	discovered in epididymis		

hsa-miR-67 15b-5p	3134	4155	discovered in epididymis		
hsa-miR-67 1-5p	3135	4156		rectal cancer, prolactinomas	
hsa-miR-67 16-3p	3136	4157	discovered in epididymis		
hsa-miR-67 16-5p	3137	4158	discovered in epididymis		
hsa-miR-67 17-5p	3138	4159	discovered in epididymis		
hsa-miR-67 18-5p	3139	4160	discovered in epididymis		
hsa-miR-67 19-3p	3140	4161	discovered in epididymis		
hsa-miR-6720-3p	3141	4162	discovered in epididymis		
hsa-miR-6721-5p	3142	4163	discovered in epididymis		
hsa-miR-6722-3p	3143	4164	discovered in epididymis		
hsa-miR-6722-5p	3144	4165	discovered in epididymis		
hsa-miR-6723-5p	3145	4166	discovered in epididymis		
hsa-miR-6724-5p	3146	4167	discovered in epididymis		
hsa-miR-675-3p	3147	4168		adrenocortical tumor	
hsa-miR-675-5p	3148	4169		adrenocortical tumor	
hsa-miR-676-3p	3149	4170	discovered in female reproductive tract		
hsa-miR-676-5p	3150	4171	discovered in female reproductive tract		
hsa-miR-708-3p	3151	4172		Various cancers (lung, bladder, pancreatic , ALL)	
hsa-miR-708-5p	3152	4173		Various cancers (lung, bladder, pancreatic , ALL)	
hsa-miR-7 11	3153	4174		cutaneous T-cell lymphomas	
hsa-miR-7-l-3p	3154	4175	Glioblast, brain, pancreas		
hsa-miR-7 18	3155	4176	blood		
hsa-miR-7-2-3p	3156	4177	brain, pancreas		

hsa-miR-744-3p	3157	4178	heart		
hsa-miR-744-5p	3158	4179	embryonic stem cells, heart		
hsa-miR-758-3p	3159	4180	cholesterol regulation and brain		
hsa-miR-758-5p	3160	4181	cholesterol regulation and brain		
hsa-miR-759	3161	4182			
hsa-miR-7-5p	3162	4183	brain		
hsa-miR-760	3163	4184		colonrectal and breast cancer	
hsa-miR-761	3164	4185			
hsa-miR-762	3165	4186	corneal epithelial cells		
hsa-miR-764	3166	4187	osteoblast		
hsa-miR-765	3167	4188		rectal cancer	
hsa-miR-766-3p	3168	4189	embryonic stem cells		
hsa-miR-766-5p	3169	4190	embryonic stem cells		
hsa-miR-767-3p	3170	4191			
hsa-miR-767-5p	3171	4192			
hsa-miR-769-3p	3172	4193			
hsa-miR-769-5p	3173	4194			
hsa-miR-770-5p	3174	4195			
hsa-miR-802	3175	4196	brain , epithelial cells, hepatocytes	down syndrome	
hsa-miR-873-3p	3176	4197			
hsa-miR-873-5p	3177	4198			
hsa-miR-874	3178	4199		cervical cancer, lung cancer, carcinoma	
hsa-miR-875-3p	3179	4200			
hsa-miR-875-5p	3180	4201			
hsa-miR-876-3p	3181	4202			
hsa-miR-876-5p	3182	4203			
hsa-miR-877-3p	3183	4204			
hsa-miR-877-5p	3184	4205			

hsa-miR-885-3p	3185	4206	embryonic stem cells		
hsa-miR-885-5p	3186	4207	embryonic stem cells		
hsa-miR-887	3187	4208			
hsa-miR-888-3p	3188	4209			
hsa-miR-888-5p	3189	4210			
hsa-miR-889	3190	4211			
hsa-miR-890	3191	4212	epididymis		
hsa-miR-891a	3192	4213	epididymis	osteosarcoma	
hsa-miR-891b	3193	4214	epididymis		
hsa-miR-892a	3194	4215	epididymis		
hsa-miR-892b	3195	4216	epididymis		
hsa-miR-892c-3p	3196	4217	discovered in epididymis		
hsa-miR-892c-5p	3197	4218	discovered in epididymis		
hsa-miR-920	3198	4219	human testis		
hsa-miR-921	3199	4220	human testis	muscle invasive bladder cancer	
hsa-miR-922	3200	4221	human testis, neuronal tissues	multiple sclerosis, Alcoholic liver disease	
hsa-miR-924	3201	4222	human testis		
hsa-miR-92a-1-5p	3202	4223	endothelial cells		
hsa-miR-92a-2-5p	3203	4224	endothelial cells		
hsa-miR-92a-3p	3204	4225	endothelial cells, CNS		
hsa-miR-92b-3p	3205	4226	endothelial cells, heart		
hsa-miR-92b-5p	3206	4227	endothelial cells, heart		
hsa-miR-933	3207	4228	discovered in cervical cancer		
hsa-miR-93-3p	3208	4229	embryonic stem cells	basal cell carcinoma	
hsa-miR-934	3209	4230	discovered in cervical cancer		
hsa-miR-935	3210	4231	blood monoclear cells	energy metabolism/ obesity, medullablastoma/n eural stem cells	

hsa-miR-93-5p	3211	4232	embryonic stem cells		
hsa-miR-936	3212	4233	skin		
hsa-miR-937-3p	3213	4234		cervical cancer	
hsa-miR-937-5p	3214	4235		cervical cancer	
hsa-miR-938	3215	4236		Various cancer cells	
hsa-miR-939-3p	3216	4237	hepatocytes		
hsa-miR-939-5p	3217	4238	hepatocytes		
hsa-miR-9-3p	3218	4239	brain	Cancers and brain diseases	
hsa-miR-940	3219	4240	identified in Cervical cancer		
hsa-miR-941	3220	4241	Embryonic stem cells		
hsa-miR-942	3221	4242		lung cancer	
hsa-miR-943	3222	4243	identified in Cervical cancer		
hsa-miR-944	3223	4244		various cancers (cervical, pancreatic, colonrectal)	
hsa-miR-95	3224	4245		various cancers (pancreatic, glioblastoma, colorectal etc)	
hsa-miR-9-5p	3225	4246	brain	Cancers and brain disease	
hsa-miR-96-3p	3226	4247	stem cells	various cancers (prostate, lymphoma, HCC, etc) and inflammation	
hsa-miR-96-5p	3227	4248	stem cells	various cancers (prostate, lymphoma, HCC, etc) and inflammation	
hsa-miR-98-3p	3228	4249		various cancer cells	apoptosis
hsa-miR-98-5p	3229	4250		various cancer cells	apoptosis
hsa-miR-99a-3p	3230	4251	hemapoietic cells		
hsa-miR-99a-5p	3231	4252	hemapoietic cells		
hsa-miR-99b-3p	3232	4253	hemapoietic cells, embryonic stem		

			cells		
hsa-miR-99b-5p	3233	4254	hemapoietic cells, embryonic stem cells		

[00351] MicroRNAs that are enriched in specific types of immune cells are listed in Table 13. Furthermore, novel miroRNAs are discovered in the immune cells in the art through micro-array hybridization and microtome analysis (Jima **DD** et al, Blood, 2010, 116:el 18-el27; Vaz C et al, BMC Genomics, 2010, 11,288, the content of each of which is incorporated herein by reference in its entirety). In Table 13, "HCC" represents hepatocellular carcinoma, "ALL" stands for acute lymphoblastic leukemia and "CLL" stands for chronic lymphocytic leukemia.

Table 13. microRNAs in immune cells

microRNA	mir SEQ ID	BS SEQ ID	tissues/cells with MicroRNAs	associated diseases	biological functions/targets
hsa-let-7a-2-3p	171	1192	embryonic stem cells, lung, myeloid cells	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor, target to c-myc
hsa-let-7a-3p	172	1193	embryonic stem cell, lung, myeloid cells	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor, target to c-myc
hsa-let-7a-5p	173	1194	embryonic stem cells, lung, myeloid cells	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor, target to c-myc
hsa-let-7c	176	1197	dendritic cells	various cancers (cervical, pancreatic, lung , esophageal, etc)	tumor suppressor apoptosis (target to BCL-xl)
hsa-let-7e-3p	179	1200	immune cells	various cancer cells, autoimmunity TLR signal pathway in endotoxin tolerance	tumor suppressor
hsa-let-7e-5p	180	1201	immune cells	associated with	tumor suppressor

				various cancer cells	
hsa-let-7f-1-3p	181	1202	immune cells (T cells)	associated with various cancer cells	tumor suppressor
hsa-let-7f-2-3p	182	1203	immune cells (T cells)	associated with various cancer cells	tumor suppressor
hsa-let-7f-5p	183	1204	immune cells (T cells)	associated with various cancer cells	tumor suppressor
hsa-let-7g-3p	184	1205	hematopoietic cells, adipose, smooth muscle cells	various cancer cells (lung, breast, etc)	tumor suppressor (target to NFkB, LOX1)
hsa-let-7g-5p	185	1206	hematopoietic cells, adipose, smooth muscle cells	various cancer cells (lung, breast, etc)	tumor suppressor (target to NFkB, LOX1)
hsa-let-7i-3p	186	1207	immune cells	chronic lymphocyte leukemia	tumor suppressor
hsa-let-7i-5p	187	1208	immune cells	chronic lymphocyte leukemia	tumor suppressor
hsa-miR- 10a-3p	203	1224	hematopoietic cells	acute myeloid leukemia	oncogene, cell growth
hsa-miR- 10a-5p	204	1225	hematopoietic cells	acute myeloid leukemia	oncogene, cell growth
hsa-miR- 1184	214	1235	Hematopoietic cells	downregulated in oral leukoplakia (OLK)	predicted in the intron 22 of F8 gene
hsa-miR- 125b-1-3p	279	1300	hematopoietic cells (monocytes), brain (neuron)	various cancer (ALL, prostate, HCC, etc); TLR signal pathway in endotoxin tolerance	oncogene, cell differentiation
hsa-miR- 125b-2-3p	280	1301	hematopoietic cells (monocytes), brain (neuron)	various cancer (ALL, prostate, HCC etc); TLR signal pathway in endotoxin tolerance	oncogene cell differentiation
hsa-miR- 125b-5p	281	1302	hematopoietic cells, brain (neuron)	various cancer (Cutaneous T cell lymphomas, prostate, HCC, etc); TLR signal pathway in	oncogene cell differentiation

				endotoxin tolerance	
hsa-miR-1279	315	1336	monocytes		
hsa-miR-130a-3p	353	1374	lung, monocytes, vascular endothelial cells	various cancers (basal cell carcinoma, HCC, ovarian, etc), drug resistance	pro-angiogenic
hsa-miR-130a-5p	354	1375	lung, monocytes, vascular endothelial cells	various cancers (basal cell carcinoma, HCC, ovarian, etc), drug resistance	pro-angiogenic
hsa-miR-132-3p	360	1381	brain(neuron), immune cells		
hsa-miR-132-5p	362	1383	brain(neuron), immune cells		
hsa-miR-142-3p	383	1404	myeloid cells, hematopoiesis, APC cells		tumor suppressor, immune response
hsa-miR-142-5p	384	1405	myeloid cells, hematopoiesis, APC cells		immune response
hsa-miR-143-5p	386	1407	vascular smooth muscle, T-cells	increased in serum after virus infection	
hsa-miR-146a-3p	393	1414	immune cells, hematopoiesis, cartilage,	associated with CLL, TLR signal pathway in endotoxin tolerance	
hsa-miR-146a-5p	394	1415	immune cells, hematopoiesis, cartilage,	associated with CLL, TLR signal pathway in endotoxin tolerance	
hsa-miR-146b-3p	395	1416	immune cells	cancers (thyroid carcinoma)	immune response
hsa-miR-146b-5p	396	1417	embryoid body cells	thyroid cancer, associated with CLL	tumor invasion, migration
hsa-miR-147a	399	1420	Macrophage	inflammatory response	
hsa-miR-147b	400	1421	Macrophage	inflammatory response	
hsa-miR-148a-	401	1422	hematopoietic	associated with	

3p			cells	CLL, T-lineage ALL	
hsa-miR-148a-5p	402	1423	hematopoietic cells	associated with CLL, T-lineage ALL	
hsa-miR-150-3p	407	1428	hematopoietic cells (lymphoid)	circulating plasma (acute myeloid leukemia)	
hsa-miR-150-5p	408	1429	hematopoietic cells (lymphoid)	circulating plasma (acute myeloid leukemia)	
hsa-miR-151b	411	1432	immune cells (B-cells)		
hsa-miR-155-3p	419	1440	T/B cells, monocytes, breast	associated with CLL, TLR signal pathway in endotoxin tolerance ; upregulated in B cell lymphoma (CLL) and other cancers (breast, lung, ovarian, cervical, colorectal, prostate)	
hsa-miR-155-5p	420	1441	T/B cells, monocytes, breast	associated with CLL, TLR signal pathway in endotoxin tolerance, upregulated in B cell lymphoma (CLL) and other cancers (breast, lung, ovarian, cervical, colorectal, prostate)	
hsa-miR-15a-3p	422	1443	blood, lymphocyte, hematopoietic tissues (spleen)	chronic lymphocytic leukemia	
hsa-miR-15a-5p	423	1444	blood, lymphocyte, hematopoietic tissues (spleen)	chronic lymphocytic leukemia	
hsa-miR-15b-	424	1445	blood,		cell cycle,

3p			lymphocyte, hematopoietic tissues (spleen)		proliferation
hsa-miR- 15b-5p	425	1446	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR- 16-1-3p	426	1447	embryonic stem cells, blood, hematopoietic tissues (spleen)	chronic lymphocytic leukemia	
hsa-miR- 16-2-3p	427	1448	blood, lymphocyte, hematopoietic tissues (spleen)		
hsa-miR- 16-5p	428	1449	blood, lymphocyte, hematopoietic tissues		
hsa-miR- 181a-3p	432	1453	glioblast, myeloid cells, Embryonic stem cells		
hsa-miR- 181a-5p	433	1454	glioblast, myeloid cells, Embryonic stem cells		
hsa-miR- 182-3p	439	1460	immune cells	colonrectal cancer, autoimmune	immune response
hsa-miR- 182-5p	441	1462	lung, immune cells	autoimmune	immune response
hsa-miR- 197-3p	490	151 1	blood (myeloid), other tissues	various cancers (thyroid tumor, leukemia, etc)	
hsa-miR- 197-5p	491	1512	blood (myeloid), other tissues	various cancers (thyroid tumor, leukemia, etc)	
hsa-miR-21-3p	542	1563	glioblast, Blood (meyloid cells), liver, vascular endothelial cells	autoimmune, heart diseases, cancers	
hsa-miR-214-3p	543	1564	immune cells, pancreas	varioua cancers (melanoma, pancreatic, ovarian)	immune response
hsa-miR-214-5p	544	1565	immune cells, pancreas	varioua cancers (melanoma, pancreatic, ovarian)	immune response

hsa-miR-2 1-5p	546	1567	blood (myeloid cells), liver, endothelial cells	autoimmune, heart diseases, cancers	
hsa-miR-221-3p	557	1578	endothelial cells, immune cells	breast cancer, upregulated in thyroid cell transformation induced by HMGA1, TLR signal pathway in endotoxin tolerance, upregulated in T cell ALL	angiogenesis/vasculogenesis
hsa-miR-221-5p	558	1579	endothelial cells, immune cells	breast cancer, upregulated in thyroid cell transformation induced by HMGA1, TLR signal pathway in endotoxin tolerance , upregulated in T cell ALL	angiogenesis/vasculogenesis
hsa-miR-223-3p	561	1582	myeloid cells	associated with CLL	
hsa-miR-223-5p	562	1583	myeloid cells	associated with CLL	
hsa-miR-23b-3p	576	1597	blood, myeloid cells	cancers (renal cancer, glioblastoma, prostate, etc) and autoimmune	
hsa-miR-23b-5p	577	1598	blood, myeloid cells	cancers (glioblastoma, prostate, etc) and autoimmune	
hsa-miR-24-1-5p	579	1600	lung, myeloid cells		
hsa-miR-24-2-5p	580	1601	lung, myeloid cells		
hsa-miR-24-3p	581	1602	lung, myeloid cells		
hsa-miR-26a-1-3p	590	1611	embryonic stem cells, blood (T cells)	chronic lymphocyte leukemia and other cancers	cell cycle and differentiation
hsa-miR-26a-2-3p	591	1612	blood (T cells), other tissues	chronic lymphocyte	cell cycle and differentiation

				leukemia and other cancers	
hsa-miR-26a-5p	592	1613	blood (Tcells), other tissues	chronic lymphocyte leukemia and other cancers	cell cycle and differentiation
hsa-miR-26b-3p	593	1614	hematopoietic cells		
hsa-miR-26b-5p	594	1615	hematopoietic cells		
hsa-miR-27a-3p	595	1616	myeloid cells	various cancer cells	
hsa-miR-27a-5p	596	1617	myeloid cells	various cancer cells	
hsa-miR-27b-3p	597	1618	myeloid cells, vascular endothelial cells	various cancer cells	pro-angiogenic
hsa-miR-28-3p	599	1620	blood(immune cells)	B/T cell lymphoma	
hsa-miR-28-5p	600	1621	blood(immune cells)	B/T cell lymphoma	
hsa-miR-2909	602	1623	T-Lymphocytes		
hsa-miR-29a-3p	611	1632	immuno system, colonrectun	various cancers, neurodegenative disease	tumor suppression, immune modulation (mir-29 family)
hsa-miR-29a-5p	612	1633	immuno system, colonrectun	various cancers, neurodegenative disease	adaptive immunity
hsa-miR-29b-1-5p	613	1634	immuno system	associated with CLL, other cancers, neurodegenative disease	adaptive immunity
hsa-miR-29b-2-5p	614	1635	immuno system	associated with CLL, other cancers,	adaptive immunity
hsa-miR-29b-3p	615	1636	immuno system	associated with CLL, other cancers	adaptive immunity
hsa-miR-29c-3p	616	1637	immuno system	associated with CLL, other cancers	adaptive immunity
hsa-miR-29c-5p	617	1638	immuno system	associated with CLL, other cancers	adaptive immunity
hsa-miR-30e-3p	647	1668	myeloid cells, glia cells		

hsa-miR-30e-5p	648	1669	myeloid cells, glia cells		
hsa-miR-331-5p	793	1814	lymphocytes		
hsa-miR-339-3p	800	1821	immune cells		
hsa-miR-339-5p	801	1822	immune cells		
hsa-miR-345-3p	810	1831	hematopoietic cells	increased in follicular lymphoma(53), other cancers	
hsa-miR-345-5p	811	1832	hematopoietic cells	increased in follicular lymphoma(53)	
hsa-miR-346	812	1833	immune cells	cancers and autoimmune	
hsa-miR-34a-3p	813	1834	breast, myeloid cells, ciliated epithelial cells	gastric cancer, CLL, other	tumor suppressor, p53 inducible
hsa-miR-34a-5p	814	1835	breast, myeloid cells, ciliated epithelial cells	gastric cancer, CLL, other	tumor suppressor, p53 inducible
hsa-miR-363-3p	856	1877	kidney stem cell, blood cells		
hsa-miR-363-5p	857	1878	kidney stem cell, blood cells		
hsa-miR-372	940	1961	hematopoietic cells, lung, placental (blood)		
hsa-miR-377-3p	957	1978	hematopoietic cells		
hsa-miR-377-5p	958	1979	hematopoietic cells		
hsa-miR-493-3p	2610	3631	myeloid cells, pancreas (islet)		
hsa-miR-493-5p	2611	3632	myeloid cells, pancreas (islet)		
hsa-miR-542-3p	2769	3790	monocytes		targets to survivin , introduce growth arrest
hsa-miR-548b-5p	2820	3841	immune cells frontal cortex		
hsa-miR-548c-5p	2822	3843	immune cells frontal cortex		
hsa-miR-548i	2831	3852	embryonic stem cells (41), immune cells		

hsa-miR-548j	2832	3853	immune cells		
hsa-miR-548n	2836	3857	embryonic stem cells , immune cells		
hsa-miR-574-3p	2942	3963	blood (myeloid cells)	increased in follicular lymphoma(53)	
hsa-miR-598	2973	3994	in blood lymphocytes (PBL)		
hsa-miR-935	3210	4231	identified in human cervical cancer blood mononuclear cells	associated with energy metabolism/obesity, medullablastoma/neural stem cells	
hsa-miR-99a-3p	3230	4251	hemapoietic cells		
hsa-miR-99a-5p	3231	4252	hemapoietic cells, plasma (exosome)		
hsa-miR-99b-3p	3232	4253	hemapoietic cells, Embryonic stem cells,		
hsa-miR-99b-5p	3233	4254	hemapoietic cells, Embryonic stem cells , plasma(exosome)		

III. Modifications

[00352] Herein, in a nucleotide, nucleoside polynucleotide (such as the nucleic acids of the invention, e.g., modified RNA, modified nucleic acid molecule, modified RNAs, nucleic acid and modified nucleic acids), the terms "modification" or, as appropriate, "modified" refer to modification with respect to A, G, U or C ribonucleotides. Generally, herein, these terms are not intended to refer to the ribonucleotide modifications in naturally occurring 5'-terminal mRNA cap moieties. In a polypeptide, the term "modification" refers to a modification as compared to the canonical set of 20 amino acids.

[00353] The modifications may be various distinct modifications. In some embodiments, where the nucleic acids or modified RNA, the coding region, the flanking regions and/or the terminal regions may contain one, two, or more (optionally different) nucleoside or nucleotide modifications. In some embodiments, a modified nucleic acids

or modified RNA introduced to a cell may exhibit reduced degradation in the cell, as compared to an unmodified nucleic acid or modified RNA.

[00354] The polynucleotide, primary construct, nucleic acids or modified RNA can include any useful modification, such as to the sugar, the nucleobase, or the internucleoside linkage (*e.g.* to a linking phosphate / to a phosphodiester linkage / to the phosphodiester backbone). One or more atoms of a pyrimidine nucleobase may be replaced or substituted with optionally substituted amino, optionally substituted thiol, optionally substituted alkyl (*e.g.*, methyl or ethyl), or halo (*e.g.*, chloro or fluoro). In certain embodiments, modifications (*e.g.*, one or more modifications) are present in each of the sugar and the internucleoside linkage. Modifications according to the present invention may be modifications of ribonucleic acids (RNAs) to deoxyribonucleic acids (DNAs), *e.g.*, the substitution of the 2'OH of the ribofuranosyl ring to 2'H, threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs) or hybrids thereof). Additional modifications are described herein.

[00355] As described herein, the polynucleotides, primary construct, nucleic acids or modified RNA of the invention do not substantially induce an innate immune response of a cell into which the polynucleotides, primary constructs, nucleic acids or modified RNA (*e.g.*, mRNA) is introduced. Features of an induced innate immune response include 1) increased expression of pro-inflammatory cytokines, 2) activation of intracellular PRRs (RIG-I, MDA5, etc, and/or 3) termination or reduction in protein translation.

[00356] In certain embodiments, it may be desirable for a modified nucleic acid molecule introduced into the cell to be degraded intracellularly. For example, degradation of a modified nucleic acid molecule may be preferable if precise timing of protein production is desired. Thus, in some embodiments, the invention provides a modified nucleic acid molecule containing a degradation domain, which is capable of being acted on in a directed manner within a cell. In another aspect, the present disclosure provides polynucleotides, primary constructs, nucleic acids or modified RNA comprising a nucleoside or nucleotide that can disrupt the binding of a major groove interacting, *e.g.* binding, partner with the polynucleotides, primary constructs, nucleic acids or modified RNA (*e.g.*, where the modified nucleotide has decreased binding affinity to major groove interacting partner, as compared to an unmodified nucleotide).

[00357] The polynucleotides, primary constructs, nucleic acids or modified RNA can optionally include other agents (e.g., RNAi-inducing agents, RNAi agents, siRNAs, shRNAs, miRNAs, antisense RNAs, ribozymes, catalytic DNA, tRNA, RNAs that induce triple helix formation, aptamers, vectors, etc.). In some embodiments, the polynucleotides, primary constructs, nucleic acids or modified RNA may include one or more messenger RNAs (mRNAs) having one or more modified nucleoside or nucleotides (i.e., modified mRNA molecules). Details for these nucleic acids or modified RNA follow.

Modified mRNA Molecules

[00358] The polynucleotides, primary constructs, nucleic acids or modified RNA of the invention includes a first region of linked nucleosides encoding a polypeptide of interest, a first flanking region located at the 5' terminus of the first region, and a second flanking region located at the 3' terminus of the first region. The first region of linked nucleosides may be a translatable region.

[00359] In some embodiments, the polynucleotide, primary construct, or mmRNA (e.g., the first region, first flanking region, or second flanking region) includes n number of linked nucleosides having any base, sugar, backbone, building block or other structure or formula, including but not limited to those of Formulas I through IX or any substructures thereof as described in International Application PCT/US12/58519 filed October 3, 2012 (Attorney Docket Number: M009.20), the contents of which are incorporated herein by reference in their entirety. Such structures include modifications to the sugar, nucleobase, internucleoside linkage, or combinations thereof.

[00360] Combinations of chemical modifications include those taught in including but not limited to those described in International Application PCT/US12/58519 filed October 3, 2012 (Attorney Docket Number: M009.20), the contents of which are incorporated herein by reference in their entirety.

[00361] The synthesis of polynucleotides, primary constructs or mmRNA of the present invention may be according to the methods described in International Application PCT/US12/58519 filed October 3, 2012 (Attorney Docket Number: M009.20), the contents of which are incorporated herein by reference in their entirety.

[00362] In some embodiments, the nucleobase selected from the group consisting of cytosine, guanine, adenine, and uracil.

[00363] In some embodiments, the modified nucleobase is a modified uracil.

Exemplary nucleobases and nucleosides having a modified uracil include pseudouridine (ψ), pyridin-4-one ribonucleoside, 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thiouridine (s^2U), 4-thio-uridine (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxyuridine (ho^5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridine or 5-bromo-uridine), 3-methyluridine (m^3U), 5-methoxy-uridine (mo^5U), uridine 5-oxyacetic acid (cmo^5U), uridine 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uridine (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm^5U), 5-carboxyhydroxymethyl-uridine methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uridine (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm^5s^2U), 5-aminomethyl-2-thio-uridine (**nm⁵VU**), 5-methylaminomethyl-uridine (mnm^5U), 5-methylaminomethyl-2-thio-uridine (mnm^5s^2U), 5-methylaminomethyl-2-seleno-uridine (mnm^5se^2U), 5-carbamoylmethyl-uridine (ncm^5U), 5-carboxymethylaminomethyl-uridine ($cmnm^5U$), 5-carboxymethylaminomethyl-2-thio-uridine ($cmnm^5s^2U$), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-aurinomethyluridine (**τm^5U**), 1-aurinomethyl-pseudouridine, 5-aurinomethyl-2-thio-uridine (τm^5s^2U), 1-aurinomethyl-4-thio-pseudouridine, 5-methyl-uridine (m^5U , i.e., having the nucleobase deoxythymine), 1-methyl-pseudouridine ($m^1\psi$), 5-methyl-2-thio-uridine (m^5s^2U), 1-methyl-4-thio-pseudouridine (**$m^1V \psi$**), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m^5D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp^3U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\psi$), 5-(isopentenylaminomethyl)uridine (inm^5U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm^5s^2U), a-thio-uridine, 2'-O-methyl-uridine (Um), 5,2'-O-dimethyl-uridine (m^5Um), 2'-O-methyl-pseudouridine (ψm), 2-thio-2'-O-methyl-uridine (s^2Um), 5-methoxycarbonylmethyl-2'-O-methyl-uridine (mcm^5Um), 5-carbamoylmethyl-2'-O-methyl-uridine (ncm^5Um), 5-carboxymethylaminomethyl-2'-O-

methyl-uridine (cmnm⁵Um), 3,2'-O-dimethyl-uridine (m³Um), and 5-(isopentenylaminomethyl)-2'-O-methyl-uridine (inm⁵Um), 1-thio-uridine, deoxythymidine, 2'-F-ara-uridine, 2'-F-uridine, 2'-OH-ara-uridine, 5-(2-carbomethoxyvinyl) uridine, and 5-[3-(1-E-propenylamino)uridine.

[00364] In some embodiments, the modified nucleobase is a modified cytosine. Exemplary nucleobases and nucleosides having a modified cytosine include 5-azacytidine, 6-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine (m³C), N4-acetyl-cytidine (ac⁴C), 5-formylcytidine (f⁵C), N4-methylcytidine (m⁴C), 5-methyl-cytidine (m⁵C), 5-halo-cytidine (e.g., 5-iodo-cytidine), 5-hydroxymethylcytidine (hm⁵C), 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine (s²C), 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 1-methyl-1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, 4-methoxy-1-methyl-pseudoisocytidine, lysidine (k₂C), a-thio-cytidine, 2'-O-methyl-cytidine (Cm), 5,2'-O-dimethyl-cytidine (m⁵Cm), N4-acetyl-2'-O-methyl-cytidine (ac⁴Cm), N4,2'-O-dimethyl-cytidine (m⁴Cm), 5-formyl-2'-O-methyl-cytidine (f⁵Cm), N4,N4,2'-O-trimethyl-cytidine (m⁴₂Cm), 1-thio-cytidine, 2'-F-ara-cytidine, 2'-F-cytidine, and 2'-OH-ara-cytidine.

[00365] In some embodiments, the modified nucleobase is a modified adenine. Exemplary nucleobases and nucleosides having a modified adenine include 2-aminopurine, 2,6-diaminopurine, 2-amino-6-halo-purine (e.g., 2-amino-6-chloro-purine), 6-halo-purine (e.g., 6-chloro-purine), 2-amino-6-methyl-purine, 8-azido-adenosine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-amino-purine, 7-deaza-8-aza-2-amino-purine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyladenosine (rr⁶A), 2-methyl-adenine (m²A), N6-methyladenosine (m⁶A), 2-methylthio-N6-methyladenosine (ms²m⁶A), N6-isopentenyladenosine (i⁶A), 2-methylthio-N6-isopentenyladenosine (ms²i⁶A), N6-(cis-hydroxyisopentenyl)adenosine (io⁶A), 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine (ms²io⁶A), N6-glycinylocarbamoyladenosine (g⁶A), N6-threonylocarbamoyladenosine (t⁶A), N6-methyl-N6-threonylocarbamoyl-adenosine (m⁶t⁶A), 2-methylthio-N6-threonyl carbamoyladenosine (ms²g⁶A), N6,N6-dimethyl-

adenosine (m^6_2A), N6-hydroxynorvalylcarbamoyl-adenosine (hn^6A), 2-methylthio-N6-hydroxynorvalylcarbamoyl-adenosine (ms^2hn^6A), N6-acetyl-adenosine (ac^6A), 7-methyladenine, 2-methylthio-adenine, 2-methoxy-adenine, α -thio-adenosine, 2'-O-methyl-adenosine (Am), N6,2'-O-dimethyl-adenosine (m^6Am), N6,N6,2'-O-trimethyl-adenosine (m^6_2Am), 1,2'-O-dimethyl-adenosine ($rr^{\wedge}Am$), 2'-O-ribosyladenosine (phosphate) ($Ar(p)$), 2-amino-N6-methyl-purine, 1-thio-adenosine, 8-azido-adenosine, 2'-F-ara-adenosine, 2'-F-adenosine, 2'-OH-ara-adenosine, and N6-(19-amino-pentaoxanonadecyl)-adenosine.

[00366] In some embodiments, the modified nucleobase is a modified guanine.

Exemplary nucleobases and nucleosides having a modified guanine include inosine (I), 1-methyl-inosine (m^1I), wyosine (imG), methylwyosine ($mimG$), 4-demethyl-wyosine ($imG-14$), isowyosine ($imG2$), wybutosine (yW), peroxywybutosine (o_2yW), hydroxywybutosine ($OHyW$), undermodified hydroxywybutosine ($OHyW^*$), 7-deaza-guanosine, queuosine (Q), epoxyqueuosine (oQ), galactosyl-queuosine ($galQ$), mannosyl-queuosine ($manQ$), 7-cyano-7-deaza-guanosine ($preQo$), 7-aminomethyl-7-deaza-guanosine ($preQi$), archaeosine (G^+), 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methylguanosine (m^7G), 6-thio-7-methyl-guanosine, 7-methyl-inosine, 6-methoxy-guanosine, 1-methylguanosine ($n^{\wedge}G$), N2-methyl-guanosine (m^2G), N2,N2-dimethyl-guanosine (m^2_2G), N2,7-dimethyl-guanosine ($m^{2,7}G$), N2, N2,7-dimethyl-guanosine ($m^{2,2,7}G$), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, N2,N2-dimethyl-6-thio-guanosine, α -thio-guanosine, 2'-O-methyl-guanosine (Gm), N2-methyl-2'-O-methyl-guanosine (m^2Gm), N2,N2-dimethyl-2'-O-methyl-guanosine (m^2_2Gm), 1-methyl-2'-O-methyl-guanosine ($m^{\wedge}m$), N2,7-dimethyl-2'-O-methyl-guanosine ($m^{2,7}Gm$), 2'-O-methyl-inosine (Im), 1,2'-O-dimethyl-inosine ($m^{\wedge}m$), 2'-O-ribosylguanosine (phosphate) ($Gr(p)$), 1-thio-guanosine, 06-methyl-guanosine, 2'-F-ara-guanosine, and 2'-F-guanosine.

[00367] Phosphorothioate DNA and RNA have increased nuclease resistance and subsequently a longer half-life in a cellular environment. Phosphorothioate linked nucleic acids are expected to also reduce the innate immune response through weaker binding/activation of cellular innate immune molecules.

[00368] The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine or pyrimidine analog. For example, the nucleobase can each be independently selected from adenine, cytosine, guanine, uracil, or hypoxanthine. In another embodiment, the nucleobase can also include, for example, naturally-occurring and synthetic derivatives of a base, including pyrazolo[3,4-d]pyrimidines, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo (*e.g.*, 8-bromo), 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, deazaguanine, 7-deazaguanine, 3-deazaguanine, deazaadenine, 7-deazaadenine, 3-deazaadenine, pyrazolo[3,4-d]pyrimidine, imidazo[1,5-a] 1,3,5 triazinones, 9-deazapurines, imidazo[4,5-d]pyrazines, thiazolo[4,5-d]pyrimidines, pyrazin-2-ones, 1,2,4-triazine, pyridazine; and 1,3,5 triazine. When the nucleotides are depicted using the shorthand A, G, C, T or U, each letter refers to the representative base and/or derivatives thereof, *e.g.*, A includes adenine or adenine analogs, *e.g.*, 7-deaza adenine).

Modifications on the Internucleoside Linkage

[00369] The modified nucleotides, which may be incorporated into a nucleic acid or modified RNA molecule, can be modified on the internucleoside linkage (*e.g.*, phosphate backbone). Herein, in the context of the polynucleotides, primary constructs, nucleic acids or modified RNA backbone, the phrases "phosphate" and "phosphodiester" are used interchangeably. Backbone phosphate groups can be modified by replacing one or more of the oxygen atoms with a different substituent. Further, the modified nucleosides and nucleotides can include the wholesale replacement of an unmodified phosphate moiety with another internucleoside linkage as described herein. Examples of modified phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, boranophosphates, boranophosphate esters, hydrogen phosphonates, phosphoramidates, phosphorodiamidates, alkyl or aryl phosphonates, and phosphotriesters.

Phosphorodithioates have both non-linking oxygens replaced by sulfur. The phosphate linker can also be modified by the replacement of a linking oxygen with nitrogen (bridged phosphoramidates), sulfur (bridged phosphorothioates), and carbon (bridged methylene-phosphonates) .

[00370] The α -thio substituted phosphate moiety is provided to confer stability to RNA and DNA polymers through the unnatural phosphorothioate backbone linkages. Phosphorothioate DNA and RNA have increased nuclease resistance and subsequently a longer half-life in a cellular environment. While not wishing to be bound by theory, phosphorothioate linked polynucleotides, primary constructs, nucleic acids or modified RNA molecules are expected to also reduce the innate immune response through weaker binding/activation of cellular innate immune molecules.

[00371] In specific embodiments, a modified nucleoside includes an α -thio-nucleoside (e.g., 5'-O-(1-thiophosphate)-adenosine, 5'-O-(1-thiophosphate)-cytidine (α -thio-cytidine), 5'-O-(1-thiophosphate)-guanosine, 5'-O-(1-thiophosphate)-uridine, or 5'-O-(1-thiophosphate)-pseudouridine) .

[00372] Other internucleoside linkages that may be employed according to the present invention, including internucleoside linkages which do not contain a phosphorous atom, are described herein below.

Combinations of Modified Sugars, Nucleobases, and Internucleoside Linkages

[00373] The nucleic acids or modified RNA of the invention can include a combination of modifications to the sugar, the nucleobase, and/or the internucleoside linkage. These combinations can include any one or more modifications described herein. For examples, any of the nucleotides described herein in Formulas (Ia), (Ia-1)-(Ia-3), (Ib)-(If), (IIa)-(IIp), (IIb-1), (IIb-2), (IIc-1)-(IIc-2), (IIIn-1), (IIIn-2), (IVa)-(IVl), and (IXa)-(IXr) can be combined with any of the nucleobases described herein (e.g., in Formulas (bl)-(b43) or any other described herein).

Synthesis of Nucleic Acids or Modified RNA Molecules (modified RNAs)

[00374] Nucleic acids for use in accordance with the invention may be prepared according to any useful technique as described herein or any available technique including, but not limited to chemical synthesis, enzymatic synthesis, which is generally termed *in vitro* transcription, enzymatic or chemical cleavage of a longer precursor, etc.

Methods of synthesizing RNAs are known in the art (see, *e.g.*, Gait, M.J. (ed.) *Oligonucleotide synthesis: a practical approach*, Oxford [Oxfordshire], Washington, DC: IRL Press, 1984; and Herdewijn, P. (ed.) *Oligonucleotide synthesis: methods and applications*, Methods in Molecular Biology, v. 288 (Clifton, N.J.) Totowa, N.J.: Humana Press, 2005; both of which are incorporated herein by reference).

[00375] The modified nucleosides and nucleotides used in the synthesis of modified RNAs disclosed herein can be prepared from readily available starting materials using the following general methods and procedures. It is understood that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given; other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[00376] The processes described herein can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (*e.g.*, ^1H or ^{13}C) infrared spectroscopy, spectrophotometry (*e.g.*, UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

[00377] Preparation of modified nucleosides and nucleotides used in the manufacture or synthesis of modified RNAs of the present invention can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups can be readily determined by one skilled in the art.

[00378] The chemistry of protecting groups can be found, for example, in Greene, et al, *Protective Groups in Organic Synthesis*, 2d. Ed., Wiley & Sons, 1991, which is incorporated herein by reference in its entirety.

[00379] The reactions of the processes described herein can be carried out in suitable solvents, which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out,

i.e., temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected.

[00380] Resolution of racemic mixtures of modified nucleosides and nucleotides can be carried out by any of numerous methods known in the art. An example method includes fractional recrystallization using a "chiral resolving acid" which is an optically active, salt-forming organic acid. Suitable resolving agents for fractional recrystallization methods are, for example, optically active acids, such as the D and L forms of tartaric acid, diacetyltartaric acid, dibenzoyltartaric acid, mandelic acid, malic acid, lactic acid or the various optically active camphorsulfonic acids. Resolution of racemic mixtures can also be carried out by elution on a column packed with an optically active resolving agent (*e.g.*, dinitrobenzoylphenylglycine). Suitable elution solvent composition can be determined by one skilled in the art.

[00381] Modified nucleosides and nucleotides (*e.g.*, building block molecules) can be prepared according to the synthetic methods described in Ogata et al, J. Org. Chem. 74:2585-2588 (2009); Purmal et al, Nucl. Acids Res. 22(1): 72-78, (1994); Fukuhara et al, Biochemistry, 1(4): 563-568 (1962); and Xu et al, Tetrahedron, 48(9): 1729-1740 (1992), each of which are incorporated by reference in their entirety.

[00382] Modified nucleosides and nucleotides (*e.g.*, building block molecules) can be prepared according to the synthetic methods described in Ogata et al, J. Org. Chem. 74:2585-2588 (2009); Purmal et al, Nucl. Acids Res. 22(1): 72-78, (1994); Fukuhara et al, Biochemistry, 1(4): 563-568 (1962); and Xu et al, Tetrahedron, 48(9): 1729-1740 (1992), each of which are incorporated by reference in their entirety.

[00383] The modified nucleic acids of the invention may or may not be uniformly modified along the entire length of the molecule. Different nucleotide modifications and/or backbone structures may exist at various positions in the nucleic acid. One of ordinary skill in the art will appreciate that the nucleotide analogs or other modification(s) may be located at any position(s) of a nucleic acid such that the function of the nucleic acid is not substantially decreased. A modification may also be a 5' or 3' terminal modification. The nucleic acids may contain at a minimum one and at

maximum 100% modified nucleotides, or any intervening percentage, such as at least 50% modified nucleotides, at least 80% modified nucleotides, or at least 90% modified nucleotides. For example, one or more or all types of nucleotide (*e.g.*, purine or pyrimidine, or any one or more or all of A, G, U, C) may or may not be uniformly modified in a nucleic acids or modified RNA of the invention, or in a given predetermined sequence region thereof. In some embodiments, all nucleotides X in a nucleic acids or modified RNA of the invention (or in a given sequence region thereof) are modified, wherein X may any one of nucleotides A, G, U, C, or any one of the combinations A+G, A+U, A+C, G+U, G+C, U+C, A+G+U, A+G+C, G+U+C or A+G+C.

[00384] Different sugar modifications, nucleotide modifications, and/or internucleoside linkages (*e.g.*, backbone structures) may exist at various positions in the nucleic acids or modified RNA. One of ordinary skill in the art will appreciate that the nucleotide analogs or other modification(s) may be located at any position(s) of a nucleic acid or modified RNA such that the function of the nucleic acids or modified RNA is not substantially decreased. A modification may also be a 5' or 3' terminal modification. The nucleic acids or modified RNA may contain from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, *i.e.* any one or more of A, G, U or C) or any intervening percentage (*e.g.*, from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%).

[00385] In some embodiments, the nucleic acids or modified RNA includes a modified pyrimidine (*e.g.*, a modified uracil/uridine/U or modified cytosine/cytidine/C). In some

embodiments, the uracil or uridine (generally: U) in the nucleic acids or modified RNA molecule may be replaced with from about 1% to about 100% of a modified uracil or modified uridine (e.g., from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100% of a modified uracil or modified uridine). The modified uracil or uridine can be replaced by a compound having a single unique structure or by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures, as described herein). In some embodiments, the cytosine or cytidine (generally: C) in the nucleic acid or modified RNA molecule may be replaced with from about 1% to about 100% of a modified cytosine or modified cytidine (e.g., from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100% of a modified cytosine or modified cytidine). The modified cytosine or cytidine can be replaced by a compound having a single unique structure or by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures, as described herein).

[00386] Other components of the nucleic acid are optional, and are beneficial in some embodiments. For example, a 5' untranslated region (UTR) and/or a 3'UTR are provided,

wherein either or both may independently contain one or more different nucleotide modifications. In such embodiments, nucleotide modifications may also be present in the translatable region. Also provided are nucleic acids containing a Kozak sequence which may include an IRES sequence or not include an IRES sequence (See e.g., the polynucleotides described in Table 30 in Example 31).

[00387] Additionally, provided are nucleic acids containing one or more intronic nucleotide sequences capable of being excised from the nucleic acid.

Combinations of Nucleotides

[00388] Further examples of modified nucleotides and modified nucleotide combinations are provided below in Table 14. These combinations of modified nucleotides can be used to form the nucleic acids or modified RNA of the invention. Unless otherwise noted, the modified nucleotides may be completely substituted for the natural nucleotides of the nucleic acids or modified RNA of the invention. As a non-limiting example, the natural nucleotide uridine may be substituted with a modified nucleoside described herein. In another non-limiting example, the natural nucleotide uridine may be partially substituted (e.g., about 0.1%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99.9%o) with at least one of the modified nucleoside disclosed herein.

Table 14. Chemical Modifications

<u>Modified Nucleotide</u>	<u>Modified Nucleotide Combination</u>
6-aza-cytidine	α -thio-cytidine/5-iodo-uridine
2-thio-cytidine	α -thio-cytidine/N1-methyl-pseudo-uridine
α -thio-cytidine	α -thio-cytidine/ α -thio-uridine
Pseudo-iso-cytidine	α -thio-cytidine/5-methyl-uridine
5-aminoallyl-uridine	α -thio-cytidine/pseudo-uridine
5-iodo-uridine	Pseudo-iso-cytidine/5-iodo-uridine
N1-methyl-pseudouridine	Pseudo-iso-cytidine/N1-methyl-pseudo-uridine
5,6-dihydrouridine	Pseudo-iso-cytidine/ α -thio-uridine
α -thio-uridine	Pseudo-iso-cytidine/5-methyl-uridine
4-thio-uridine	Pseudo-iso-cytidine/Pseudo-uridine
6-aza-uridine	
5-hydroxy-uridine	Pyrrolo-cytidine/5-iodo-uridine
Deoxy-thymidine	Pyrrolo-cytidine/N1-methyl-pseudo-uridine
Pseudo-uridine	Pyrrolo-cytidine/ α -thio-uridine
Inosine	Pyrrolo-cytidine/5-methyl-uridine

α -thio-guanosine	Pyrrolo-cytidine/Pseudo-uridine
8-oxo-guanosine	5-methyl-cytidine/ 5-iodo-uridine
06-methyl-guanosine	5-methyl-cytidine/N 1-methyl-pseudo-uridine
7-deaza-guanosine	5-methyl-cytidine/ a-thio-uridine
No modification	5-methyl-cytidine/ 5-methyl-uridine
N 1-methyl-adenosine	5-methyl-cytidine/Pseudo-uridine
2-amino-6-Chloro-purine	
N6-methyl-2-amino-purine	about 25% of cytosines are Pseudo-iso-cytidine
6-Chloro-purine	about 25% of uridines are N1-methyl-pseudo-uridine
N6-methyl-adenosine	25% N 1-Methyl-pseudo-uridine/75 % pseudo-uridine
a-thio-adenosine	
8-azido-adenosine	
7-deaza-adenosine	about 50% of the cytosines are pyrrolo-cytidine
Pyrrolo-cytidine	5-methyl-cytidine/ 5-iodo-uridine
5-methyl-cytidine	5-methyl-cytidine/N 1-methyl-pseudouridine
N4-acetyl-cytidine	5-methyl-cytidine/ a-thio-uridine
5-methyl-uridine	5-methyl-cytidine/ 5-methyl-uridine
5-iodo-cytidine	5-methyl-cytidine/pseudouridine
	about 25% of cytosines are 5-methyl-cytidine
	about 50% of cytosines are 5-methyl-cytidine
	5-methyl-cytidine/ 5-methoxy-uridine
	5-methyl-cytidine/ 5-bromo-uridine
	5-methyl-cytidine/2-thio-uridine
	5-methyl-cytidine/about 50% of uridines are 2-thio-uridine
	about 50% of uridines are 5-methyl-cytidine/ about 50% of uridines are 2-thio-uridine
	N4-acetyl-cytidine 15-iodo-uridine
	N4-acetyl-cytidine /N 1-methyl-pseudouridine
	N4-acetyl-cytidine /a-thio-uridine
	N4-acetyl-cytidine /5-methyl-uridine
	N4-acetyl-cytidine /pseudouridine
	about 50% of cytosines are N4-acetyl-cytidine
	about 25% of cytosines are N4-acetyl-cytidine
	N4-acetyl-cytidine /5-methoxy-uridine
	N4-acetyl-cytidine /5-bromo-uridine
	N4-acetyl-cytidine /2-thio-uridine
	about 50% of cytosines are N4-acetyl-cytidine/ about 50% of uridines are 2-thio-uridine
	pseudoisocytidine/about 50% of uridines are N1-methyl-pseudouridine and about 50% of uridines are pseudouridine
	pseudoisocytidine/about 25% of uridines are N1-methyl-pseudouridine and about 25% of uridines are pseudouridine

	(e.g., 25% N1-methyl-pseudouridine/75% pseudouridine)
	about 50% of the cytosines are a-thio-cytidine

[00389] Certain modified nucleotides and nucleotide combinations have been explored by the current inventors. These findings are described in U.S. Provisional Application No 61/404,413, filed on October 1, 2010, entitled Engineered Nucleic Acids and Methods of Use Thereof, U.S. Patent Application No 13/251,840, filed on October 3, 2011, entitled Modified Nucleotides, and Nucleic Acids, and Uses Thereof, now abandoned, U.S. Patent Application No 13/481,127, filed on May 25, 2012, entitled Modified Nucleotides, and Nucleic Acids, and Uses Thereof, International Patent Publication No WO2012045075, filed on October 3, 2011, entitled Modified Nucleosides, Nucleotides, And Nucleic Acids, and Uses Thereof, U.S. Patent Publication No US20120237975 filed on October 3, 2011, entitled Engineered Nucleic Acids and Method of Use Thereof, and International Patent Publication No WO2012045082, which are incorporated by reference in their entireties.

[00390] Further examples of modified nucleotide combinations are provided below in Table 15. These combinations of modified nucleotides can be used to form the nucleic acids of the invention.

Table 15. Chemical Modifications

Modified Nucleotide	Modified Nucleotide Combination
modified cytidine having one or more nucleobases of Formula (b10)	modified cytidine with (b10)/pseudouridine
	modified cytidine with (b10)/N1-methyl-pseudouridine
	modified cytidine with (b10)/5-methoxy-uridine
	modified cytidine with (b10)/5-methyl-uridine
	modified cytidine with (b10)/5-bromo-uridine
	modified cytidine with (b10)/2-thio-uridine
	about 50% of cytidine substituted with modified cytidine (b10)/ about 50% of uridines are 2-thio-uridine
modified cytidine having one or more nucleobases of Formula (b32)	modified cytidine with (b32)/pseudouridine
	modified cytidine with (b32)/N1-methyl-pseudouridine
	modified cytidine with (b32)/5-methoxy-

	uridine
	modified cytidine with (b32)/5-methyl-uridine
	modified cytidine with (b32)/5-bromo-uridine
	modified cytidine with (b32)/2-thio-uridine
	about 50% of cytidine substituted with modified cytidine (b32)/ about 50% of uridines are 2-thio-uridine
modified uridine having one or more nucleobases of Formula (bl)	modified uridine with (bl)/ N4-acetyl-cytidine
	modified uridine with (bl)/ 5-methyl-cytidine
modified uridine having one or more nucleobases of Formula (b8)	modified uridine with (b8)/ N4-acetyl-cytidine
	modified uridine with (b8)/ 5-methyl-cytidine
modified uridine having one or more nucleobases of Formula (b28)	modified uridine with (b28)/ N4-acetyl-cytidine
	modified uridine with (b28)/ 5-methyl-cytidine
modified uridine having one or more nucleobases of Formula (b29)	modified uridine with (b29)/ N4-acetyl-cytidine
	modified uridine with (b29)/ 5-methyl-cytidine
modified uridine having one or more nucleobases of Formula (b30)	modified uridine with (b30)/ N4-acetyl-cytidine
	modified uridine with (b30)/ 5-methyl-cytidine

[00391] In some embodiments, at least 25% of the cytosines are replaced by a compound of Formula (bl0)-(bl4), (b24), (b25), or (b32)-(b35) (e.g., at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% of, e.g., a compound of Formula (bl0) or (b32)).

[00392] In some embodiments, at least 25% of the uracils are replaced by a compound of Formula (bl)-(b9), (b21)-(b23), or (b28)-(b31) (e.g., at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% of, e.g., a compound of Formula (bl), (b8), (b28), (b29), or (b30)).

[00393] In some embodiments, at least 25% of the cytosines are replaced by a compound of Formula (M0)-(b14), (b24), (b25), or (b32)-(b35) (e.g. Formula (b10) or (b32)), and at least 25% of the uracils are replaced by a compound of Formula (b1)-(b9), (b21)-(b23), or (b28)-(b31) (e.g. Formula (b1), (b8), (b28), (b29), or (b30)) (e.g., at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%).

Modifications including Linker and a Payload

Payload

[00394] The methods and compositions described herein are useful for delivering a payload to a biological target. The payload can be used, *e.g.*, for labeling (*e.g.*, a detectable agent such as a fluorophore), or for therapeutic purposes (*e.g.*, a cytotoxin or other therapeutic agent).

Payload: Therapeutic Agents

[00395] In some embodiments the payload is a therapeutic agent such as a cytotoxin, radioactive ion, chemotherapeutic, or other therapeutic agent. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol (see U.S. Pat. No. 5,208,020), CC-1065 (see U.S. Pat. Nos. 5,475,092, 5,585,499, 5,846,545) and analogs or homologs thereof. Radioactive ions include, but are not limited to iodine (*e.g.*, iodine 125 or iodine 131), strontium 89, phosphorous, palladium, cesium, iridium, phosphate, cobalt, yttrium 90, Samarium 153 and praseodymium. Other therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, CC-1065, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II)

(DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine, vinblastine, taxol and maytansinoids).

Payload: Detectable Agents

[00396] Examples of detectable substances include various organic small molecules, inorganic compounds, nanoparticles, enzymes or enzyme substrates, fluorescent materials, luminescent materials, bioluminescent materials, chemiluminescent materials, radioactive materials, and contrast agents. Such optically-detectable labels include for example, without limitation, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid; acridine and derivatives: acridine, acridine isothiocyanate; 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS); 4-amino-N-[3-vinylsulfonyl]phenyl]naphthalimide-3,5-disulfonate; N-(4-anilino-1-naphthyl)maleimide; anthranilamide; BODIPY; Brilliant Yellow; coumarin and derivatives; coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), 7-amino-4-trifluoromethylcoumarin (Coumarin 151); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5'-5''-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; 5-[dimethylamino]-naphthalene-1-sulfonyl chloride (DNS, dansylchloride); 4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives; eosin, eosin isothiocyanate, erythrosin and derivatives; erythrosin B, erythrosin, isothiocyanate; ethidium; fluorescein and derivatives; 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF), 2',7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein, fluorescein, fluorescein isothiocyanate, QFITC, (XRITC); fluorescamine; IR144; IR1446; Malachite Green isothiocyanate; 4-methylumbelliferoneortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthaldialdehyde; pyrene and derivatives: pyrene, pyrene butyrate, succinimidyl 1-pyrene; butyrate quantum dots; Reactive Red 4 (Cibacron™ Brilliant Red 3B-A) rhodamine and derivatives: 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod),

rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red); N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); riboflavin; rosolic acid; terbium chelate derivatives; Cyanine-3 (Cy3); Cyanine-5 (Cy5); Cyanine-5.5 (Cy5.5), Cyanine-7 (Cy7); IRD 700; IRD 800; Alexa 647; La Jolla Blue; phthalocyanine; and naphthalocyanine. In some embodiments, the detectable label is a fluorescent dye, such as Cy5 and Cy3.

[00397] Examples luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin.

[00398] Examples of suitable radioactive material include ^{18}F , ^{67}Ga , $^{81\text{m}}\text{Kr}$, ^{82}Rb , ^{111}In , ^{123}I , ^{133}Xe , ^{201}Tl , ^{125}I , ^{35}S , ^{14}C , or ^3H , $^{99\text{m}}\text{Tc}$ (e.g., as pertechnetate (technetate(VII), Tc(V) either directly or indirectly, or other radioisotope detectable by direct counting of radioemission or by scintillation counting).

[00399] In addition, contrast agents, e.g., contrast agents for MRI or NMR, for X-ray CT, Raman imaging, optical coherence tomography, absorption imaging, ultrasound imaging, or thermal imaging can be used. Exemplary contrast agents include gold (e.g., gold nanoparticles), gadolinium (e.g., chelated Gd), iron oxides (e.g., superparamagnetic iron oxide (SPIO), monocrystalline iron oxide nanoparticles (MIONs), and ultrasmall superparamagnetic iron oxide (USPIO)), manganese chelates (e.g., Mn-DPDP), barium sulfate, iodinated contrast media (iohexol), microbubbles, or perfluorocarbons can also be used.

[00400] In some embodiments, the detectable agent is a non-detectable pre-cursor that becomes detectable upon activation. Examples include fluorogenic tetrazine-fluorophore constructs (e.g., tetrazine-BODIPY FL, tetrazine-Oregon Green 488, or tetrazine-BODIPY TMR-X) or enzyme activatable fluorogenic agents (e.g., PROSENSE (VisEn Medical)).

[00401] When the compounds are enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, the enzymatic label is detected by determination of conversion of an appropriate substrate to product.

[00402] *In vitro* assays in which these compositions can be used include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis.

[00403] Labels other than those described herein are contemplated by the present disclosure, including other optically-detectable labels. Labels can be attached to the modified nucleotide of the present disclosure at any position using standard chemistries such that the label can be removed from the incorporated base upon cleavage of the cleavable linker.

Payload:Cell Penetrating Payloads

[00404] In some embodiments, the modified nucleotides and modified nucleic acids can also include a payload that can be a cell penetrating moiety or agent that enhances intracellular delivery of the compositions. For example, the compositions can include a cell-penetrating peptide sequence that facilitates delivery to the intracellular space, *e.g.*, HIV-derived TAT peptide, penetratins, transportans, or hCT derived cell-penetrating peptides, see, *e.g.*, Caron et al, (2001) Mol Ther. 3(3):3 10-8; Langel, Cell-Penetrating Peptides: Processes and Applications (CRC Press, Boca Raton FL 2002); El-Andaloussi et al, (2005) Curr Pharm Des. 11(28):3597-61 1; and Deshayes et al, (2005) Cell Mol Life Sci. 62(16): 1839-49. The compositions can also be formulated to include a cell penetrating agent, *e.g.*, liposomes, which enhance delivery of the compositions to the intracellular space.

Payload:Biological Targets

[00405] The modified nucleotides and modified nucleic acids described herein can be used to deliver a payload to any biological target for which a specific ligand exists or can be generated. The ligand can bind to the biological target either covalently or non-covalently.

[00406] Exemplary biological targets include biopolymers, *e.g.*, antibodies, nucleic acids such as RNA and DNA, proteins, enzymes; exemplary proteins include enzymes, receptors, and ion channels. In some embodiments the target is a tissue- or cell-type specific marker, *e.g.*, a protein that is expressed specifically on a selected tissue or cell type. In some embodiments, the target is a receptor, such as, but not limited to, plasma membrane receptors and nuclear receptors; more specific examples include G-protein-

coupled receptors, cell pore proteins, transporter proteins, surface-expressed antibodies, HLA proteins, MHC proteins and growth factor receptors.

Synthesis of Modified Nucleotides

[00407] The modified nucleosides and nucleotides disclosed herein can be prepared from readily available starting materials using the following general methods and procedures. It is understood that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given; other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[00408] The processes described herein can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (*e.g.*, ^1H or ^{13}C) infrared spectroscopy, spectrophotometry (*e.g.*, UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

[00409] Preparation of modified nucleosides and nucleotides can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups can be readily determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Greene, et al, *Protective Groups in Organic Synthesis*, 2d. Ed., Wiley & Sons, 1991, which is incorporated herein by reference in its entirety.

[00410] The reactions of the processes described herein can be carried out in suitable solvents, which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, i.e., temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected.

[00411] Resolution of racemic mixtures of modified nucleosides and nucleotides can be carried out by any of numerous methods known in the art. An example method includes fractional recrystallization using a "chiral resolving acid" which is an optically active, salt-forming organic acid. Suitable resolving agents for fractional recrystallization methods are, for example, optically active acids, such as the D and L forms of tartaric acid, diacetyltartaric acid, dibenzoyltartaric acid, mandelic acid, malic acid, lactic acid or the various optically active camphorsulfonic acids. Resolution of racemic mixtures can also be carried out by elution on a column packed with an optically active resolving agent (*e.g.*, dinitrobenzoylphenylglycine). Suitable elution solvent composition can be determined by one skilled in the art.

Length

[00412] Generally, the length of a modified mRNA of the present invention is greater than 30 nucleotides in length. In another embodiment, the RNA molecule is greater than 35 nucleotides in length. In another embodiment, the length is at least 40 nucleotides. In another embodiment, the length is at least 45 nucleotides. In another embodiment, the length is at least 55 nucleotides. In another embodiment, the length is at least 60 nucleotides. In another embodiment, the length is at least 60 nucleotides. In another embodiment, the length is at least 80 nucleotides. In another embodiment, the length is at least 90 nucleotides. In another embodiment, the length is at least 100 nucleotides. In another embodiment, the length is at least 120 nucleotides. In another embodiment, the length is at least 140 nucleotides. In another embodiment, the length is at least 160 nucleotides. In another embodiment, the length is at least 180 nucleotides. In another embodiment, the length is at least 200 nucleotides. In another embodiment, the length is at least 250 nucleotides. In another embodiment, the length is at least 300 nucleotides. In another embodiment, the length is at least 350 nucleotides. In another embodiment, the length is at least 400 nucleotides. In another embodiment, the length is at least 450 nucleotides. In another embodiment, the length is at least 500 nucleotides. In another embodiment, the length is at least 600 nucleotides. In another embodiment, the length is at least 700 nucleotides. In another embodiment, the length is at least 800 nucleotides. In another embodiment, the length is at least 900 nucleotides. In another embodiment, the length is at least 1000 nucleotides. In another embodiment, the length is at least 1100

nucleotides. In another embodiment, the length is at least 1200 nucleotides. In another embodiment, the length is at least 1300 nucleotides. In another embodiment, the length is at least 1400 nucleotides. In another embodiment, the length is at least 1500 nucleotides. In another embodiment, the length is at least 1600 nucleotides. In another embodiment, the length is at least 1800 nucleotides. In another embodiment, the length is at least 2000 nucleotides. In another embodiment, the length is at least 2500 nucleotides. In another embodiment, the length is at least 3000 nucleotides. In another embodiment, the length is at least 4000 nucleotides. In another embodiment, the length is at least 5000 nucleotides, or greater than 5000 nucleotides. In another embodiment, the length is at least 5000 nucleotides, or greater than 6000 nucleotides. In another embodiment, the length is at least 7000 nucleotides, or greater than 7000 nucleotides. In another embodiment, the length is at least 8000 nucleotides, or greater than 8000 nucleotides. In another embodiment, the length is at least 9000 nucleotides, or greater than 9000 nucleotides. In another embodiment, the length is at least 10,000 nucleotides, or greater than 10,000 nucleotides.

Use of modified RNAs

Prevention or reduction of innate cellular immune response activation

[00413] The term "innate immune response" includes a cellular response to exogenous single stranded nucleic acids, generally of viral or bacterial origin, which involves the induction of cytokine expression and release, particularly the interferons, and cell death. Protein synthesis is also reduced during the innate cellular immune response. While it is advantageous to eliminate the innate immune response in a cell, the invention provides modified mRNAs that substantially reduce the immune response, including interferon signaling, without entirely eliminating such a response. In some embodiments, the immune response is reduced by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or greater than 99.9% as compared to the immune response induced by a corresponding unmodified nucleic acid. Such a reduction can be measured by expression or activity level of Type 1 interferons or the expression of interferon-regulated genes such as the toll-like receptors (e.g., TLR7 and TLR8). Reduction of innate immune response can also be measured by decreased cell death following one or more administrations of modified RNAs to a cell population; e.g., cell death is 10%>, 25%,

50%, 75%, 85%, 90%, 95%, or over 95% less than the cell death frequency observed with a corresponding unmodified nucleic acid. Moreover, cell death may affect fewer than 50%, 40%, 30%, 20%, 10%, 5%, 1%, 0.1%, 0.01% or fewer than 0.01% of cells contacted with the modified nucleic acids.

[00414] The invention provides for the repeated introduction (e.g., transfection) of modified nucleic acids into a target cell population, e.g., *in vitro*, *ex vivo*, or *in vivo*. The step of contacting the cell population may be repeated one or more times (such as two, three, four, five or more than five times). In some embodiments, the step of contacting the cell population with the modified nucleic acids is repeated a number of times sufficient such that a predetermined efficiency of protein translation in the cell population is achieved. Given the reduced cytotoxicity of the target cell population provided by the nucleic acid modifications, such repeated transfections are achievable in a diverse array of cell types.

Major Groove Interacting Partners

[00415] As described herein, the phrase "major groove interacting partner" refers to RNA recognition receptors that detect and respond to RNA ligands through interactions, e.g. binding, with the major groove face of a nucleotide or nucleic acid. As such, RNA ligands comprising modified nucleotides or nucleic acids such as the modified RNAs as described herein decrease interactions with major groove binding partners, and therefore decrease an innate immune response.

[00416] Example major groove interacting, e.g. binding, partners include, but are not limited to the following nucleases and helicases. Within membranes, TLRs (Toll-like Receptors) 3, 7, and 8 can respond to single- and double-stranded RNAs. Within the cytoplasm, members of the superfamily 2 class of DEX(D/H) helicases and ATPases can sense RNAs to initiate antiviral responses. These helicases include the RIG-I (retinoic acid-inducible gene 1) and MDA5 (melanoma differentiation-associated gene 5). Other examples include laboratory of genetics and physiology 2 (LGP2), HIN-200 domain containing proteins, or Helicase-domain containing proteins.

RNA binding proteins

[00417] In some embodiments of the present invention, RNA binding proteins are provided. RNA binding proteins may be provided as proteins and/or as nucleic acids

encoding such proteins. RNA binding proteins play a multitude of roles in regulating RNA stability and protein translation. A/U rich elements in the 3' UTR of mRNAs leads to formation of secondary structures which are bound by A/U Rich Binding Proteins (AREBPs) resulting in increased or decreased mRNA stability (Fan, X.C. et al., Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. EMBO J. 1998 Jun 15;17(12):3448-60). HuR is a stabilizing AREBP. To increase the stability of the mRNA of interest, an mRNA encoding HuR can be co-transfected or co-injected along with the mRNA of interest into the cells or into the tissue. These proteins can also be tethered to the mRNA of interest in vitro and then administered to the cells together. Poly A tail binding protein, PABP interacts with eukaryotic translation initiation factor eIF4G to stimulate translational initiation. Co-administration of mRNAs encoding these RBPs along with the mRNA drug and/or tethering these proteins to the mRNA drug in vitro and administering the protein-bound mRNA into the cells can increase the translational efficiency of the mRNA. The same concept can be extended to co-administration of mRNA along with mRNAs encoding various translation factors and facilitators as well as with the proteins themselves to influence RNA stability and/or translational efficiency.

Polypeptide variants

[00418] Provided are nucleic acids that encode variant polypeptides, which have a certain identity with a reference polypeptide sequence. The term "identity" as known in the art, refers to a relationship between the sequences of two or more peptides, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between peptides, as determined by the number of matches between strings of two or more amino acid residues.

[00419] "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms"). Identity of related peptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence

Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al, SIAM J. Applied Math. 48, 1073 (1988).

[00420] In some embodiments, the polypeptide variant has the same or a similar activity as the reference polypeptide. Alternatively, the variant has an altered activity (e.g., increased or decreased) relative to a reference polypeptide. Generally, variants of a particular polynucleotide or polypeptide of the invention will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art.

[00421] As recognized by those skilled in the art, protein fragments, functional protein domains, and homologous proteins are also considered to be within the scope of this invention. For example, provided herein is any protein fragment of a reference protein (meaning a polypeptide sequence at least one amino acid residue shorter than a reference polypeptide sequence but otherwise identical) 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or greater than 100 amino acids in length. In another example, any protein that includes a stretch of about 20, about 30, about 40, about 50, or about 100 amino acids which are about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or about 100% identical to any of the sequences described herein can be utilized in accordance with the invention. In certain embodiments, a protein sequence to be utilized in accordance with the invention includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations as shown in any of the sequences provided or referenced herein.

Polypeptide libraries

[00422] Also provided are polynucleotide libraries containing nucleoside modifications, wherein the polynucleotides individually contain a first nucleic acid sequence encoding a polypeptide, such as an antibody, protein binding partner, scaffold protein, and other polypeptides known in the art. Preferably, the polynucleotides are mRNA in a form suitable for direct introduction into a target cell host, which in turn synthesizes the encoded polypeptide.

[00423] In certain embodiments, multiple variants of a protein, each with different amino acid modification(s), are produced and tested to determine the best variant in terms of pharmacokinetics, stability, biocompatibility, and/or biological activity, or a biophysical property such as expression level. Such a library may contain 10 , 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , or over 10^9 possible variants (including substitutions, deletions of one or more residues, and insertion of one or more residues).

Polypeptide-nucleic acid complexes

[00424] Proper protein translation involves the physical aggregation of a number of polypeptides and nucleic acids associated with the mRNA. Provided by the invention are complexes containing conjugates of protein and nucleic acids, containing a translatable mRNA having one or more nucleoside modifications (e.g., at least two different nucleoside modifications) and one or more polypeptides bound to the mRNA. Generally, the proteins are provided in an amount effective to prevent or reduce an innate immune response of a cell into which the complex is introduced.

Targeting Moieties

[00425] In embodiments of the invention, modified nucleic acids are provided to express a protein-binding partner or a receptor on the surface of the cell, which functions to target the cell to a specific tissue space or to interact with a specific moiety, either *in vivo* or *in vitro*. Suitable protein-binding partners include antibodies and functional fragments thereof, scaffold proteins, or peptides. Additionally, modified nucleic acids can be employed to direct the synthesis and extracellular localization of lipids, carbohydrates, or other biological moieties.

[00426] As described herein, a useful feature of the modified nucleic acids of the invention is the capacity to reduce the innate immune response of a cell to an exogenous nucleic acid. Provided are methods for performing the titration, reduction or elimination of the immune response in a cell or a population of cells. In some embodiments, the cell is contacted with a first composition that contains a first dose of a first exogenous nucleic acid including a translatable region and at least one nucleoside modification, and the level of the innate immune response of the cell to the first exogenous nucleic acid is determined. Subsequently, the cell is contacted with a second composition, which

includes a second dose of the first exogenous nucleic acid, the second dose containing a lesser amount of the first exogenous nucleic acid as compared to the first dose.

[00427] Alternatively, the cell is contacted with a first dose of a second exogenous nucleic acid. The second exogenous nucleic acid may contain one or more modified nucleosides, which may be the same or different from the first exogenous nucleic acid or, alternatively, the second exogenous nucleic acid may not contain modified nucleosides. The steps of contacting the cell with the first composition and/or the second composition may be repeated one or more times.

[00428] Additionally, efficiency of protein production (e.g., protein translation) in the cell is optionally determined, and the cell may be re-transfected with the first and/or second composition repeatedly until a target protein production efficiency is achieved.

[00429] In one embodiment, the 3' end of the modified nucleic acids described herein may include a sequence for targeting the modified nucleic acid to a desired location within the cell such as, but not limited to, microvesicles within a cell. The sequence for targeting may be "zip code-like" in its function as it can be recognized by the cellular machinery that can traffic molecules to various places within the cell. Non-limiting examples of sequences for targeting nucleic acids are described in International Patent Publication No. WO2013 109713, the contents of which are herein incorporated by reference in its entirety. Zip-code like sequences and miR-1289 have been shown by Bolukbasi et al. to enrich mRNA in microvesicles (Mol. Ther. Nuc. Acid 2012 1, e10; the contents of which are herein incorporated by reference in its entirety) as both zipcodes and microRNA have a role in post-transcriptional regulation of mRNA.

[00430] In one embodiment, the sequence for targeting the modified nucleic acid is SEQ ID NO: 22, SEQ ID NO: 38 or a concatomer of at least one SEQ ID NO: 22 and at least one SEQ ID NO: 38 as described in International Patent Publication No. WO2013 109713, the contents of which are herein incorporated by reference in its entirety.

Vaccines

[00431] As described herein, provided are mRNAs having sequences that are substantially not translatable. Such mRNA is effective as a vaccine when administered to a mammalian subject.

[00432] Also provided are modified nucleic acids that contain one or more noncoding regions. Such modified nucleic acids are generally not translated, but are capable of binding to and sequestering one or more translational machinery component such as a ribosomal protein or a transfer RNA (tRNA), thereby effectively reducing protein expression in the cell. The modified nucleic acid may contain a small nucleolar RNA (sno-RNA), micro RNA (miRNA), small interfering RNA (siRNA) or Piwi-interacting RNA (piRNA).

[00433] Additionally, certain modified nucleosides, or combinations thereof, when introduced into modified nucleic acids activate the innate immune response. Such activating modified nucleic acids, e.g., modified RNAs, are useful as adjuvants when combined with polypeptide or other vaccines. In certain embodiments, the activated modified mRNAs contain a translatable region which encodes for a polypeptide sequence useful as a vaccine, thus providing the ability to be a self-adjuvant.

Therapeutic Agents

[00434] The modified nucleic acids (modified RNAs) and the proteins translated from the modified nucleic acids described herein can be used as therapeutic agents. For example, a modified nucleic acid described herein can be administered to a subject, wherein the modified nucleic acid is translated in vivo to produce a therapeutic peptide in the subject. Provided are compositions, methods, kits, and reagents for treatment or prevention of disease or conditions in humans and other mammals. The active therapeutic agents of the invention include modified nucleic acids, cells containing modified nucleic acids or polypeptides translated from the modified nucleic acids, polypeptides translated from modified nucleic acids, and cells contacted with cells containing modified nucleic acids or polypeptides translated from the modified nucleic acids.

[00435] In certain embodiments, provided are combination therapeutics containing one or more modified nucleic acids containing translatable regions that encode for a protein or proteins that boost a mammalian subject's immunity along with a protein that induces antibody-dependent cellular toxicity. For example, provided are therapeutics containing one or more nucleic acids that encode trastuzumab and granulocyte-colony stimulating factor (G-CSF). In particular, such combination therapeutics are useful in Her2+ breast

cancer patients who develop induced resistance to trastuzumab. (See, e.g., Albrecht, Immunotherapy. 2(6):795-8 (2010)).

[00436] Provided are methods of inducing translation of a recombinant polypeptide in a cell population using the modified nucleic acids described herein. Such translation can be *in vivo*, *ex vivo*, *in culture*, or *in vitro*. The cell population is contacted with an effective amount of a composition containing a nucleic acid that has at least one nucleoside modification, and a translatable region encoding the recombinant polypeptide. The population is contacted under conditions such that the nucleic acid is localized into one or more cells of the cell population and the recombinant polypeptide is translated in the cell from the nucleic acid.

[00437] An effective amount of the composition is provided based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the nucleic acid (e.g., size, and extent of modified nucleosides), and other determinants. In general, an effective amount of the composition provides efficient protein production in the cell, preferably more efficient than a composition containing a corresponding unmodified nucleic acid. Increased efficiency may be demonstrated by increased cell transfection (i.e., the percentage of cells transfected with the nucleic acid), increased protein translation from the nucleic acid, decreased nucleic acid degradation (as demonstrated, e.g., by increased duration of protein translation from a modified nucleic acid), or reduced innate immune response of the host cell.

[00438] Aspects of the invention are directed to methods of inducing *in vivo* translation of a recombinant polypeptide in a mammalian subject in need thereof. Therein, an effective amount of a composition containing a nucleic acid that has at least one nucleoside modification and a translatable region encoding the recombinant polypeptide is administered to the subject using the delivery methods described herein. The nucleic acid is provided in an amount and under other conditions such that the nucleic acid is localized into a cell of the subject and the recombinant polypeptide is translated in the cell from the nucleic acid. The cell in which the nucleic acid is localized, or the tissue in which the cell is present, may be targeted with one or more than one rounds of nucleic acid administration.

[00439] Other aspects of the invention relate to transplantation of cells containing modified nucleic acids to a mammalian subject. Administration of cells to mammalian subjects is known to those of ordinary skill in the art, such as local implantation (e.g., topical or subcutaneous administration), organ delivery or systemic injection (e.g., intravenous injection or inhalation), as is the formulation of cells in pharmaceutically acceptable carrier. Compositions containing modified nucleic acids are formulated for administration intramuscularly, transarterially, intraocularly, vaginally, rectally, intraperitoneally, intravenously, intranasally, subcutaneously, endoscopically, transdermally, or intrathecally. In some embodiments, the composition is formulated for extended release.

[00440] The subject to whom the therapeutic agent is administered suffers from or is at risk of developing a disease, disorder, or deleterious condition. Provided are methods of identifying, diagnosing, and classifying subjects on these bases, which may include clinical diagnosis, biomarker levels, genome-wide association studies (GWAS), and other methods known in the art.

[00441] In certain embodiments, the administered modified nucleic acid directs production of one or more recombinant polypeptides that provide a functional activity which is substantially absent in the cell in which the recombinant polypeptide is translated. For example, the missing functional activity may be enzymatic, structural, or gene regulatory in nature. In related embodiments, the administered modified nucleic acid directs production of one or more recombinant polypeptides that increases (e.g., synergistically) a functional activity which is present but substantially deficient in the cell in which the recombinant polypeptide is translated.

[00442] In other embodiments, the administered modified nucleic acid directs production of one or more recombinant polypeptides that replace a polypeptide (or multiple polypeptides) that is substantially absent in the cell in which the recombinant polypeptide is translated. Such absence may be due to genetic mutation of the encoding gene or regulatory pathway thereof. In some embodiments, the recombinant polypeptide increases the level of an endogenous protein in the cell to a desirable level; such an increase may bring the level of the endogenous protein from a subnormal level to a normal level, or from a normal level to a super-normal level.

[00443] Alternatively, the recombinant polypeptide functions to antagonize the activity of an endogenous protein present in, on the surface of, or secreted from the cell. Usually, the activity of the endogenous protein is deleterious to the subject, for example, do to mutation of the endogenous protein resulting in altered activity or localization. Additionally, the recombinant polypeptide antagonizes, directly or indirectly, the activity of a biological moiety present in, on the surface of, or secreted from the cell. Examples of antagonized biological moieties include lipids (e.g., cholesterol), a lipoprotein (e.g., low density lipoprotein), a nucleic acid, a carbohydrate, a protein toxin such as shiga and tetanus toxins, or a small molecule toxin such as botulinum, cholera, and diphtheria toxins. Additionally, the antagonized biological molecule may be an endogenous protein that exhibits an undesirable activity, such as a cytotoxic or cytostatic activity. The recombinant proteins described herein are engineered for localization within the cell, potentially within a specific compartment such as the nucleus, or are engineered for secretion from the cell or translocation to the plasma membrane of the cell.

Therapeutics

[00444] Provided are methods for treating or preventing a symptom of diseases characterized by missing or aberrant protein activity, by replacing the missing protein activity or overcoming the aberrant protein activity. Because of the rapid initiation of protein production following introduction of modified mRNAs, as compared to viral DNA vectors, the compounds of the present invention are particularly advantageous in treating acute diseases such as sepsis, stroke, and myocardial infarction. Moreover, the lack of transcriptional regulation of the modified mRNAs of the invention is advantageous in that accurate titration of protein production is achievable.

[00445] In some embodiments, modified mRNAs and their encoded polypeptides in accordance with the present invention may be used for therapeutic purposes. In some embodiments, modified mRNAs and their encoded polypeptides in accordance with the present invention may be used for treatment of any of a variety of diseases, disorders, and/or conditions, including but not limited to one or more of the following: autoimmune disorders (e.g. diabetes, lupus, multiple sclerosis, psoriasis, rheumatoid arthritis); inflammatory disorders (e.g. arthritis, pelvic inflammatory disease); infectious diseases (e.g. viral infections (e.g., HIV, HCV, RSV), bacterial infections, fungal infections,

sepsis); neurological disorders (*e.g.* Alzheimer's disease, Huntington's disease; autism; Duchenne muscular dystrophy); cardiovascular disorders (*e.g.* atherosclerosis, hypercholesterolemia, thrombosis, clotting disorders, angiogenic disorders such as macular degeneration); proliferative disorders (*e.g.* cancer, benign neoplasms); respiratory disorders (*e.g.* chronic obstructive pulmonary disease); digestive disorders (*e.g.* inflammatory bowel disease, ulcers); musculoskeletal disorders (*e.g.* fibromyalgia, arthritis); endocrine, metabolic, and nutritional disorders (*e.g.* diabetes, osteoporosis); urological disorders (*e.g.* renal disease); psychological disorders (*e.g.* depression, schizophrenia); skin disorders (*e.g.* wounds, eczema); blood and lymphatic disorders (*e.g.* anemia, hemophilia); *etc.*

[00446] Diseases characterized by dysfunctional or aberrant protein activity include cystic fibrosis, sickle cell anemia, epidermolysis bullosa, amyotrophic lateral sclerosis, and glucose-6-phosphate dehydrogenase deficiency. The present invention provides a method for treating such conditions or diseases in a subject by introducing nucleic acid or cell-based therapeutics containing the modified nucleic acids provided herein, wherein the modified nucleic acids encode for a protein that antagonizes or otherwise overcomes the aberrant protein activity present in the cell of the subject. Specific examples of a dysfunctional protein are the missense mutation variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which produce a dysfunctional protein variant of CFTR protein, which causes cystic fibrosis.

[00447] Diseases characterized by missing (or substantially diminished such that proper protein function does not occur) protein activity include cystic fibrosis, Niemann-Pick type C, β thalassemia major, Duchenne muscular dystrophy, Hurler Syndrome, Hunter Syndrome, and Hemophilia A. Such proteins may not be present, or are essentially non-functional. The present invention provides a method for treating such conditions or diseases in a subject by introducing nucleic acid or cell-based therapeutics containing the modified nucleic acids provided herein, wherein the modified nucleic acids encode for a protein that replaces the protein activity missing from the target cells of the subject. Specific examples of a dysfunctional protein are the nonsense mutation variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which produce a nonfunctional protein variant of CFTR protein, which causes cystic fibrosis.

[00448] Thus, provided are methods of treating cystic fibrosis in a mammalian subject by contacting a cell of the subject with a modified nucleic acid having a translatable region that encodes a functional CFTR polypeptide, under conditions such that an effective amount of the CFTR polypeptide is present in the cell. Preferred target cells are epithelial, endothelial and mesothelial cells, such as the lung, and methods of administration are determined in view of the target tissue; i.e., for lung delivery, the RNA molecules are formulated for administration by inhalation.

[00449] In another embodiment, the present invention provides a method for treating hyperlipidemia in a subject, by introducing into a cell population of the subject with a modified mRNA molecule encoding Sortilin, a protein recently characterized by genomic studies, thereby ameliorating the hyperlipidemia in a subject. The *SORT1* gene encodes a trans-Golgi network (TGN) transmembrane protein called Sortilin. Genetic studies have shown that one of five individuals has a single nucleotide polymorphism, rs12740374, in the *lpl3* locus of the *SORT1* gene that predisposes them to having low levels of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL). Each copy of the minor allele, present in about 30% of people, alters LDL cholesterol by 8 mg/dL, while two copies of the minor allele, present in about 5% of the population, lowers LDL cholesterol 16 mg/dL. Carriers of the minor allele have also been shown to have a 40% decreased risk of myocardial infarction. Functional *in vivo* studies in mice describes that overexpression of *SORT1* in mouse liver tissue led to significantly lower LDL-cholesterol levels, as much as 80% lower, and that silencing *SORT1* increased LDL cholesterol approximately 200% (Musunuru K et al. From noncoding variant to phenotype via *SORT1* at the *lpl3* cholesterol locus. *Nature* 2010; 466: 714-721, herein incorporated by reference in its entirety.).

Modulation of cell fate

[00450] Provided are methods of inducing an alteration in cell fate in a target mammalian cell. The target mammalian cell may be a precursor cell and the alteration may involve driving differentiation into a lineage, or blocking such differentiation. Alternatively, the target mammalian cell may be a differentiated cell, and the cell fate alteration includes driving de-differentiation into a pluripotent precursor cell, or blocking such de-differentiation, such as the dedifferentiation of cancer cells into cancer stem

cells. In situations where a change in cell fate is desired, effective amounts of mRNAs encoding a cell fate inductive polypeptide is introduced into a target cell under conditions such that an alteration in cell fate is induced. In some embodiments, the modified mRNAs are useful to reprogram a subpopulation of cells from a first phenotype to a second phenotype. Such a reprogramming may be temporary or permanent.

[00451] Optionally, the reprogramming induces a target cell to adopt an intermediate phenotype.

[00452] Additionally, the methods of the present invention are particularly useful to generate induced pluripotent stem cells (iPS cells) because of the high efficiency of transfection, the ability to re-transfect cells, and the tenability of the amount of recombinant polypeptides produced in the target cells. Further, the use of iPS cells generated using the methods described herein is expected to have a reduced incidence of teratoma formation.

[00453] Also provided are methods of reducing cellular differentiation in a target cell population. For example, a target cell population containing one or more precursor cell types is contacted with a composition having an effective amount of a modified mRNA encoding a polypeptide, under conditions such that the polypeptide is translated and reduces the differentiation of the precursor cell. In non-limiting embodiments, the target cell population contains injured tissue in a mammalian subject or tissue affected by a surgical procedure. The precursor cell is, e.g., a stromal precursor cell, a neural precursor cell, or a mesenchymal precursor cell.

[00454] In a specific embodiment, provided are modified nucleic acids that encode one or more differentiation factors Gata4, Mef2c and Tbx4. These mRNA-generated factors are introduced into fibroblasts and drive the reprogramming into cardiomyocytes. Such a reprogramming can be performed *in vivo*, by contacting an mRNA-containing patch or other material to damaged cardiac tissue to facilitate cardiac regeneration. Such a process promotes cardiomyocyte genesis as opposed to fibrosis.

Targeting of pathogenic organisms; purification of biological materials

[00455] Provided herein are methods for targeting pathogenic microorganisms, such as bacteria, yeast, protozoa, helminthes and the like, using modified mRNAs that encode cytostatic or cytotoxic polypeptides. Preferably the mRNA introduced into the target

pathogenic organism contains modified nucleosides or other nucleic acid sequence modifications that the mRNA is translated exclusively, or preferentially, in the target pathogenic organism, to reduce possible off-target effects of the therapeutic. Such methods are useful for removing pathogenic organisms from biological material, including blood, semen, eggs, and transplant materials including embryos, tissues, and organs.

Targeting diseased cells

[00456] Provided herein are methods for targeting pathogenic or diseased cells, particularly cancer cells, using modified mRNAs that encode cytostatic or cytotoxic polypeptides. Preferably the mRNA introduced into the target pathogenic cell contains modified nucleosides or other nucleic acid sequence modifications that the mRNA is translated exclusively, or preferentially, in the target pathogenic cell, to reduce possible off-target effects of the therapeutic. Alternatively, the invention provides targeting moieties that are capable of targeting the modified mRNAs to preferentially bind to and enter the target pathogenic cell.

Protein production

[00457] The methods provided herein are useful for enhancing protein product yield in a cell culture process. In a cell culture containing a plurality of host cells, introduction of the modified mRNAs described herein results in increased protein production efficiency relative to a corresponding unmodified nucleic acid. Such increased protein production efficiency can be demonstrated, e.g., by showing increased cell transfection, increased protein translation from the nucleic acid, decreased nucleic acid degradation, and/or reduced innate immune response of the host cell. Protein production can be measured by ELISA, and protein activity can be measured by various functional assays known in the art. The protein production may be generated in a continuous or a fed-batch mammalian process.

[00458] Additionally, it is useful to optimize the expression of a specific polypeptide in a cell line or collection of cell lines of potential interest, particularly an engineered protein such as a protein variant of a reference protein having a known activity. In one embodiment, provided is a method of optimizing expression of an engineered protein in a target cell, by providing a plurality of target cell types, and independently contacting with

each of the plurality of target cell types a modified mRNA encoding an engineered polypeptide. Additionally, culture conditions may be altered to increase protein production efficiency. Subsequently, the presence and/or level of the engineered polypeptide in the plurality of target cell types is detected and/or quantitated, allowing for the optimization of an engineered polypeptide's expression by selection of an efficient target cell and cell culture conditions relating thereto. Such methods are particularly useful when the engineered polypeptide contains one or more post-translational modifications or has substantial tertiary structure, situations which often complicate efficient protein production.

Gene silencing

[00459] The modified mRNAs described herein are useful to silence (i.e., prevent or substantially reduce) expression of one or more target genes in a cell population. A modified mRNA encoding a polypeptide capable of directing sequence-specific histone H3 methylation is introduced into the cells in the population under conditions such that the polypeptide is translated and reduces gene transcription of a target gene via histone H3 methylation and subsequent heterochromatin formation. In some embodiments, the silencing mechanism is performed on a cell population present in a mammalian subject. By way of non-limiting example, a useful target gene is a mutated Janus Kinase-2 family member, wherein the mammalian subject expresses the mutant target gene suffers from a myeloproliferative disease resulting from aberrant kinase activity.

[00460] Co-administration of modified mRNAs and siRNAs are also provided herein. As demonstrated in yeast, sequence-specific *trans* silencing is an effective mechanism for altering cell function. Fission yeast require two RNAi complexes for siRNA-mediated heterochromatin assembly: the RNA-induced transcriptional silencing (RITS) complex and the RNA-directed RNA polymerase complex (RDRC) (Motamedi et al. Cell 2004, 119, 789-802). In fission yeast, the RITS complex contains the siRNA binding Argonaute family protein Agol, a chromodomain protein Chpl, and Tas3. The fission yeast RDRC complex is composed of an RNA-dependent RNA Polymerase Rdpl, a putative RNA helicase Hrrl, and a polyA polymerase family protein Cid12. These two complexes require the Dicer ribonuclease and Clr4 histone H3 methyltransferase for activity. Together, Agol binds siRNA molecules generated through Dicer-mediated

cleavage of Rdpl co-transcriptionally generated dsRNA transcripts and allows for the sequence-specific direct association of Chpl, Tas3, Hrrl, and Clr4 to regions of DNA destined for methylation and histone modification and subsequent compaction into transcriptionally silenced heterochromatin. While this mechanism functions in *cis*- with centromeric regions of DNA, sequence-specific *trans* silencing is possible through co-transfection with double-stranded siRNAs for specific regions of DNA and concomitant RNAi-directed silencing of the siRNA ribonuclease Eril (Buhler et al. Cell 2006, 125, 873-886, herein incorporated by reference in its entirety.).

Modulation of biological pathways

[00461] The rapid translation of modified mRNAs introduced into cells provides a desirable mechanism of modulating target biological pathways. Such modulation includes antagonism or agonism of a given pathway. In one embodiment, a method is provided for antagonizing a biological pathway in a cell by contacting the cell with an effective amount of a composition comprising a modified nucleic acid encoding a recombinant polypeptide, under conditions such that the nucleic acid is localized into the cell and the recombinant polypeptide is capable of being translated in the cell from the nucleic acid, wherein the recombinant polypeptide inhibits the activity of a polypeptide functional in the biological pathway. Exemplary biological pathways are those defective in an autoimmune or inflammatory disorder such as multiple sclerosis, rheumatoid arthritis, psoriasis, lupus erythematosus, ankylosing spondylitis colitis, or Crohn's disease; in particular, antagonism of the IL-12 and IL-23 signaling pathways are of particular utility. (See Kikly K, Liu L, Na S, Sedgwick JD (2006) Curr. Opin. Immunol. 18 (6): 670-5, herein incorporated by reference in its entirety.).

[00462] Further, provided are modified nucleic acids encoding an antagonist for chemokine receptors; chemokine receptors CXCR-4 and CCR-5 are required for, e.g., HIV entry into host cells (Arenzana-Seisdedos F et al, (1996) Nature. Oct 3;383(6599):400, herein incorporated by reference in its entirety.).

[00463] Alternatively, provided are methods of agonizing a biological pathway in a cell by contacting the cell with an effective amount of a modified nucleic acid encoding a recombinant polypeptide under conditions such that the nucleic acid is localized into the cell and the recombinant polypeptide is capable of being translated in the cell from the

nucleic acid, and the recombinant polypeptide induces the activity of a polypeptide functional in the biological pathway. Exemplary agonized biological pathways include pathways that modulate cell fate determination. Such agonization is reversible or, alternatively, irreversible.

Cellular nucleic acid delivery

[00464] Methods of the present invention enhance nucleic acid delivery into a cell population, *in vivo*, *ex vivo*, or *in culture*. For example, a cell culture containing a plurality of host cells (e.g., eukaryotic cells such as yeast or mammalian cells) is contacted with a composition that contains an enhanced nucleic acid having at least one nucleoside modification and, optionally, a translatable region. The composition also generally contains a transfection reagent or other compound that increases the efficiency of enhanced nucleic acid uptake into the host cells. The enhanced nucleic acid exhibits enhanced retention in the cell population, relative to a corresponding unmodified nucleic acid. The retention of the enhanced nucleic acid is greater than the retention of the unmodified nucleic acid. In some embodiments, it is at least about 50%, 75%, 90%>, 95%, 100%, 150%, 200% or more than 200% greater than the retention of the unmodified nucleic acid. Such retention advantage may be achieved by one round of transfection with the enhanced nucleic acid, or may be obtained following repeated rounds of transfection.

[00465] In some embodiments, the enhanced nucleic acid is delivered to a target cell population with one or more additional nucleic acids. Such delivery may be at the same time, or the enhanced nucleic acid is delivered prior to delivery of the one or more additional nucleic acids. The additional one or more nucleic acids may be modified nucleic acids or unmodified nucleic acids. It is understood that the initial presence of the enhanced nucleic acids does not substantially induce an innate immune response of the cell population and, moreover, that the innate immune response will not be activated by the later presence of the unmodified nucleic acids. In this regard, the enhanced nucleic acid may not itself contain a translatable region, if the protein desired to be present in the target cell population is translated from the unmodified nucleic acids.

IV. Pharmaceutical Compositions

Formulation, Administration, Delivery and Dosing

[00466] The present invention provides polynucleotides, modified nucleic acid, enhanced modified RNA and ribonucleic acid compositions and complexes in combination with one or more pharmaceutically acceptable excipients. Pharmaceutical compositions may optionally comprise one or more additional active substances, e.g. therapeutically and/or prophylactically active substances. General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in *Remington: The Science and Practice of Pharmacy* 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

[00467] In one embodiment, provided are formulations containing an effective amount of a ribonucleic acid (e.g., an mRNA or a nucleic acid containing an mRNA) engineered to avoid an innate immune response of a cell into which the ribonucleic acid enters. The ribonucleic acid generally includes a nucleotide sequence encoding a polypeptide of interest.

[00468] In some embodiments, compositions are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase "active ingredient" generally refers to a modified nucleic acid, an enhanced nucleic acid or a ribonucleic acid to be delivered as described herein.

[00469] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, e.g., to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[00470] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00471] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00472] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient

Formulations

[00473] The polynucleotides, modified nucleic acid, enhanced modified RNA and ribonucleic acid of the invention can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (e.g., from a depot formulation of the modified nucleic acids, enhanced modified RNA or ribonucleic acids); (4) alter the biodistribution (e.g., target the modified nucleic acids, enhanced modified RNA or ribonucleic acids to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo*; and/or (6) alter the release profile of encoded protein *in vivo*. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients

of the present invention can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with polynucleotides, modified nucleic acid, enhanced modified RNA and ribonucleic acid (e.g., for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof.

[00474] Accordingly, the formulations of the invention can include one or more excipients, each in an amount that together increases the stability of the polynucleotide, modified nucleic acid, enhanced modified RNA or ribonucleic acid, increases cell transfection by the polynucleotides, modified nucleic acid, enhanced modified RNA or ribonucleic acid, increases the expression of polynucleotides, modified nucleic acid, enhanced modified RNA or ribonucleic acid encoded protein, and/or alters the release profile of the polynucleotides, modified nucleic acid, enhanced modified RNA or ribonucleic acid encoded proteins. Further, the polynucleotides, modified nucleic acid, enhanced modified RNA or ribonucleic acid of the present invention may be formulated using self-assembled nucleic acid nanoparticles.

[00475] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients.

[00476] The polynucleotides, modified nucleic acid, enhanced modified RNA and ribonucleic acid of the invention may be formulated for delivery in the tissues and/or organs of a subject. Organs may include, but are not limited to, the heart, lung, brain, liver, basal ganglia, brain stem medulla, midbrain, pons, cerebellum, cerebral cortex, hypothalamus, eye, pituitary, thyroid, parathyroid, esophagus, thymus, adrenal glands, appendix, bladder, gallbladder, intestines (e.g., large intestine and small intestine), kidney, pancreas, spleen, stomach, skin, prostate, testes, ovaries, uterus, adrenal glands, anus, bronchi, ears, esophagus, genitals, larynx (voice box), lymph nodes, meninges, mouth, nose, parathyroid glands, pituitary gland, rectum, salivary glands, spinal cord, thymus gland, tongue, trachea, ureters, urethra, colon. Tissues may include, but are not limited to, heart valves, bone, vein, middle ear, muscle (cardiac, smooth or skeletal) cartilage, tendon or ligaments. As a non-limiting example, the polynucleotides, modified

nucleic acid, enhanced modified RNA and ribonucleic acid may be formulated in a lipid nanoparticle and delivered to an organ such as, but not limited, to the liver, spleen, kidney or lung. In another non-limiting example, the polynucleotides, modified nucleic acids, enhanced modified RNA and ribonucleic acid may be formulated in a lipid nanoparticle comprising the cationic lipid DLin-KC2-DMA and delivered to an organ such as, but not limited to, the liver, spleen, kidney or lung.

[00477] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient may generally be equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage including, but not limited to, one-half or one-third of such a dosage.

[00478] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient.

[00479] In some embodiments, the modified mRNA formulations described herein may contain at least one modified mRNA. The formulations may contain 1, 2, 3, 4 or 5 modified mRNA. In one embodiment the formulation may contain modified mRNA encoding proteins selected from categories such as, but not limited to, human proteins, veterinary proteins, bacterial proteins, biological proteins, antibodies, immunogenic proteins, therapeutic peptides and proteins, secreted proteins, plasma membrane proteins, cytoplasmic and cytoskeletal proteins, intracellular membrane bound proteins, nuclear proteins, proteins associated with human disease and/or proteins associated with non-human diseases. In one embodiment, the formulation contains at least three modified mRNA encoding proteins. In one embodiment, the formulation contains at least five modified mRNA encoding proteins.

[00480] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes, but is not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

[00481] In some embodiments, the particle size of the lipid nanoparticle may be increased and/or decreased. The change in particle size may be able to help counter biological reaction such as, but not limited to, inflammation or may increase the biological effect of the modified mRNA delivered to mammals.

[00482] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, surface active agents and/or emulsifiers, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in the pharmaceutical formulations of the invention

Lipidoid

[00483] The synthesis of lipidoids has been extensively described and formulations containing these compounds are particularly suited for delivery of polynucleotides, modified nucleic acids, enhanced modified RNA and ribonucleic acids (see Mahon et al., Bioconjug Chem. 2010 21:1448-1454; Schroeder et al, J Intern Med. 2010 267:9-21; Akinc et al, Nat Biotechnol. 2008 26:561-569; Love et al, Proc Natl Acad Sci U S A. 2010 107:1864- 1869; Siegwart et al., Proc Natl Acad Sci U S A. 2011 108:12996-3001; all of which are incorporated herein in their entirety).

[00484] While these lipidoids have been used to effectively deliver double stranded small interfering RNA molecules in rodents and non-human primates (see Akinc et al, Nat Biotechnol. 2008 26:561-569; Frank-Kamenetsky et al, Proc Natl Acad Sci U S A. 2008 105:1 1915-1 1920; Akinc et al, Mol Ther. 2009 17:872-879; Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869; Leuschner et al, Nat Biotechnol. 2011 29:1005-1010; all of which is incorporated herein in their entirety), the present disclosure describes their formulation and use in delivering single stranded polynucleotide, modified nucleic acids, enhanced modified RNA and ribonucleic acids. Complexes, micelles, liposomes or particles can be prepared containing these lipidoids and therefore, can result in an effective delivery of the polynucleotides, modified nucleic acids, enhanced modified RNA and ribonucleic acids, as judged by the production of an encoded protein, following the injection of a lipidoid formulation via localized and/or systemic routes of administration. Lipidoid complexes of polynucleotides, modified nucleic acids, enhanced modified RNA and ribonucleic acids can be administered by various means including, but not limited to, intravenous, intramuscular, or subcutaneous routes.

[00485] *In vivo* delivery of nucleic acids may be affected by many parameters, including, but not limited to, the formulation composition, nature of particle PEGylation, degree of loading, oligonucleotide to lipid ratio, and biophysical parameters such as particle size (Akinc et al., Mol Ther. 2009 17:872-879; herein incorporated by reference in its entirety). As an example, small changes in the anchor chain length of poly(ethylene glycol) (PEG) lipids may result in significant effects on *in vivo* efficacy. Formulations with the different lipidoids, including, but not limited to penta[3-(1-laurylamino)propionyl]-triethylenetetramine hydrochloride (TETA-5LAP; aka 98N12-5, see Murugaiah et al., Analytical Biochemistry, 401:61 (2010)), C12-200 (including derivatives and variants), and MD1, can be tested for *in vivo* activity.

[00486] The lipidoid referred to herein as "98N12-5" is disclosed by Akinc et al, Mol Ther. 2009 17:872-879 and is incorporated by reference in its entirety.

[00487] The lipidoid referred to herein as "CI 2-200" is disclosed by Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869 and Liu and Huang, Molecular Therapy. 2010 669-670; both of which are herein incorporated by reference in their entirety. The lipidoid formulations can include particles comprising either 3 or 4 or more components in

addition to polynucleotide, modified nucleic acids, enhanced modified RNA and ribonucleic acids. As an example, formulations with certain lipidoids, include, but are not limited to, 98N12-5 and may contain 42% lipidoid, 48% cholesterol and 10% PEG (C14 alkyl chain length). As another example, formulations with certain lipidoids, include, but are not limited to, C12-200 and may contain 50% lipidoid, 10% disteoylphosphatidyl choline, 38.5% cholesterol, and 1.5% PEG-DMG.

[00488] In one embodiment, a modified nucleic acids, enhanced modified RNA or ribonucleic acids formulated with a lipidoid for systemic intravenous administration can target the liver. For example, a final optimized intravenous formulation using modified nucleic acids, enhanced modified RNA or ribonucleic acids, and comprising a lipid molar composition of 42% 98N12-5, 48% cholesterol, and 10% PEG-lipid with a final weight ratio of about 7.5 to 1 total lipid to polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids, and a C14 alkyl chain length on the PEG lipid, with a mean particle size of roughly 50-60 nm, can result in the distribution of the formulation to be greater than 90% to the liver.(see, Akinc et al, Mol Ther. 2009 17:872-879; herein incorporated in its entirety). In another example, an intravenous formulation using a C12-200 (see US provisional application 61/175,770 and published international application WO2010129709, each of which is herein incorporated by reference in their entirety) lipidoid may have a molar ratio of 50/10/38.5/1.5 of C12-200/disteoylphosphatidyl choline/cholesterol/PEG-DMG, with a weight ratio of 7 to 1 total lipid to polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids, and a mean particle size of 80 nm may be effective to deliver polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids to hepatocytes (see, Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869 herein incorporated by reference). In another embodiment, an MD1 lipidoid-containing formulation may be used to effectively deliver polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids to hepatocytes *in vivo*. The characteristics of optimized lipidoid formulations for intramuscular or subcutaneous routes may vary significantly depending on the target cell type and the ability of formulations to diffuse through the extracellular matrix into the blood stream. While a particle size of less than 150 nm may be desired for effective hepatocyte delivery due to the size of the endothelial fenestrae (see, Akinc et al., Mol

Ther. 2009 17:872-879 herein incorporated by reference), use of a lipidoid-formulated polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids to deliver the formulation to other cells types including, but not limited to, endothelial cells, myeloid cells, and muscle cells may not be similarly size-limited. Use of lipidoid formulations to deliver siRNA in vivo to other non-hepatocyte cells such as myeloid cells and endothelium has been reported (see Akinc et al., Nat Biotechnol. 2008 26:561-569; Leuschner et al, Nat Biotechnol. 2011 29:1005-1010; Cho et al. Adv. Funct. Mater. 2009 19:3112-3118; 8th International Judah Folkman Conference, Cambridge, MA October 8-9, 2010 herein incorporated by reference in its entirety). Effective delivery to myeloid cells, such as monocytes, lipidoid formulations may have a similar component molar ratio. Different ratios of lipidoids and other components including, but not limited to, distearylphosphatidyl choline, cholesterol and PEG-DMG, may be used to optimize the formulation of the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids for delivery to different cell types including, but not limited to, hepatocytes, myeloid cells, muscle cells, etc. For example, the component molar ratio may include, but is not limited to, 50% C12-200, 10% distearylphosphatidyl choline, 38.5% cholesterol, and %1.5 PEG-DMG (see Leuschner et al, Nat Biotechnol 2011 29:1005-1010; herein incorporated by reference in its entirety). The use of lipidoid formulations for the localized delivery of nucleic acids to cells (such as, but not limited to, adipose cells and muscle cells) via either subcutaneous or intramuscular delivery, may not require all of the formulation components desired for systemic delivery, and as such may comprise only the lipidoid and the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids.

[00489] Combinations of different lipidoids may be used to improve the efficacy of polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids directed protein production as the lipidoids may be able to increase cell transfection by the polynucleotides, modified nucleic acid, or modified nucleic acids, enhanced modified RNA or ribonucleic acids; and/or increase the translation of encoded protein (see Whitehead et al., Mol. Ther. 2011, 19:1688-1694, herein incorporated by reference in its entirety).

Liposomes, Lipoplexes, and Lipid Nanoparticles

[00490] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention can be formulated using one or more liposomes, lipoplexes, or lipid nanoparticles. In one embodiment, pharmaceutical compositions of polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids include liposomes. Liposomes are artificially-prepared vesicles which may primarily be composed of a lipid bilayer and may be used as a delivery vehicle for the administration of nutrients and pharmaceutical formulations. Liposomes can be of different sizes such as, but not limited to, a multilamellar vesicle (MLV) which may be hundreds of nanometers in diameter and may contain a series of concentric bilayers separated by narrow aqueous compartments, a small unicellular vesicle (SUV) which may be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) which may be between 50 and 500 nm in diameter. Liposome design may include, but is not limited to, opsonins or ligands in order to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to, endocytosis. Liposomes may contain a low or a high pH in order to improve the delivery of the pharmaceutical formulations.

[00491] The formation of liposomes may depend on the physicochemical characteristics such as, but not limited to, the pharmaceutical formulation entrapped and the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimization size, polydispersity and the shelf-life of the vesicles for the intended application, and the batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

[00492] In one embodiment, pharmaceutical compositions described herein may include, without limitation, liposomes such as those formed from 1,2-dioleoyloxy -*N,N*-dimethylaminopropane (DODMA) liposomes, DiLa2 liposomes from Marina Biotech (Bothell, WA), 1,2-dilinoleoyloxy-3-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), and MC3 (US20 100324 120; herein incorporated by reference in its entirety) and liposomes which may deliver small molecule drugs such as, but not limited to, DOXIL® from Janssen Biotech, Inc. (Horsham, PA). In one embodiment, pharmaceutical compositions described

herein may include, without limitation, liposomes such as those formed from the synthesis of stabilized plasmid-lipid particles (SPLP) or stabilized nucleic acid lipid particle (SNALP) that have been previously described and shown to be suitable for oligonucleotide delivery in vitro and in vivo (see Wheeler et al. *Gene Therapy*. 1999 6:271-281; Zhang et al. *Gene Therapy*. 1999 6:1438-1447; Jeffs et al. *Pharm Res*. 2005 22:362-372; Morrissey et al., *Nat Biotechnol*. 2005 2:1002-1007; Zimmermann et al., *Nature*. 2006 441:11-14; Heyes et al. *J Contr Rel*. 2005 107:276-287; Semple et al. *Nature Biotech*. 2010 28:172-176; Judge et al. *J Clin Invest*. 2009 119:661-673; deFougerolles *Hum Gene Ther*. 2008 19:125-132; all of which are incorporated herein in their entireties.) The original manufacture method by Wheeler et al. was a detergent dialysis method, which was later improved by Jeffs et al. and is referred to as the spontaneous vesicle formation method. The liposome formulations are composed of 3 to 4 lipid components in addition to the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids. As an example a liposome can contain, but is not limited to, 55% cholesterol, 20% distearylphosphatidyl choline (DSPC), 10% PEG-S-DSG, and 15% 1,2-dioleoyloxy -N,N-dimethylaminopropane (DODMA), as described by Jeffs et al. As another example, certain liposome formulations may contain, but are not limited to, 48% cholesterol, 20% DSPC, 2% PEG-c-DMA, and 30% cationic lipid, where the cationic lipid can be 1,2-distearoyloxy -N,N-dimethylaminopropane (DSDMA), DODMA, DLin-DMA, or 1,2-dilinolenyloxy-3-dimethylaminopropane (DLenDMA), as described by Heyes et al.

[00493] In one embodiment, pharmaceutical compositions may include liposomes which may be formed to deliver polynucleotides, modified nucleic acids, enhanced modified RNA and ribonucleic acids which may encode at least one immunogen. The polynucleotides, modified nucleic acids, enhanced modified RNA and ribonucleic acids may be encapsulated by the liposome and/or it may be contained in an aqueous core which may then be encapsulated by the liposome (see International Pub. Nos. WO2012031046, WO2012031043, WO2012030901 and WO2012006378; each of which is herein incorporated by reference in their entirety). In another embodiment, the modified nucleic acids, enhanced modified RNA and ribonucleic acids which may encode an immunogen may be formulated in a cationic oil-in-water emulsion

where the emulsion particle comprises an oil core and a cationic lipid which can interact with the polynucleotides, modified nucleic acids, enhanced modified RNA and ribonucleic acids anchoring the molecule to the emulsion particle (see International Pub. No. WO2012006380). In yet another embodiment, the lipid formulation may include at least cationic lipid, a lipid which may enhance transfection and at least one lipid which contains a hydrophilic head group linked to a lipid moiety (International Pub. No. WO2011076807 and U.S. Pub. No. 20110200582; each of which is herein incorporated by reference in their entirety). In another embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids encoding an immunogen may be formulated in a lipid vesicle which may have crosslinks between functionalized lipid bilayers (see U.S. Pub. No. 20120177724, herein incorporated by reference in its entirety).

[00494] In one embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be formulated in a lipid vesicle which may have crosslinks between functionalized lipid bilayers.

[00495] In one embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be formulated in a lipid-polycation complex. The formation of the lipid-polycation complex may be accomplished by methods known in the art and/or as described in U.S. Pub. No. 20120178702, herein incorporated by reference in its entirety. As a non-limiting example, the polycation may include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine. In another embodiment, the polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be formulated in a lipid-polycation complex which may further include a neutral lipid such as, but not limited to, cholesterol or dioleoyl phosphatidylethanolamine (DOPE).

[00496] The liposome formulation may be influenced by, but not limited to, the selection of the cationic lipid component, the degree of cationic lipid saturation, the nature of the PEGylation, ratio of all components and biophysical parameters such as size. In one example by Semple et al. (Semple et al. Nature Biotech. 2010 28:172-176), the liposome formulation was composed of 57.1 % cationic lipid, 7.1% dipalmitoylphosphatidylcholine, 34.3 % cholesterol, and 1.4% PEG-c-DMA. As another

example, changing the composition of the cationic lipid could more effectively deliver siRNA to various antigen presenting cells (Basha et al. Mol Ther. 2011 19:2186-2200; herein incorporated by reference in its entirety).

[00497] In some embodiments, the ratio of PEG in the LNP formulations may be increased or decreased and/or the carbon chain length of the PEG lipid may be modified from C14 to C18 to alter the pharmacokinetics and/or biodistribution of the LNP formulations. As a non-limiting example, LNP formulations may contain 1-5% of the lipid molar ratio of PEG-c-DOMG as compared to the cationic lipid, DSPC and cholesterol. In another embodiment the PEG-c-DOMG may be replaced with a PEG lipid such as, but not limited to, PEG-DSG (1,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol) or PEG-DPG (1,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol). The cationic lipid may be selected from any lipid known in the art such as, but not limited to, DLin-MC3-DMA, DLin-DMA, C12-200 and DLin-KC2-DMA.

[00498] In one embodiment, the cationic lipid may be selected from, but not limited to, a cationic lipid described in International Publication Nos. WO2012040184, WO2011153120, WO2011149733, WO2011090965, WO2011043913, WO2011022460, WO2012061259, WO2012054365, WO2012044638, WO2010080724, WO201021865 and WO2008103276, US Patent Nos. 7,893,302 and 7,404,969 and US Patent Publication No. US20100036115; each of which is herein incorporated by reference in their entirety. In another embodiment, the cationic lipid may be selected from, but not limited to, formula A described in International Publication Nos. WO2012040184, WO2011153120, WO2011149733, WO2011090965, WO2011043913, WO2011022460, WO2012061259, WO2012054365 and WO2012044638; each of which is herein incorporated by reference in their entirety. In yet another embodiment, the cationic lipid may be selected from, but not limited to, formula CLI-CLXXIX of International Publication No. WO2008103276, formula CLI-CLXXIX of US Patent No. 7,893,302, formula CLI-CLXXXII of US Patent No. 7,404,969 and formula I-VI of US Patent Publication No. US20100036115; each of which is herein incorporated by reference in their entirety. As a non-limiting example, the cationic lipid may be selected from (20Z,23Z)-N,N-dimethylnonacosan-20,23-dien-10-amine, (17Z,20Z)-N,N-dimethylhexacosan-17,20-dien-9-amine, (1Z,19Z)-N,N-dimethylpentacosan-16,19-dien-8-amine, (13Z,16Z)-N,N-dimethyldocosan-13,16-

dien-5-amine, (12Z, 15Z)-N,N-dimethylhenicosan-12,15-dien-4-amine, (14Z, 17Z)-N,N-dimethyltricosan-14,17-dien-6-amine, (15Z, 18Z)-N,N-dimethyltetracosan-15,18-dien-7-amine, (18Z,21Z)-N,N-dimethylheptacosan-18,21-dien-10-amine, (15Z, 18Z)-N,N-dimethyltetracosan-15,18-dien-5-amine, (14Z, 17Z)-N,N-dimethyltricosan-14,17-dien-4-amine, (19Z,22Z)-N,N-dimethyloctacosan-19,22-dien-9-amine, (18Z,21Z)-N,N-dimethylheptacosan-18,21-dien-8-amine, (17Z,20Z)-N,N-dimethylhexacosan-17,20-dien-7-amine, (16Z; 19Z)-N,N-dimethylpentacosan-16,19-dien-6-amine, (22Z,25Z)-N,N-dimethylhentriacontan-22,25-dien-10-amine, (21Z,24Z)-N,N-dimethyltriacontan-21,24-dien-9-amine, (18Z)-N,N-dimethylheptacosan-18-en-10-amine, (17Z)-N,N-dimethylhexacosan-17-en-9-amine, (19Z,22Z)-N,N-dimethyloctacosan-19,22-dien-7-amine, N,N-dimethylheptacosan-10-amine, (20Z,23Z)-N-ethyl-N-methylnonacosan-20,23-dien-10-amine, 1-[(1Z,14Z)-1-nonylicosyl]-1,14-dien-1-yl]pyrrolidine, (20Z)-N,N-dimethylheptacosan-20-en-10-amine, (15Z)-N,N-dimethylheptacosan-15-en-10-amine, (14Z)-N,N-dimethylnonacosan-14-en-10-amine, (17Z)-N,N-dimethylnonacosan-17-en-10-amine, (24Z)-N,N-dimethyltriacontan-24-en-10-amine, (20Z)-N,N-dimethylnonacosan-20-en-10-amine, (22Z)-N,N-dimethylhentriacontan-22-en-10-amine, (16Z)-N,N-dimethylpentacosan-16-en-8-amine, (12Z, 15Z)-N,N-dimethyl-2-nonylhenicosan-12,15-dien-1-amine, (13Z, 16Z)-N,N-dimethyl-3-nonyldocosan-13,16-dien-1-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 1-[(1S,2R)-2-hexylcyclopropyl]-N,N-dimethylnonadecan-10-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]nonadecan-10-amine, N,N-dimethyl-21~[(1S,2R)-2-octylcyclopropyl]henicosan-10-amine, N,N-dimethyl-1-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl}cyclopropyl]nonadecan-10-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]hexadecan-8-amine, N,N-dimethyl-1-[(1R,2S)-2-undecylcyclopropyl]tetradecan-5-amine, N,N-dimethyl-3-{7-[(1S,2R)-2-octylcyclopropyl]heptyl}dodecan-1-amine, 1-[(1R,2S)-2-heptylcyclopropyl]-N,N-dimethyloctadecan-9-amine, 1-[(1S,2R)-2-decylcyclopropyl]-N,N-dimethylpentadecan-6-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]pentadecan-8-amine, R-N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-(octyloxy)propan-2-amine, S-N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-(octyloxy)propan-2-amine, 1-{2-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-1-[(octyloxy)]

methyl]ethyl}pyrrolidine, (2S)-N,N-dimethyl- 1-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy]-3-[(5Z)-oct -5-en-1-yloxy]propan-2-amine, 1-{2-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-1-[(octyloxy) methyl]ethyl}azetidine, (2S)-1-(hexyloxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9, 12-dien- 1-yloxy]propan-2-amine, (2S)- 1-(heptyloxy)-N,N-dimethyl-3-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy]propan-2-amine, N,N-dimethyl- 1-(nonyloxy)-3-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy]propan-2-amine, N,N-dimethyl- 1-[(9Z)-octadec-9-en-1-yloxy]-3-(octyloxy)propan-2-amine (Compound 9); (2S)-N,N-dimethyl-1-[(6Z,9Z, 12Z)-octadeca-6,9, 12-trien- 1-yloxy]-3-(octyloxy)propan-2-amine, (2S)- 1-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethyl-3-(pentyloxy)propan-2-amine, (2S)-1-(hexyloxy)-3-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethylpropan-2-amine, 1-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, 1-[(13Z, 16Z)-docosa-13, 16-dien-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, (2S)-1-[(13Z, 16Z)-docosa-13,16-dien- 1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, (2S)- 1-[(13Z)-docos-13-en- 1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, 1-[(13Z)-docos-13-en- 1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, 1-[(9Z)-hexadec-9-en- 1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, (2R)-N,N-dimethyl-H(1-methyloctyl)oxy]-3-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy]propan-2-amine, (2R)-1-[(3,7-dimethyloctyl)oxy]-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl- 1-(octyloxy)-3-[(8-[(1S,2S)-2-[(1R,2R)-2-pentylcyclopropyl]methyl} cyclopropyl]octyl)oxy]propan-2-amine, N,N-dimethyl- 1-[[8-(2-oxolylcyclopropyl)octyl]oxy]-3-(octyloxy)propan-2-amine and (11E,20Z,23Z)-N,N-dimethylnonacos-11,20,2-trien-10-amine or a pharmaceutically acceptable salt or stereoisomer thereof.

[00499] In one embodiment, the cationic lipid may be synthesized by methods known in the art and/or as described in International Publication Nos. WO20 12040 184, WO201 1153120, WO201 1149733, WO201 1090965, WO201 1043913, WO201 1022460, WO2012061259, WO2012054365, WO2012044638, WO2010080724 and WO20 102 1865; each of which is herein incorporated by reference in their entirety.

[00500] In one embodiment, the LNP formulation may contain PEG-c-DOMG 3% lipid molar ratio. In another embodiment, the LNP formulation may contain PEG-c-DOMG 1.5% lipid molar ratio.

[00501] In one embodiment, the LNP formulation may contain PEG-DMG 2000 (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]). In one embodiment, the LNP formulation may contain PEG-DMG 2000, a cationic lipid known in the art and at least one other component. In another embodiment, the LNP formulation may contain PEG-DMG 2000, a cationic lipid known in the art, DSPC and cholesterol. As a non-limiting example, the LNP formulation may contain PEG-DMG 2000, DLin-DMA, DSPC and cholesterol. As another non-limiting example the LNP formulation may contain PEG-DMG 2000, DLin-DMA, DSPC and cholesterol in a molar ratio of 2:40:10:48 (see Geall et al., Nonviral delivery of self-amplifying RNA vaccines, PNAS 2012; PMID: 22908294).

[00502] In one embodiment, the LNP formulation may be formulated by the methods described in International Publication Nos. WO201 1127255 or WO2008103276, each of which is herein incorporated by reference in their entirety. As a non-limiting example, modified RNA described herein may be encapsulated in LNP formulations as described in WO201 1127255 and/or WO2008 103276; each of which is herein incorporated by reference in their entirety.

[00503] In one embodiment, LNP formulations described herein may comprise a polycationic composition. As a non-limiting example, the polycationic composition may be selected from formula 1-60 of US Patent Publication No. US20050222064; herein incorporated by reference in its entirety. In another embodiment, the LNP formulations comprising a polycationic composition may be used for the delivery of the modified RNA described herein *in vivo* and/or *in vitro*.

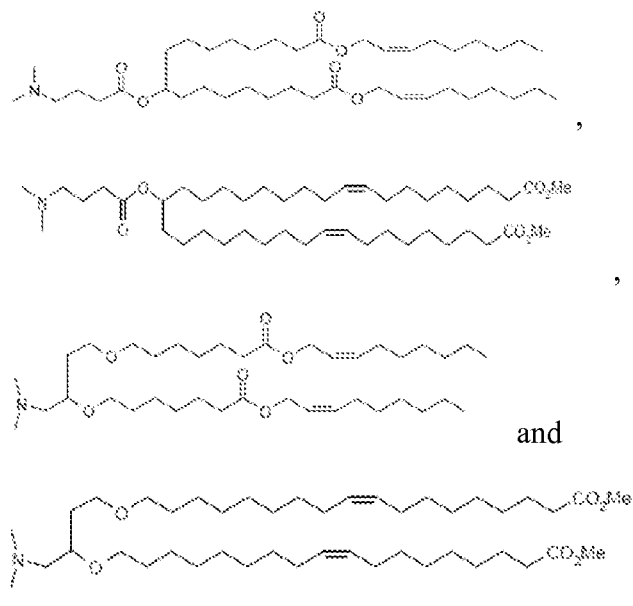
[00504] In one embodiment, the LNP formulations described herein may additionally comprise a permeability enhancer molecule. Non-limiting permeability enhancer molecules are described in US Patent Publication No. US20050222064; herein incorporated by reference in its entirety.

[00505] In one embodiment, the pharmaceutical compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, WA), SMARTICLES® (Marina Biotech, Bothell, WA), neutral DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) based liposomes (e.g., siRNA delivery for ovarian cancer

(Landen et al. Cancer Biology & Therapy 2006 5(12)1708-1713)) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

[00506] Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin-MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

[00507] In one embodiment, the internal ester linkage may be located on either side of the saturated carbon. Non-limiting examples of reLNPs include,



[00508] In one embodiment, an immune response may be elicited by delivering a lipid nanoparticle which may include a nanospecies, a polymer and an immunogen. (U.S. Publication No. 20120189700 and International Publication No. WO2012099805; each of which is herein incorporated by reference in their entirety). The polymer may

encapsulate the nanospecies or partially encapsulate the nanospecies. The immunogen may be a recombinant protein, a modified RNA described herein. In one embodiment, the lipid nanoparticle may be formulated for use in a vaccine such as, but not limited to, against a pathogen.

[00509] Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limited to, oral (e.g., the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (e.g., stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (e.g., nasal, pharyngeal, tracheal and bronchial membranes), genital (e.g., vaginal, cervical and urethral membranes).

Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers. Mucus is continuously secreted, shed, discarded or digested and recycled so most of the trapped particles may be removed from the mucosal tissue within seconds or within a few hours. Large polymeric nanoparticles (200nm -500nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai et al. PNAS 2007 104(5): 1482-487; Lai et al. Adv Drug Deliv Rev. 2009 61(2): 158-171; each of which is herein incorporated by reference in their entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT).

[00510] The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (i.e. a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-limiting examples of specific polymers include poly(caprolactone)

(PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a co-polymer such as, but not limited to, a block co-polymer, and (poly(ethylene glycol))-(poly(propylene oxide))- (poly(ethylene glycol)) triblock copolymer (see US Publication 20120121718 and US Publication 20100003337; each of which is herein incorporated by reference in their entirety). The co-polymer may be a polymer that is generally regarded as safe (GRAS) and the formation of the lipid nanoparticle may be in such a way that no new chemical entities are created. For example, the lipid nanoparticle may comprise poloxamers coating PLGA nanoparticles without forming new chemical entities which are still able to

rapidly penetrate human mucus (Yang et al. *Angew. Chem. Int. Ed.* 2011 50:2597-2600; herein incorporated by reference in its entirety).

[00511] The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic component of other surfactants (e.g., sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains).

[00512] The lipid nanoparticle engineered to penetrate mucus may include surface altering agents such as, but not limited to, polynucleotides, modified nucleic acids, enhanced modified RNA, ribonucleic acids, anionic protein (e.g., bovine serum albumin), surfactants (e.g., cationic surfactants such as for example dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (e.g., cyclodextrin), nucleic acids, polymers (e.g., heparin, polyethylene glycol and poloxamer), mucolytic agents (e.g., N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocysteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β 4 dornase alfa, neltexine, erdosteine) and various DNases including rhDNase.. The surface altering agent may be embedded or enmeshed in the particle's surface or disposed (e.g., by coating, adsorption, covalent linkage, or other process) on the surface of the lipid nanoparticle. (see US Publication 20100215580 and US Publication 20080166414; each of which is herein incorporated by reference in their entirety).

[00513] The mucus penetrating lipid nanoparticles may comprise at least one polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein. The modified nucleic acids, enhanced modified RNA or ribonucleic acids may be encapsulated in the lipid nanoparticle and/or disposed on the surface of the particle. The polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be covalently coupled to the lipid nanoparticle. Formulations of mucus penetrating lipid nanoparticles may comprise a plurality of nanoparticles. Further, the formulations may contain particles which may interact with the mucus and alter the structural and/or adhesive properties of the surrounding mucus to decrease mucoadhesion

which may increase the delivery of the mucus penetrating lipid nanoparticles to the mucosal tissue.

[00514] In one embodiment, the polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids is formulated as a lipoplex, such as, without limitation, the ATUPLEX™ system, the DACC system, the DBTC system and other siPvNA-lipoplex technology from Silence Therapeutics (London, United Kingdom), STEMFECT™ from STEMGENT® (Cambridge, MA), and polyethylenimine (PEI) or protamine-based targeted and non-targeted delivery of nucleic acids (Aleku et al. Cancer Res. 2008 68:9788-9798; Strumberg et al. Int J Clin Pharmacol Ther 2012 50:76-78; Santel et al, Gene Ther 2006 13:1222-1234; Santel et al, Gene Ther 2006 13:1360-1370; Gutbier et al, Pulm Pharmacol. Ther. 2010 23:334-344; Kaufmann et al. Microvasc Res 2010 80:286-293; Weide et al. J Immunother. 2009 32:498-507; Weide et al. J Immunother. 2008 31:180-188; Pascolo Expert Opin. Biol. Ther. 4:1285-1294; Fotin-Mleczek et al., 2011 J. Immunother. 34:1-15; Song et al., Nature Biotechnol. 2005, 23:709-717; Peer et al, Proc Natl Acad Sci U S A. 2007 6;104:4095-4100; deFougero Ues Hum Gene Ther. 2008 19:125-132; all of which are incorporated herein by reference in its entirety).

[00515] In one embodiment such formulations may also be constructed or compositions altered such that they passively or actively are directed to different cell types *in vivo*, including but not limited to hepatocytes, immune cells, tumor cells, endothelial cells, antigen presenting cells, and leukocytes (Akinc et al. Mol Ther. 2010 18:1357-1364; Song et al, Nat Biotechnol. 2005 23:709-717; Judge et al, J Clin Invest. 2009 119:661-673; Kaufmann et al, Microvasc Res 2010 80:286-293; Santel et al, Gene Ther 2006 13:1222-1234; Santel et al, Gene Ther 2006 13:1360-1370; Gutbier et al, Pulm Pharmacol. Ther. 2010 23:334-344; Basha et al, Mol. Ther. 2011 19:2186-2200; Fenske and Cullis, Expert Opin Drug Deliv. 2008 5:25-44; Peer et al, Science. 2008 319:627-630; Peer and Lieberman, Gene Ther. 2011 18:127-133; all of which are incorporated herein by reference in its entirety). One example of passive targeting of formulations to liver cells includes the DLin-DMA, DLin-KC2-DMA and MC3-based lipid nanoparticle formulations which have been shown to bind to apolipoprotein E and promote binding and uptake of these formulations into hepatocytes *in vivo* (Akinc et al.

Mol Ther. 2010 18:1357-1364; herein incorporated by reference in its entirety).

Formulations can also be selectively targeted through expression of different ligands on their surface as exemplified by, but not limited by, folate, transferrin, N-acetylgalactosamine (GalNAc), and antibody targeted approaches (Kolhatkar et al., Curr Drug Discov Technol. 2011 8:197-206; Musacchio and Torchilin, *Front Biosci.* 2011 16:1388-1412; Yu et al, Mol Membr Biol. 2010 27:286-298; Patil et al, Crit Rev Ther Drug Carrier Syst. 2008 25:1-61; Benoit et al., Biomacromolecules. 2011 12:2708-2714; Zhao et al, Expert Opin Drug Deliv. 2008 5:309-319; Akinc et al, Mol Ther. 2010 18:1357-1364; Srinivasan et al, Methods Mol Biol. 2012 820:105-116; Ben-Arie et al, Methods Mol Biol. 2012 757:497-507; Peer 2010 J Control Release. 20:63-68; Peer et al, Proc Natl Acad Sci U S A. 2007 104:4095-4100; Kim et al, Methods Mol Biol. 2011 721:339-353; Subramanya et al, Mol Ther. 2010 18:2028-2037; Song et al, Nat Biotechnol. 2005 23:709-717; Peer et al, Science. 2008 319:627-630; Peer and Lieberman, Gene Ther. 2011 18:1127-1133; all of which are incorporated herein by reference in its entirety)..

[00516] In one embodiment, the polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids is formulated as a solid lipid nanoparticle. A solid lipid nanoparticle (SLN) may be spherical with an average diameter between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic molecules and may be stabilized with surfactants and/or emulsifiers. In a further embodiment, the lipid nanoparticle may be a self-assembly lipid-polymer nanoparticle (see Zhang et al., ACS Nano, 2008, 2 (8), pp 1696-1702; herein incorporated by reference in its entirety).

[00517] Liposomes, lipoplexes, or lipid nanoparticles may be used to improve the efficacy of polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids directed protein production as these formulations may be able to increase cell transfection by the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids; and/or increase the translation of encoded protein. One such example involves the use of lipid encapsulation to enable the effective systemic delivery of polyplex plasmid DNA (Heyes et al, Mol Ther. 2007 15:713-720; herein incorporated by reference in its entirety). The liposomes, lipoplexes, or lipid

nanoparticles may also be used to increase the stability of the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids.

[00518] In one embodiment, the polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention can be formulated for controlled release and/or targeted delivery. As used herein, "controlled release" refers to a pharmaceutical composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome. In one embodiment, the polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be encapsulated into a delivery agent described herein and/or known in the art for controlled release and/or targeted delivery. As used herein, the term "encapsulate" means to enclose, surround or encase. As it relates to the formulation of the compounds of the invention, encapsulation may be substantial, complete or partial. The term "substantially encapsulated" means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.9 or greater than 99.999% of the pharmaceutical composition or compound of the invention may be enclosed, surrounded or encased within the delivery agent. "Partially encapsulation" means that less than 10, 10, 20, 30, 40 50 or less of the pharmaceutical composition or compound of the invention may be enclosed, surrounded or encased within the delivery agent. Advantageously, encapsulation may be determined by measuring the escape or the activity of the pharmaceutical composition or compound of the invention using fluorescence and/or electron micrograph. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the pharmaceutical composition or compound of the invention are encapsulated in the delivery agent.

[00519] In another embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be encapsulated into a lipid nanoparticle or a rapidly eliminating lipid nanoparticle and the lipid nanoparticles or a rapidly eliminating lipid nanoparticle may then be encapsulated into a polymer, hydrogel and/or surgical sealant described herein and/or known in the art. As a non-limiting example, the polymer, hydrogel or surgical sealant may be PLGA, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, FL), HYLENEX® (Halozyme Therapeutics, San Diego CA), surgical sealants such as fibrinogen polymers (Ethicon

Inc. Cornelia, GA), TISSELL® (Baxter International, Inc Deerfield, IL), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, IL).

[00520] In one embodiment, the lipid nanoparticle may be encapsulated into any polymer or hydrogel known in the art which may form a gel when injected into a subject. As another non-limiting example, the lipid nanoparticle may be encapsulated into a polymer matrix which may be biodegradable.

[00521] In one embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids formulation for controlled release and/or targeted delivery may also include at least one controlled release coating. Controlled release coatings include, but are not limited to, OPADRY®, polyvinylpyrrolidone/vinyl acetate copolymer, polyvinylpyrrolidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, EUDRAGIT RL®, EUDRAGIT RS® and cellulose derivatives such as ethylcellulose aqueous dispersions (AQUACOAT® and SURELEASE®).

[00522] In one embodiment, the controlled release and/or targeted delivery formulation may comprise at least one degradable polyester which may contain polycationic side chains. Degradable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another embodiment, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

[00523] In one embodiment, the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention may be encapsulated in a therapeutic nanoparticle. Therapeutic nanoparticles may be formulated by methods described herein and known in the art such as, but not limited to, International Pub Nos. WO20 10005740, WO2010030763, WO2010005721, WO2010005723, WO2012054923, US Pub. Nos. US201 10262491, US20100104645, US20100087337, US20100068285, US20 110274759, US20100068286, and US Pat No. 8,206,747; each of which is herein incorporated by reference in their entirety. In another embodiment, therapeutic polymer nanoparticles may be identified by the methods described in US Pub No. US20 120 140790, herein incorporated by reference in its entirety.

[00524] In one embodiment, the therapeutic nanoparticle may be formulated for sustained release. As used herein, "sustained release" refers to a pharmaceutical composition or compound that conforms to a release rate over a specific period of time. The period of time may include, but is not limited to, hours, days, weeks, months and years. As a non-limiting example, the sustained release nanoparticle may comprise a polymer and a therapeutic agent such as, but not limited to, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention (see International Pub No. 2010075072 and US Pub No. US20 1002 16804 and US201 10217377, each of which is herein incorporated by reference in their entirety).

[00525] In one embodiment, the therapeutic nanoparticles may be formulated to be target specific. As a non-limiting example, the thereapeutic nanoparticles may include a corticosteroid (see International Pub. No. WO201 1084518). In one embodiment, the therapeutic nanoparticles may be formulated to be cancer specific. As a non-limiting example, the therapeutic nanoparticles may be formulated in nanoparticles described in International Pub No. WO2008121949, WO2010005726, WO2010005725, WO201 1084521 and US Pub No. US20 100069426, US20120004293 and US20100104655, each of which is herein incorporated by reference in their entirety.

[00526] In one embodiment, the nanoparticles of the present invention may comprise a polymeric matrix. As a non-limiting example, the nanoparticle may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester) or combinations thereof.

[00527] In one embodiment, the diblock copolymer may include PEG in combination with a polymer such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates,

polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester) or combinations thereof.

[00528] In one embodiment, the therapeutic nanoparticle comprises a diblock copolymer. As a non-limiting example the therapeutic nanoparticle comprises a PLGA-PEG block copolymer (see US Pub. No. US20120004293 and US Pat No. 8,236,330, each of which is herein incorporated by reference in their entirety). In another non-limiting example, the therapeutic nanoparticle is a stealth nanoparticle comprising a diblock copolymer of PEG and PLA or PEG and PLGA (see US Pat No 8,246,968, herein incorporated by reference in its entirety).

[00529] In one embodiment, the therapeutic nanoparticle may comprise at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

[00530] In one embodiment, the therapeutic nanoparticles may comprise at least one cationic polymer described herein and/or known in the art.

[00531] In one embodiment, the therapeutic nanoparticles may comprise at least one amine-containing polymer such as, but not limited to polylysine, polyethylene imine, poly(amidoamine) dendrimers and combinations thereof.

[00532] In one embodiment, the therapeutic nanoparticles may comprise at least one degradable polyester which may contain polycationic side chains. Degradable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another embodiment, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

[00533] In another embodiment, the therapeutic nanoparticle may include a conjugation of at least one targeting ligand.

[00534] In one embodiment, the therapeutic nanoparticle may be formulated in an aqueous solution which may be used to target cancer (see International Pub No.

WO201 1084513 and US Pub No. US201 10294717, each of which is herein incorporated by reference in their entirety).

[00535] In one embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be encapsulated in, linked to and/or associated with synthetic nanocarriers. The synthetic nanocarriers may be formulated using methods known in the art and/or described herein. As a non-limiting example, the synthetic nanocarriers may be formulated by the methods described in International Pub Nos. WO20 10005740, WO20 10030763 and US Pub. Nos. US201 10262491, US20100104645 and US20100087337, each of which is herein incorporated by reference in their entirety. In another embodiment, the synthetic nanocarrier formulations may be lyophilized by methods described in International Pub. No. WO201 1072218 and US Pat No. 8,21 1,473; each of which is herein incorporated by reference in their entirety.

[00536] In one embodiment, the synthetic nanocarriers may contain reactive groups to release the modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein (see International Pub. No. WO20120952552 and US Pub No. US20120171229, each of which is herein incorporated by reference in their entirety).

[00537] In one embodiment, the synthetic nanocarriers may contain an immunostimulatory agent to enhance the immune response from delivery of the synthetic nanocarrier. As a non-limiting example, the synthetic nanocarrier may comprise a Th1 immunostimulatory agent which may enhance a Th1 -based response of the immune system (see International Pub No. WO2010123569 and US Pub. No. US201 10223201, each of which is herein incorporated by reference in its entirety).

[00538] In one embodiment, the synthetic nanocarriers may be formulated for targeted release. In one embodiment, the synthetic nanocarrier is formulated to release the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids at a specified pH and/or after a desired time interval. As a non-limiting example, the synthetic nanoparticle may be formulated to release the modified nucleic acids, enhanced modified RNA or ribonucleic acids after 24 hours and/or at a pH of 4.5 (see International Pub. Nos. WO2010138193 and WO2010138194 and US Pub Nos. US201 10020388 and US201 10027217, each of which is herein incorporated by reference in their entirety).

[00539] In one embodiment, the synthetic nanocarriers may be formulated for controlled and/or sustained release of the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein. As a non-limiting example, the synthetic nanocarriers for sustained release may be formulated by methods known in the art, described herein and/or as described in International Pub No. WO2010138192 and US Pub No. 20100303850, each of which is herein incorporated by reference in their entirety.

[00540] In one embodiment, the synthetic nanocarrier may be formulated for use as a vaccine. In one embodiment, the synthetic nanocarrier may encapsulate at least one modified nucleic acids, enhanced modified RNA or ribonucleic acids which encodes at least one antigen. As a non-limiting example, the synthetic nanocarrier may include at least one antigen and an excipient for a vaccine dosage form (see International Pub No. WO201 1150264 and US Pub No. US201 10293723, each of which is herein incorporated by reference in their entirety). As another non-limiting example, a vaccine dosage form may include at least two synthetic nanocarriers with the same or different antigens and an excipient (see International Pub No. WO201 1150249 and US Pub No. US201 10293701, each of which is herein incorporated by reference in their entirety). The vaccine dosage form may be selected by methods described herein, known in the art and/or described in International Pub No. WO201 1150258 and US Pub No. US20120027806, each of which is herein incorporated by reference in their entirety).

[00541] In one embodiment, the synthetic nanocarrier may comprise at least one polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids which encodes at least one adjuvant. In another embodiment, the synthetic nanocarrier may comprise at least one modified nucleic acids, enhanced modified RNA or ribonucleic acids and an adjuvant. As a non-limiting example, the synthetic nanocarrier comprising and adjuvant may be formulated by the methods described in International Pub No. WO201 1150240 and US Pub No. US201 10293700, each of which is herein incorporated by reference in its entirety.

[00542] In one embodiment, the synthetic nanocarrier may encapsulate at least one polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids which encodes a peptide, fragment or region from a virus. As a non-limiting example,

the synthetic nanocarrier may include, but is not limited to, the nanocarriers described in International Pub No. WO20 12024621, WO201202629, WO2012024632 and US Pub No. US201200641 10, US20120058153 and US20120058154, each of which is herein incorporated by reference in their entirety.

Polymers, Biodegradable Nanoparticles, and Core-Shell Nanoparticles

[00543] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention can be formulated using natural and/or synthetic polymers. Non-limiting examples of polymers which may be used for delivery include, but are not limited to, Dynamic POLYCONJUGATE™ formulations from MIRUS® Bio (Madison, WI) and Roche Madison (Madison, WI), PHASERX™ polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGY™ (Seattle, WA), DMRI/DOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, CA), chitosan, cyclodextrin from Calando Pharmaceuticals (Pasadena, CA), dendrimers and poly(lactic-co-glycolic acid) (PLGA) polymers, RONDEL™ (RNAi/Oligonucleotide Nanoparticle Delivery) polymers (Arrowhead Research Corporation, Pasadena, CA) and pH responsive co-block polymers such as, but not limited to, PHASERX™ (Seattle, WA).

[00544] A non-limiting example of PLGA formulations include, but are not limited to, PLGA injectable depots (e.g., ELIGARD® which is formed by dissolving PLGA in 66% N-methyl-2-pyrrolidone (NMP) and the remainder being aqueous solvent and leuprolide. Once injected, the PLGA and leuprolide peptide precipitates into the subcutaneous space).

[00545] Many of these polymer approaches have demonstrated efficacy in delivering oligonucleotides *in vivo* into the cell cytoplasm (reviewed in deFougerolles *Hum Gene Ther.* 2008 19:125-132; herein incorporated by reference in its entirety). Two polymer approaches that have yielded robust *in vivo* delivery of nucleic acids, in this case with small interfering RNA (siRNA), are dynamic polyconjugates and cyclodextrin-based nanoparticles. The first of these delivery approaches uses dynamic polyconjugates and has been shown *in vivo* in mice to effectively deliver siRNA and silence endogenous target mRNA in hepatocytes (Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887). This particular approach is a multicomponent polymer system whose key

features include a membrane-active polymer to which nucleic acid, in this case siRNA, is covalently coupled via a disulfide bond and where both PEG (for charge masking) and *N*-acetylgalactosamine (for hepatocyte targeting) groups are linked via pH-sensitive bonds (Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887). On binding to the hepatocyte and entry into the endosome, the polymer complex disassembles in the low-pH environment, with the polymer exposing its positive charge, leading to endosomal escape and cytoplasmic release of the siRNA from the polymer. Through replacement of the Δ -acetylgalactosamine group with a mannose group, it was shown one could alter targeting from asialoglycoprotein receptor-expressing hepatocytes to sinusoidal endothelium and Kupffer cells. Another polymer approach involves using transferrin-targeted cyclodextrin-containing polycation nanoparticles. These nanoparticles have demonstrated targeted silencing of the *EWS-FLI1* gene product in transferrin receptor-expressing Ewing's sarcoma tumor cells (Hu-Lieskovan *et al.*, Cancer Res. 2005 65: 8984-8982) and siRNA formulated in these nanoparticles was well tolerated in non-human primates (Heidel *et al.*, Proc Natl Acad Sci USA 2007 104:5715-21). Both of these delivery strategies incorporate rational approaches using both targeted delivery and endosomal escape mechanisms.

[00546] The polymer formulation can permit the sustained or delayed release of polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids (e.g., following intramuscular or subcutaneous injection). The altered release profile for the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids can result in, for example, translation of an encoded protein over an extended period of time. The polymer formulation may also be used to increase the stability of the polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids. Biodegradable polymers have been previously used to protect nucleic acids other than modified nucleic acids, enhanced modified RNA or ribonucleic acids from degradation and been shown to result in sustained release of payloads in vivo (Rozema et al., Proc Natl Acad Sci U S A. 2007 104:12982-12887; Sullivan et al, Expert Opin Drug Deliv. 2010 7:1433-1446; Convertine et al, Biomacromolecules. 2010 Oct 1; Chu et al, Acc Chem Res. 2012 Jan 13; Manganiello et al., Biomaterials. 2012 33:2301-2309; Benoit et al., Biomacromolecules. 2011 12:2708-2714; Singha et al., Nucleic Acid Ther. 2011

2:133-147; deFougerolles Hum Gene Ther. 2008 19:125-132; Schaffert and Wagner, Gene Ther. 2008 16:1 131-1 138; Chaturvedi et al, Expert Opin Drug Deliv. 2011 8:1455-1468; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464:1067-1070; herein incorporated by reference in its entirety).

[00547] In one embodiment, the pharmaceutical compositions may be sustained release formulations. In a further embodiment, the sustained release formulations may be for subcutaneous delivery. Sustained release formulations may include, but are not limited to, PLGA microspheres, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, FL), HYLENEX® (Halozyme Therapeutics, San Diego CA), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, GA), TISSELL® (Baxter International, Inc Deerfield, IL), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, IL).

[00548] As a non-limiting example modified mRNA may be formulated in PLGA microspheres by preparing the PLGA microspheres with tunable release rates (e.g., days and weeks) and encapsulating the modified mRNA in the PLGA microspheres while maintaining the integrity of the modified mRNA during the encapsulation process. EVAc are non-biodegradable, biocompatible polymers which are used extensively in pre-clinical sustained release implant applications (e.g., extended release products Ocusert a pilocarpine ophthalmic insert for glaucoma or progestasert a sustained release progesterone intrauterine device; transdermal delivery systems Testoderm, Duragesic and Selegiline; catheters). Poloxamer F-407 NF is a hydrophilic, non-ionic surfactant triblock copolymer of polyoxyethylene-polyoxypropylene-polyoxyethylene having a low viscosity at temperatures less than 5°C and forms a solid gel at temperatures greater than 15°C. PEG-based surgical sealants comprise two synthetic PEG components mixed in a delivery device which can be prepared in one minute, seals in 3 minutes and is reabsorbed within 30 days. GELSITE® and natural polymers are capable of in-situ gelation at the site of administration. They have been shown to interact with protein and peptide therapeutic candidates through ionic interaction to provide a stabilizing effect.

[00549] Polymer formulations can also be selectively targeted through expression of different ligands as exemplified by, but not limited by, folate, transferrin, and N-acetylgalactosamine (GalNAc) (Benoit et al., Biomacromolecules. 2011 12:2708-2714;

Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464:1067-1070; each of which is herein incorporated by reference in its entirety).

[00550] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be formulated with or in a polymeric compound. The polymer may include at least one polymer such as, but not limited to, polyethenes, polyethylene glycol (PEG), poly(L-lysine)(PLL), PEG grafted to PLL, cationic lipopolymer, biodegradable cationic lipopolymer, polyethyleneimine (PEI), cross-linked branched poly(alkylene imines), a polyamine derivative, a modified poloxamer, a biodegradable polymer, biodegradable block copolymer, biodegradable random copolymer, biodegradable polyester copolymer, biodegradable polyester block copolymer, biodegradable polyester block random copolymer, linear biodegradable copolymer, poly[α-(4-aminobutyl)-L-glycolic acid] (PAGA), biodegradable cross-linked cationic multi-block copolymers, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), acrylic polymers, amine-containing polymers or combinations thereof .

[00551] As a non-limiting example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be formulated with the polymeric compound of PEG grafted with PLL as described in U.S. Pat. No. 6,177,274 herein incorporated by reference in its entirety. The formulation may be used for transfecting cells *in vitro* or for *in vivo* delivery of the modified nucleic acids, enhanced modified RNA or ribonucleic acids. In another example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be suspended in a solution or medium with a cationic polymer, in a dry pharmaceutical composition or in a solution that is capable of being dried as described in U.S. Pub. Nos. 20090042829 and 20090042825 each of which are herein incorporated by reference in their entireties.

[00552] As another non-limiting example the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be formulated with a PLGA-PEG block copolymer (see US Pub. No. US20120004293 and US Pat No. 8,236,330, each of which are herein incorporated by reference in their entireties). As a non-limiting example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be formulated with a diblock copolymer of PEG and PLA or PEG and PLGA (see US Pat No 8,246,968, herein incorporated by reference in its entirety).

[00553] A polyamine derivative may be used to deliver nucleic acids or to treat and/or prevent a disease or to be included in an implantable or injectable device (U.S. Pub. No. 20100260817 herein incorporated by reference in its entirety). As a non-limiting example, a pharmaceutical composition may include the modified nucleic acids, enhanced modified RNA or ribonucleic acids and the polyamine derivative described in U.S. Pub. No. 20100260817 (the contents of which are incorporated herein by reference in its entirety).

[00554] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be formulated with at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

[00555] In one embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention may be formulated with at least one polymer described in International Publication Nos. WO201 1115862, WO2012082574 and WO2012068187, each of which are herein incorporated by reference in their entireties. In another embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention may be formulated with a polymer of formula Z as described in WO201 1115862, herein incorporated by reference in its entirety. In yet another embodiment, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be formulated with a polymer of formula Z, Z' or Z" as described in WO2012082574 or

WO2012068187, each of which are herein incorporated by reference in their entireties. The polymers formulated with the modified RNA of the present invention may be synthesized by the methods described in WO2012082574 or WO2012068187, each of which are herein incorporated by reference in their entireties.

[00556] Formulations of polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may include at least one amine-containing polymer such as, but not limited to polylysine, polyethylene imine, poly(amidoamine) dendrimers or combinations thereof.

[00557] For example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be formulated in a pharmaceutical compound including a poly(alkylene imine), a biodegradable cationic lipopolymer, a biodegradable block copolymer, a biodegradable polymer, or a biodegradable random copolymer, a biodegradable polyester block copolymer, a biodegradable polyester polymer, a biodegradable polyester random copolymer, a linear biodegradable copolymer, PAGA, a biodegradable cross-linked cationic multi-block copolymer or combinations thereof. The biodegradable cationic lipopolymer may be made by methods known in the art and/or described in U.S. Pat. No. 6,696,038, U.S. App. Nos. 20030073619 and 20040142474 each of which is herein incorporated by reference in their entireties. The poly(alkylene imine) may be made using methods known in the art and/or as described in U.S. Pub. No. 20100004315, herein incorporated by reference in its entirety. The biodegradable polymer, biodegradable block copolymer, the biodegradable random copolymer, biodegradable polyester block copolymer, biodegradable polyester polymer, or biodegradable polyester random copolymer may be made using methods known in the art and/or as described in U.S. Pat. Nos. 6,517,869 and 6,267,987, the contents of which are each incorporated herein by reference in its entirety. The linear biodegradable copolymer may be made using methods known in the art and/or as described in U.S. Pat. No. 6,652,886. The PAGA polymer may be made using methods known in the art and/or as described in U.S. Pat. Nos. 6,217,912 herein incorporated by reference in its entirety. The PAGA polymer may be copolymerized to form a copolymer or block copolymer with polymers such as but not limited to, poly-L-lysine, polyarginine, polyornithine, histones, avidin, protamines, polylactides and

poly(lactide-co-glycolides). The biodegradable cross-linked cationic multi-block copolymers may be made by methods known in the art and/or as described in U.S. Pat. No. 8,057,821 or U.S. Pub. No. 2012009145 each of which are herein incorporated by reference in their entireties. For example, the multi-block copolymers may be synthesized using linear polyethyleneimine (LPEI) blocks which have distinct patterns as compared to branched polyethyleneimines. Further, the composition or pharmaceutical composition may be made by the methods known in the art, described herein, or as described in U.S. Pub. No. 20100004315 or U.S. Pat. Nos. 6,267,987 and 6,217,912 each of which are herein incorporated by reference in their entireties.

[00558] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be formulated with at least one degradable polyester which may contain polycationic side chains. Degradable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another embodiment, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

[00559] In one embodiment, the polymers described herein may be conjugated to a lipid-terminating PEG. As a non-limiting example, PLGA may be conjugated to a lipid-terminating PEG forming PLGA-DSPE-PEG. As another non-limiting example, PEG conjugates for use with the present invention are described in International Publication No. WO2008 103276, herein incorporated by reference in its entirety.

[00560] In one embodiment, the polynucleotides, modified RNA described herein may be conjugated with another compound. Non-limiting examples of conjugates are described in US Patent Nos. 7,964,578 and 7,833,992, each of which are herein incorporated by reference in their entireties. In another embodiment, modified RNA of the present invention may be conjugated with conjugates of formula 1-122 as described in US Patent Nos. 7,964,578 and 7,833,992, each of which are herein incorporated by reference in their entireties.

[00561] As described in U.S. Pub. No. 201000043 13, herein incorporated by reference in its entirety, a gene delivery composition may include a nucleotide sequence and a poloxamer. For example, the polynucleotide, modified nucleic acids, enhanced modified

RNA or ribonucleic acids of the present invention may be used in a gene delivery composition with the poloxamer described in U.S. Pub. No. 20100004313.

[00562] In one embodiment, the polymer formulation of the present invention may be stabilized by contacting the polymer formulation, which may include a cationic carrier, with a cationic lipopolymer which may be covalently linked to cholesterol and polyethylene glycol groups. The polymer formulation may be contacted with a cationic lipopolymer using the methods described in U.S. Pub. No. 20090042829 herein incorporated by reference in its entirety. The cationic carrier may include, but is not limited to, polyethylenimine, poly(trimethylenimine), poly(tetramethylenimine), polypropylenimine, aminoglycoside-polyamine, dideoxy-diamino-b-cyclodextrin, spermine, spermidine, poly(2-dimethylamino)ethyl methacrylate, poly(lysine), poly(histidine), poly(arginine), cationized gelatin, dendrimers, chitosan, 1,2-Dioleoyl-3-Trimethylammonium-Propane (DOTAP), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolium chloride (DOTIM), 2,3-dioleoyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), 3B-[N—(N,N'-Dimethylaminoethane)-carbonyl]Cholesterol Hydrochloride (DC-Cholesterol HC1) diheptadecylamidoglycyl spermidine (DOGS), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC) and combinations thereof.

[00563] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention can also be formulated as a nanoparticle using a combination of polymers, lipids, and/or other biodegradable agents, such as, but not limited to, calcium phosphate. Components may be combined in a core-shell, hybrid, and/or layer-by-layer architecture, to allow for fine-tuning of the nanoparticle so to deliver the modified nucleic acids, enhanced modified RNA or ribonucleic acids may be enhanced (Wang et al, Nat Mater. 2006 5:791-796; Fuller et al, Biomaterials. 2008 29:1526-1532; DeKoker et al, Adv Drug Deliv Rev. 2011 63:748-761; Endres et al, Biomaterials. 2011 32:7721-7731; Su et al, Mol Pharm. 2011 Jun 6;8(3):774-87; herein incorporated by reference in its entirety).

[00564] Biodegradable calcium phosphate nanoparticles in combination with lipids and/or polymers have been shown to deliver polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids *in vivo*. In one embodiment, a lipid coated calcium phosphate nanoparticle, which may also contain a targeting ligand such as anisamide, may be used to deliver the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention. For example, to effectively deliver siRNA in a mouse metastatic lung model a lipid coated calcium phosphate nanoparticle was used (Li et al., J Contr Rel. 2010 142: 416-421; Li et al., J Contr Rel. 2012 158:108-114; Yang et al, Mol Ther. 2012 20:609-615). This delivery system combines both a targeted nanoparticle and a component to enhance the endosomal escape, calcium phosphate, in order to improve delivery of the siRNA.

[00565] In one embodiment, calcium phosphate with a PEG-polyanion block copolymer may be used to deliver polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids (Kazikawa et al., J Contr Rel. 2004 97:345-356; Kazikawa et al, J Contr Rel. 2006 111:368-370).

[00566] In one embodiment, a PEG-charge-conversional polymer (Pitella et al., Biomaterials. 2011 32:3106-3114) may be used to form a nanoparticle to deliver the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention. The PEG-charge-conversional polymer may improve upon the PEG-polyanion block copolymers by being cleaved into a polycation at acidic pH, thus enhancing endosomal escape.

[00567] The use of core-shell nanoparticles has additionally focused on a high-throughput approach to synthesize cationic cross-linked nanogel cores and various shells (Siegwart et al, Proc Natl Acad Sci U S A. 2011 108:12996-13001). The complexation, delivery, and internalization of the polymeric nanoparticles can be precisely controlled by altering the chemical composition in both the core and shell components of the nanoparticle. For example, the core-shell nanoparticles may efficiently deliver siRNA to mouse hepatocytes after they covalently attach cholesterol to the nanoparticle.

[00568] In one embodiment, a hollow lipid core comprising a middle PLGA layer and an outer neutral lipid layer containing PEG may be used to delivery of the polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present

invention. As a non-limiting example, in mice bearing a luciferase-expressing tumor, it was determined that the lipid-polymer-lipid hybrid nanoparticle significantly suppressed luciferase expression, as compared to a conventional lipoplex (Shi et al, *Angew Chem Int Ed.* 2011 50:7027-7031).

Peptides and Proteins

[00569] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention can be formulated with peptides and/or proteins in order to increase transfection of cells by the modified nucleic acids, enhanced modified RNA or ribonucleic acids. In one embodiment, peptides such as, but not limited to, cell penetrating peptides and proteins and peptides that enable intracellular delivery may be used to deliver pharmaceutical formulations. A non-limiting example of a cell penetrating peptide which may be used with the pharmaceutical formulations of the present invention includes a cell-penetrating peptide sequence attached to polycations that facilitates delivery to the intracellular space, e.g., HIV-derived TAT peptide, penetratins, transportans, or hCT derived cell-penetrating peptides (see, e.g., Caron et al, *Mol. Ther.* 3(3):310-8 (2001); Langel, *Cell-Penetrating Peptides: Processes and Applications* (CRC Press, Boca Raton FL, 2002); El-Andaloussi et al, *Curr. Pharm. Des.* 11(28):3597-611 (2003); and Deshayes et al, *Cell. Mol. Life Sci.* 62(16):1839-49 (2005), all of which are incorporated herein by reference). The compositions can also be formulated to include a cell penetrating agent, e.g., liposomes, which enhance delivery of the compositions to the intracellular space. Modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be complexed to peptides and/or proteins such as, but not limited to, peptides and/or proteins from Aileron Therapeutics (Cambridge, MA) and Permeon Biologies (Cambridge, MA) in order to enable intracellular delivery (Cronican et al., *ACS Chem. Biol.* 2010 5:747-752; McNaughton et al, *Proc. Natl. Acad. Sci. USA* 2009 106:6111-6116; Sawyer, *Chem Biol Drug Des.* 2009 73:3-6; Verdine and Hilinski, *Methods Enzymol.* 2012;503:3-33; all of which are herein incorporated by reference in its entirety).

[00570] In one embodiment, the cell-penetrating polypeptide may comprise a first domain and a second domain. The first domain may comprise a supercharged polypeptide. The second domain may comprise a protein-binding partner. As used herein,

"protein-binding partner" includes, but are not limited to, antibodies and functional fragments thereof, scaffold proteins, or peptides. The cell-penetrating polypeptide may further comprise an intracellular binding partner for the protein-binding partner. The cell-penetrating polypeptide may be capable of being secreted from a cell where the modified nucleic acids, enhanced modified RNA or ribonucleic acids may be introduced.

[00571] Formulations of the including peptides or proteins may be used to increase cell transfection by the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids, alter the biodistribution of the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids (e.g., by targeting specific tissues or cell types), and/or increase the translation of encoded protein.

Cells

[00572] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention can be transfected *ex vivo* into cells, which are subsequently transplanted into a subject. As non-limiting examples, the pharmaceutical compositions may include red blood cells to deliver modified RNA to liver and myeloid cells, virosomes to deliver modified RNA in virus-like particles (VLPs), and electroporated cells such as, but not limited to, from MAXCYTE® (Gaithersburg, MD) and from ERYTECH® (Lyon, France) to deliver modified RNA. Examples of use of red blood cells, viral particles and electroporated cells to deliver payloads other than polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids have been documented (Godfrin et al., Expert Opin Biol Ther. 2012 12:127-133; Fang et al, Expert Opin Biol Ther. 2012 12:385-389; Hu et al, Proc Natl Acad Sci U S A. 201 1 108:10980-10985; Lund et al, Pharm Res. 2010 27:400-420; Huckriede et al, J Liposome Res. 2007;17:39-47; Cusi, Hum Vaccin. 2006 2:1-7; de Jonge et al, Gene Ther. 2006 13:400-41 1; all of which are herein incorporated by reference in its entirety). The modified RNA may be delivered in synthetic VLPs synthesized by the methods described in International Pub No. WO201 1085231 and US Pub No. 201 10171248, each of which are herein incorporated by reference in their entireties.

[00573] Cell-based formulations of the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be used to ensure cell transfection (e.g., in the cellular carrier), alter the biodistribution of the modified nucleic

acids, enhanced modified RNA or ribonucleic acids (e.g., by targeting the cell carrier to specific tissues or cell types), and/or increase the translation of encoded protein.

Introduction into cells

[00574] A variety of methods are known in the art and suitable for introduction of nucleic acid into a cell, including viral and non-viral mediated techniques. Examples of typical non-viral mediated techniques include, but are not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion.

[00575] The technique of sonoporation, or cellular sonication, is the use of sound (e.g., ultrasonic frequencies) for modifying the permeability of the cell plasma membrane. Sonoporation methods are known to those in the art and are taught for example as it relates to bacteria in US Patent Publication 20100196983 and as it relates to other cell types in, for example, US Patent Publication 20100009424, each of which are incorporated herein by reference in their entirety.

[00576] Electroporation techniques are also well known in the art. In one embodiment, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be delivered by electroporation as described in Example 11.

Hyaluronidase

[00577] The intramuscular or subcutaneous localized injection of polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention can include hyaluronidase, which catalyzes the hydrolysis of hyaluronan. By catalyzing the hydrolysis of hyaluronan, a constituent of the interstitial barrier, hyaluronidase lowers the viscosity of hyaluronan, thereby increasing tissue permeability (Frost, Expert Opin. Drug Deliv. (2007) 4:427-440; herein incorporated by reference in its entirety). It is useful to speed their dispersion and systemic distribution of encoded proteins produced by transfected cells. Alternatively, the hyaluronidase can be used to increase the number of cells exposed to a modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention administered intramuscularly or subcutaneously.

Nanoparticle Mimics

[00578] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be encapsulated within and/or absorbed to a nanoparticle mimic. A nanoparticle mimic can mimic the delivery function organisms or particles such as, but not limited to, pathogens, viruses, bacteria, fungus, parasites, prions and cells. As a non-limiting example the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be encapsulated in a non-viron particle which can mimic the delivery function of a virus (see International Pub. No. WO2012006376 herein incorporated by reference in its entirety).

Nanotubes

[00579] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention can be attached or otherwise bound to at least one nanotube such as, but not limited to, rosette nanotubes, rosette nanotubes having twin bases with a linker, carbon nanotubes and/or single-walled carbon nanotubes, The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be bound to the nanotubes through forces such as, but not limited to, steric, ionic, covalent and/or other forces.

[00580] In one embodiment, the nanotube can release one or more polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids into cells. The size and/or the surface structure of at least one nanotube may be altered so as to govern the interaction of the nanotubes within the body and/or to attach or bind to the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids disclosed herein. In one embodiment, the building block and/or the functional groups attached to the building block of the at least one nanotube may be altered to adjust the dimensions and/or properties of the nanotube. As a non-limiting example, the length of the nanotubes may be altered to hinder the nanotubes from passing through the holes in the walls of normal blood vessels but still small enough to pass through the larger holes in the blood vessels of tumor tissue.

[00581] In one embodiment, at least one nanotube may also be coated with delivery enhancing compounds including polymers, such as, but not limited to, polyethylene glycol. In another embodiment, at least one nanotube and/or the modified mRNA may be mixed with pharmaceutically acceptable excipients and/or delivery vehicles.

[00582] In one embodiment, the polynucleotides or modified mRNA are attached and/or otherwise bound to at least one rosette nanotube. The rosette nanotubes may be formed by a process known in the art and/or by the process described in International Publication No. WO2012094304, herein incorporated by reference in its entirety. At least one modified mRNA may be attached and/or otherwise bound to at least one rosette nanotube by a process as described in International Publication No. WO20 12094304, herein incorporated by reference in its entirety, where rosette nanotubes or modules forming rosette nanotubes are mixed in aqueous media with at least one modified mRNA under conditions which may cause at least one modified mRNA to attach or otherwise bind to the rosette nanotubes.

Conjugates

[00583] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention include conjugates, such as a modified nucleic acids, enhanced modified RNA or ribonucleic acids covalently linked to a carrier or targeting group, or including two encoding regions that together produce a fusion protein (e.g., bearing a targeting group and therapeutic protein or peptide).

[00584] The conjugates of the invention include a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or globulin); an carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid, an oligonucleotide (e.g. an aptamer). Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolid) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

[00585] Representative U.S. patents that teach the preparation of polynucleotide conjugates, particularly to RNA, include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; each of which is herein incorporated by reference in their entireties.

[00586] In one embodiment, the conjugate of the present invention may function as a carrier for the polynucleotide, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention. The conjugate may comprise a cationic polymer such as, but not limited to, polyamine, polylysine, polyalkylenimine, and polyethylenimine which may be grafted to with poly(ethylene glycol). As a non-limiting example, the conjugate may be similar to the polymeric conjugate and the method of synthesizing the polymeric conjugate described in U.S. Pat. No. 6,586,524 herein incorporated by reference in its entirety.

[00587] The conjugates can also include targeting groups, e.g., a cell or tissue targeting agent, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B 12, biotin, an RGD peptide, an RGD peptide mimetic or an aptamer.

[00588] Targeting groups can be proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies e.g., an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell.

Targeting groups may also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, or aptamers. The ligand can be, for example, a lipopolysaccharide, or an activator of p38 MAP kinase.

[00589] The targeting group can be any ligand that is capable of targeting a specific receptor. Examples include, without limitation, folate, GalNAc, galactose, mannose, mannose-6P, aptamers, integrin receptor ligands, chemokine receptor ligands, transferrin, biotin, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL, and HDL ligands. In particular embodiments, the targeting group is an aptamer. The aptamer can be unmodified or have any combination of modifications disclosed herein.

[00590] In one embodiment, pharmaceutical compositions of the present invention may include chemical modifications such as, but not limited to, modifications similar to locked nucleic acids.

[00591] Representative U.S. Patents that teach the preparation of locked nucleic acid (LNA) such as those from Santaris, include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,670,461; 6,794,499; 6,998,484; 7,053,207; 7,084,125; and 7,399,845, each of which is herein incorporated by reference in its entirety.

[00592] Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found, for example, in Nielsen *et al.*, Science, 1991, 254, 1497-1500.

[00593] Some embodiments featured in the invention include modified nucleic acids, enhanced modified RNA or ribonucleic acids with phosphorothioate backbones and oligonucleosides with other modified backbones, and in particular $-\text{CH}_2-\text{NH}-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{O}-\text{CH}_2-$ [known as a methylene (methylimino) or MMI backbone], $-\text{CH}_2-\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{N}(\text{CH}_3)-\text{CH}_2-$ and $-\text{N}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-$ [wherein the native phosphodiester backbone is represented as $-\text{O}-\text{P}(\text{O})_2-\text{O}-\text{CH}_2-$] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some embodiments, the polynucleotides featured

herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

[00594] Modifications at the 2' position may also aid in delivery. Preferably, modifications at the 2' position are not located in a polypeptide-coding sequence, i.e., not in a translatable region. Modifications at the 2' position may be located in a 5'UTR, a 3'UTR and/or a tailing region. Modifications at the 2' position can include one of the following at the 2' position: H (i.e., 2'-deoxy); F; 0-, S-, or N-alkyl; 0-, S-, or N-alkenyl; 0-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_mO]_nCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. In other embodiments, the modified nucleic acids, enhanced modified RNA or ribonucleic acids include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3 , OCN, Cl, Br, CN, CF_3 , OCF_3 , $SOCH_3$, SO_2CH_3 , ONH_2 , NO_2 , N_3 , NH_2 , heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties, or a group for improving the pharmacodynamic properties, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O— $CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al*, *Helv. Chim. Acta*, 1995, 78:486-504) i.e., an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminoethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethyl (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O~ CH_2CH_2 ~O~ CH_2CH_2 ~N(CH_2)₂, also described in examples herein below. Other modifications include 2'-methoxy (2'- OCH_3), 2'-aminopropoxy (2'- $OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. Polynucleotides of the invention may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the

preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920 and each of which is herein incorporated by reference.

[00595] In still other embodiments, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids is covalently conjugated to a cell penetrating polypeptide. The cell-penetrating peptide may also include a signal sequence. The conjugates of the invention can be designed to have increased stability; increased cell transfection; and/or altered the biodistribution (e.g., targeted to specific tissues or cell types).

Self-Assembled Nucleic Acid Nanoparticles

[00596] Self-assembled nanoparticles have a well-defined size which may be precisely controlled as the nucleic acid strands may be easily reprogrammable. For example, the optimal particle size for a cancer-targeting nanodelivery carrier is 20-100 nm as a diameter greater than 20 nm avoids renal clearance and enhances delivery to certain tumors through enhanced permeability and retention effect. Using self-assembled nucleic acid nanoparticles a single uniform population in size and shape having a precisely controlled spatial orientation and density of cancer-targeting ligands for enhanced delivery. As a non-limiting example, oligonucleotide nanoparticles were prepared using programmable self-assembly of short DNA fragments and therapeutic siRNAs. These nanoparticles are molecularly identical with controllable particle size and target ligand location and density. The DNA fragments and siRNAs self-assembled into a one-step reaction to generate DNA/siRNA tetrahedral nanoparticles for targeted *in vivo* delivery. (Lee et al, Nature Nanotechnology 2012 7:389-393).

Excipients

[00597] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes, but are not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage

form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see *Remington: The Science and Practice of Pharmacy*, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference).. The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

[00598] In some embodiments, a pharmaceutically acceptable excipient may be at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient may be approved for use for humans and for veterinary use. In some embodiments, an excipient may be approved by United States Food and Drug Administration. In some embodiments, an excipient may be of pharmaceutical grade. In some embodiments, an excipient may meet the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00599] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in pharmaceutical formulations. The composition may also include excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents.

[00600] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and/or combinations thereof.

[00601] Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid,

guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, *etc.*, and/or combinations thereof.

[00602] Exemplary surface active agents and/or emulsifiers include, but are not limited to, natural emulsifiers (*e.g.* acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (*e.g.* bentonite [aluminum silicate] and VEEGUM[®] [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (*e.g.* stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (*e.g.* carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (*e.g.* carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (*e.g.* polyoxyethylene sorbitan monolaurate [TWEEN[®]20], polyoxyethylene sorbitan [TWEEN[®]60], polyoxyethylene sorbitan monooleate [TWEEN[®]80], sorbitan monopalmitate [SPAN[®]40], sorbitan monostearate [Span[®]60], sorbitan tristearate [Span[®]65], glyceryl monooleate, sorbitan monooleate [SPAN[®]80]), polyoxyethylene esters (*e.g.* polyoxyethylene monostearate [MYRJ[®]45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL[®]), sucrose fatty acid esters, polyethylene glycol fatty acid esters (*e.g.* CREMOPHOR[®]), polyoxyethylene ethers, (*e.g.* polyoxyethylene lauryl ether [BRIJ[®]30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLUORINC[®] F 68, POLOXAMER[®] 188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, *etc.* and/or combinations thereof.

[00603] Exemplary binding agents include, but are not limited to, starch (*e.g.* cornstarch and starch paste); gelatin; sugars (*e.g.* sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol,); natural and synthetic gums (*e.g.* acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (VEEGUM®), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; *etc.*; and combinations thereof.

[00604] Exemplary preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Exemplary antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Exemplary antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Exemplary alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Exemplary acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic

acid, dehydroacetic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, GLYDANT PLUS®, PHENONIP®, methylparaben, GERMALL® 115, GERMABEN® II, NEOLONE™, KATHON™, and/or EUXYL®.

[00605] Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, calcium gluceptate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, *etc.*, and/or combinations thereof.

[00606] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, *etc.*, and combinations thereof.

[00607] Exemplary oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mint, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasanqua, savoury,

sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and/or combinations thereof.

[00608] Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

Delivery

[00609] The present disclosure encompasses the delivery of polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids for any of therapeutic, pharmaceutical, diagnostic or imaging by any appropriate route taking into consideration likely advances in the sciences of drug delivery. Delivery may be naked or formulated.

Naked Delivery

[00610] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention may be delivered to a cell naked. As used herein in, "naked" refers to delivering polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids free from agents which promote transfection. For example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids delivered to the cell may contain no modifications. The naked polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids may be delivered to the cell using routes of administration known in the art and described herein.

Formulated Delivery

[00611] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention may be formulated, using the methods described herein. The formulations may contain polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids which may be modified and/or unmodified. The formulations may further include, but are not limited to, cell penetration agents, a pharmaceutically acceptable carrier, a delivery agent, a bioerodible or biocompatible polymer, a solvent, and a sustained-release delivery depot. The formulated

polynucleotides, modified nucleic acids or enhanced modified nucleic acids may be delivered to the cell using routes of administration known in the art and described herein.

[00612] The compositions may also be formulated for direct delivery to an organ or tissue in any of several ways in the art including, but not limited to, direct soaking or bathing, via a catheter, by gels, powder, ointments, creams, gels, lotions, and/or drops, by using substrates such as fabric or biodegradable materials coated or impregnated with the compositions, and the like.

[00613] In certain embodiments, the formulations include one or more cell penetration agents, e.g., transfection agents. In one specific embodiment, a ribonucleic acid is mixed or admixed with a transfection agent (or mixture thereof) and the resulting mixture is employed to transfect cells. Preferred transfection agents are cationic lipid compositions, particularly monovalent and polyvalent cationic lipid compositions, more particularly "LIPOFECTIN," "LIPOFECTACE," "LIPOFECTAMINE," "CELLFECTIN," DMRIE-C, DMRIE, DOTAP, DOSPA, and DOSPER, and dendrimer compositions, particularly G5-G10 dendrimers, including dense star dendrimers, PAMAM dendrimers, grafted dendrimers, and dendrimers known as dendrigrafts and "SUPERFECT." In a second specific transfection method, a ribonucleic acid is conjugated to a nucleic acid-binding group, for example a polyamine and more particularly a spermine, which is then introduced into the cell or admixed with a transfection agent (or mixture thereof) and the resulting mixture is employed to transfect cells. In a third specific embodiment, a mixture of one or more transfection-enhancing peptides, proteins, or protein fragments, including fusagenic peptides or proteins, transport or trafficking peptides or proteins, receptor-ligand peptides or proteins, or nuclear localization peptides or proteins and/or their modified analogs (e.g., spermine modified peptides or proteins) or combinations thereof are mixed with and complexed with a ribonucleic acid to be introduced into a cell, optionally being admixed with transfection agent and the resulting mixture is employed to transfect cells. Further, a component of a transfection agent (e.g., lipids, cationic lipids or dendrimers) is covalently conjugated to selected peptides, proteins, or protein fragments directly or via a linking or spacer group. Of particular interest in this embodiment are peptides or proteins that are fusagenic, membrane-permeabilizing, transport or trafficking, or which function for cell-targeting. The peptide- or protein-

transfection agent complex is combined with a ribonucleic acid and employed for transfection.

[00614] In certain embodiments, the formulations include a pharmaceutically acceptable carrier that causes the effective amount of polynucleotide, modified nucleic acid, or ribonucleic acid to be substantially retained in a target tissue containing the cell.

Administration

[00615] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to enteral, gastroenteral, epidural, oral, transdermal, epidural (peridural), intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection, (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), or in ear drops.

[00616] In one embodiment, provided are compositions for generation of an *in vivo* depot containing a polynucleotide, modified nucleic acid or engineered ribonucleotide. For example, the composition contains a bioerodible, biocompatible polymer, a solvent present in an amount effective to plasticize the polymer and form a gel therewith, and a polynucleotide, modified nucleic acid or engineered ribonucleic acid. In certain embodiments the composition also includes a cell penetration agent as described herein. In other embodiments, the composition also contains a thixotropic amount of a thixotropic agent mixable with the polymer so as to be effective to form a thixotropic composition. Further compositions include a stabilizing agent, a bulking agent, a chelating agent, or a buffering agent.

[00617] In other embodiments, provided are sustained-release delivery depots, such as for administration of a polynucleotide, modified nucleic acid, or engineered ribonucleic acid in an environment (meaning an organ or tissue site) in a patient. Such depots generally contain an engineered ribonucleic acid and a flexible chain polymer where both the engineered ribonucleic acid and the flexible chain polymer are entrapped within a porous matrix of a crosslinked matrix protein. Usually, the pore size is less than 1mm, such as 900 nm, 800 nm, 700 nm, 600 nm, 500 nm, 400 nm, 300 nm, 200 nm, 100 nm, or less than 100 nm. Usually the flexible chain polymer is hydrophilic. Usually the flexible chain polymer has a molecular weight of at least 50 kDa, such as 75 kDa, 100 kDa, 150 kDa, 200 kDa, 250 kDa, 300 kDa, 400 kDa, 500 kDa, or greater than 500 kDa. Usually the flexible chain polymer has a persistence length of less than 10%, such as 9, 8, 7, 6, 5, 4, 3, 2, 1 or less than 1% of the persistence length of the matrix protein. Usually the flexible chain polymer has a charge similar to that of the matrix protein. In some embodiments, the flexible chain polymer alters the effective pore size of a matrix of crosslinked matrix protein to a size capable of sustaining the diffusion of the engineered ribonucleic acid from the matrix into a surrounding tissue comprising a cell into which the polynucleotide, modified nucleic acid, engineered ribonucleic acid is capable of entering.

[00618] In specific embodiments, compositions may be administered in a way which allows them cross the blood-brain barrier, vascular barrier, or other epithelial barrier. Non-limiting routes of administration for the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention are described below.

[00619] The present invention provides methods comprising administering polynucleotides, modified mRNAs and their encoded proteins or complexes in accordance with the invention to a subject in need thereof. Nucleic acids, proteins or complexes, or pharmaceutical, imaging, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition (*e.g.*, a disease, disorder, and/or condition relating to working memory deficits). The exact amount required will vary from subject to subject, depending on the

species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. Compositions in accordance with the invention are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

Parenteral and Injectible Administration

[00620] Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as CREMOPHOR[®], alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

[00621] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing

agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00622] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00623] In order to prolong the effect of an active ingredient, it is often desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microcapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Rectal and Vaginal Administration

[00624] Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing compositions with suitable non-irritating excipients such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

Oral Administration

[00625] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, an active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient such as sodium citrate or dicalcium phosphate and/or fillers or extenders (*e.g.* starches, lactose, sucrose, glucose, mannitol, and silicic acid), binders (*e.g.* carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia), humectants (*e.g.* glycerol), disintegrating agents (*e.g.* agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate), solution retarding agents (*e.g.* paraffin), absorption accelerators (*e.g.* quaternary ammonium compounds), wetting agents (*e.g.* cetyl alcohol and glycerol monostearate), absorbents (*e.g.* kaolin and bentonite clay), and lubricants (*e.g.* talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

Topical or Transdermal Administration

[00626] As described herein, compositions containing the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids of the invention may be formulated for administration topically. The skin may be an ideal target site for delivery as it is readily accessible. Gene expression may be restricted not only to the skin, potentially avoiding nonspecific toxicity, but also to specific layers and cell types within the skin.

[00627] The site of cutaneous expression of the delivered compositions will depend on the route of nucleic acid delivery. Three routes are commonly considered to deliver polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids to the skin: (i) topical application (*e.g.* for local/regional treatment); (ii) intradermal injection (*e.g.* for local/regional treatment); and (iii) systemic delivery (*e.g.* for treatment of dermatologic diseases that affect both cutaneous and extracutaneous regions).

Polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids can be delivered to the skin by several different approaches known in the art. Most topical delivery approaches have been shown to work for delivery of DNA, such as but not limited to, topical application of non-cationic liposome-DNA complex, cationic

liposome-DNA complex, particle-mediated (gene gun), puncture-mediated gene transfections, and viral delivery approaches. After delivery of the nucleic acid, gene products have been detected in a number of different skin cell types, including, but not limited to, basal keratinocytes, sebaceous gland cells, dermal fibroblasts and dermal macrophages.

[00628] In one embodiment, the invention provides for a variety of dressings (e.g., wound dressings) or bandages (e.g., adhesive bandages) for conveniently and/or effectively carrying out methods of the present invention. Typically dressing or bandages may comprise sufficient amounts of pharmaceutical compositions and/or polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein to allow a user to perform multiple treatments of a subject(s).

[00629] In one embodiment, the invention provides for the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids compositions to be delivered in more than one injection.

[00630] In one embodiment, before topical and/or transdermal administration at least one area of tissue, such as skin, may be subjected to a device and/or solution which may increase permeability. In one embodiment, the tissue may be subjected to an abrasion device to increase the permeability of the skin (see U.S. Patent Publication No. 20080275468, herein incorporated by reference in its entirety). In another embodiment, the tissue may be subjected to an ultrasound enhancement device. An ultrasound enhancement device may include, but is not limited to, the devices described in U.S. Publication No. 20040236268 and U.S. Patent Nos. 6,491,657 and 6,234,990; each of which are herein incorporated by reference in their entireties. Methods of enhancing the permeability of tissue are described in U.S. Publication Nos. 20040171980 and 20040236268 and U.S. Pat. No. 6,190,315; each of which are herein incorporated by reference in their entireties.

[00631] In one embodiment, a device may be used to increase permeability of tissue before delivering formulations of modified mRNA described herein. The permeability of skin may be measured by methods known in the art and/or described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety. As a non-limiting example, a

modified mRNA formulation may be delivered by the drug delivery methods described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety.

[00632] In another non-limiting example tissue may be treated with a eutectic mixture of local anesthetics (EMLA) cream before, during and/or after the tissue may be subjected to a device which may increase permeability. Katz et al. (Anesth Analg (2004); 98:371-76; herein incorporated by reference in its entirety) showed that using the EMLA cream in combination with a low energy, an onset of superficial cutaneous analgesia was seen as fast as 5 minutes after a pretreatment with a low energy ultrasound.

[00633] In one embodiment, enhancers may be applied to the tissue before, during, and/or after the tissue has been treated to increase permeability. Enhancers include, but are not limited to, transport enhancers, physical enhancers, and cavitation enhancers. Non-limiting examples of enhancers are described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety.

[00634] In one embodiment, a device may be used to increase permeability of tissue before delivering formulations of modified mRNA described herein, which may further contain a substance that invokes an immune response. In another non-limiting example, a formulation containing a substance to invoke an immune response may be delivered by the methods described in U.S. Publication Nos. 20040171980 and 20040236268; each of which are herein incorporated by reference in their entireties.

[00635] Dosage forms for topical and/or transdermal administration of a composition may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants and/or patches. Generally, an active ingredient is admixed under sterile conditions with a pharmaceutically acceptable excipient and/or any needed preservatives and/or buffers as may be required. Additionally, the present invention contemplates the use of transdermal patches, which often have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms may be prepared, for example, by dissolving and/or dispensing the compound in the proper medium. Alternatively or additionally, rate may be controlled by either providing a rate controlling membrane and/or by dispersing the compound in a polymer matrix and/or gel.

[00636] Formulations suitable for topical administration include, but are not limited to, liquid and/or semi liquid preparations such as liniments, lotions, oil in water and/or water

in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions.

[00637] Topically-administrable formulations may, for example, comprise from about 0.1% to about 10% (w/w) active ingredient, although the concentration of active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Depot Administration

[00638] As described herein, in some embodiments, the composition is formulated in depots for extended release. Generally, a specific organ or tissue (a "target tissue") is targeted for administration.

[00639] In some aspects of the invention, the nucleic acids (particularly ribonucleic acids encoding polypeptides) are spatially retained within or proximal to a target tissue. Provided are method of providing a composition to a target tissue of a mammalian subject by contacting the target tissue (which contains one or more target cells) with the composition under conditions such that the composition, in particular the nucleic acid component(s) of the composition, is substantially retained in the target tissue, meaning that at least 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the composition is retained in the target tissue. Advantageously, retention is determined by measuring the amount of the nucleic acid present in the composition that enters one or more target cells. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the nucleic acids administered to the subject are present intracellularly at a period of time following administration. For example, intramuscular injection to a mammalian subject is performed using an aqueous composition containing a ribonucleic acid and a transfection reagent, and retention of the composition is determined by measuring the amount of the ribonucleic acid present in the muscle cells.

[00640] Aspects of the invention are directed to methods of providing a composition to a target tissue of a mammalian subject, by contacting the target tissue (containing one or more target cells) with the composition under conditions such that the composition is substantially retained in the target tissue. In another embodiment, a polynucleotide,

ribonucleic acid engineered to avoid an innate immune response of a cell into which the ribonucleic acid enters, where the ribonucleic acid contains a nucleotide sequence encoding a polypeptide of interest, under conditions such that the polypeptide of interest is produced in at least one target cell. The compositions generally contain a cell penetration agent, although "naked" nucleic acid (such as nucleic acids without a cell penetration agent or other agent) is also contemplated, and a pharmaceutically acceptable carrier.

[00641] In some circumstances, the amount of a protein produced by cells in a tissue is desirably increased. Preferably, this increase in protein production is spatially restricted to cells within the target tissue. Thus, provided are methods of increasing production of a protein of interest in a tissue of a mammalian subject. A composition is provided that contains a ribonucleic acid that is engineered to avoid an innate immune response of a cell into which the ribonucleic acid enters and encodes the polypeptide of interest and the composition is characterized in that a unit quantity of composition has been determined to produce the polypeptide of interest in a substantial percentage of cells contained within a predetermined volume of the target tissue.

[00642] In some embodiments, the composition includes a plurality of different ribonucleic acids, where one or more than one of the ribonucleic acids is engineered to avoid an innate immune response of a cell into which the ribonucleic acid enters, and where one or more than one of the ribonucleic acids encodes a polypeptide of interest. Optionally, the composition also contains a cell penetration agent to assist in the intracellular delivery of the ribonucleic acid. A determination is made of the dose of the composition required to produce the polypeptide of interest in a substantial percentage of cells contained within the predetermined volume of the target tissue (generally, without inducing significant production of the polypeptide of interest in tissue adjacent to the predetermined volume, or distally to the target tissue). Subsequent to this determination, the determined dose is introduced directly into the tissue of the mammalian subject.

[00643] In one embodiment, the invention provides for the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids to be delivered in more than one injection or by split dose injections.

[00644] In one embodiment, the invention may be retained near target tissue using a small disposable drug reservoir or patch pump. Non-limiting examples of patch pumps include those manufactured and/or sold by BD®, (Franklin Lakes, NJ), Insulet Corporation (Bedford, MA), SteadyMed Therapeutics (San Francisco, CA), Medtronic (Minneapolis, MN), UniLife (York, PA), Valeritas (Bridgewater, NJ), and SpringLeaf Therapeutics (Boston, MA).

Pulmonary Administration

[00645] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 nm to about 7 nm or from about 1 nm to about 6 nm. Such compositions are suitably in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder and/or using a self propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved and/or suspended in a low-boiling propellant in a sealed container. Such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nm and at least 95% of the particles by number have a diameter less than 7 nm. Alternatively, at least 95% of the particles by weight have a diameter greater than 1 nm and at least 90% of the particles by number have a diameter less than 6 nm. Dry powder compositions may include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

[00646] Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally the propellant may constitute 50% to 99.9% (w/w) of the composition, and active ingredient may constitute 0.1% to 20% (w/w) of the composition. A propellant may further comprise additional ingredients such as a liquid non-ionic and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

[00647] Pharmaceutical compositions formulated for pulmonary delivery may provide an active ingredient in the form of droplets of a solution and/or suspension. Such formulations may be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic

solutions and/or suspensions, optionally sterile, comprising active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, and/or a preservative such as methylhydroxybenzoate. Droplets provided by this route of administration may have an average diameter in the range from about 0.1 nm to about 200 nm.

Intranasal, Nasal and Buccal Administration

[00648] Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 μm to 500 μm . Such a formulation is administered in the manner in which snuff is taken, *i.e.* by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00649] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, 0.1% to 20% (w/w) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or atomized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

Ophthalmic Administration

[00650] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example,

be in the form of eye drops including, for example, a 0.1/1.0% (w/w) solution and/or suspension of the active ingredient in an aqueous or oily liquid excipient. Such drops may further comprise buffering agents, salts, and/or one or more other of any additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are contemplated as being within the scope of this invention.

Payload Administration: Detectable Agents and Therapeutic Agents

[00651] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein can be used in a number of different scenarios in which delivery of a substance (the "payload") to a biological target is desired, for example delivery of detectable substances for detection of the target, or delivery of a therapeutic agent. Detection methods can include, but are not limited to, both imaging *in vitro* and *in vivo* imaging methods, *e.g.*, immunohistochemistry, bioluminescence imaging (BLI), Magnetic Resonance Imaging (MRI), positron emission tomography (PET), electron microscopy, X-ray computed tomography, Raman imaging, optical coherence tomography, absorption imaging, thermal imaging, fluorescence reflectance imaging, fluorescence microscopy, fluorescence molecular tomographic imaging, nuclear magnetic resonance imaging, X-ray imaging, ultrasound imaging, photoacoustic imaging, lab assays, or in any situation where tagging/staining/imaging is required.

[00652] The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids can be designed to include both a linker and a payload in any useful orientation. For example, a linker having two ends is used to attach one end to the payload and the other end to the nucleobase, such as at the C-7 or C-8 positions of the deaza-adenosine or deaza-guanosine or to the N-3 or C-5 positions of cytosine or uracil. The polynucleotide of the invention can include more than one payload (*e.g.*, a label and a transcription inhibitor), as well as a cleavable linker.

[00653] In one embodiment, the modified nucleotide is a modified 7-deaza-adenosine triphosphate, where one end of a cleavable linker is attached to the C7 position of 7-deaza-adenine, the other end of the linker is attached to an inhibitor (*e.g.*, to the C5 position of the nucleobase on a cytidine), and a label (*e.g.*, Cy5) is attached to the center

of the linker (see, e.g., compound 1 of A*pCp C5 Parg Capless in Fig. 5 and columns 9 and 10 of U.S. Pat. No. 7,994,304, incorporated herein by reference). Upon incorporation of the modified 7-deaza-adenosine triphosphate to an encoding region, the resulting polynucleotide having a cleavable linker attached to a label and an inhibitor (e.g., a polymerase inhibitor). Upon cleavage of the linker (e.g., with reductive conditions to reduce a linker having a cleavable disulfide moiety), the label and inhibitor are released. Additional linkers and payloads (e.g., therapeutic agents, detectable labels, and cell penetrating payloads) are described herein.

[00654] For example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein can be used in reprogramming induced pluripotent stem cells (iPS cells), which can directly track cells that are transfected compared to total cells in the cluster. In another example, a drug that may be attached to the modified nucleic acids, enhanced modified RNA or ribonucleic acids via a linker and may be fluorescently labeled can be used to track the drug *in vivo*, e.g. intracellularly. Other examples include, but are not limited to, the use of polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids in reversible drug delivery into cells.

[00655] The polynucleotides, modified modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein can be used in intracellular targeting of a payload, e.g., detectable or therapeutic agent, to specific organelle. Exemplary intracellular targets can include, but are not limited to, the nuclear localization for advanced mRNA processing, or a nuclear localization sequence (NLS) linked to the mRNA containing an inhibitor.

[00656] In addition, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein can be used to deliver therapeutic agents to cells or tissues, e.g., in living animals. For example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids described herein can be used to deliver highly polar chemotherapeutics agents to kill cancer cells. The polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids attached to the therapeutic agent through a linker can facilitate member permeation allowing the therapeutic agent to travel into a cell to reach an intracellular target.

[00657] In another example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids can be attached to the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids a viral inhibitory peptide (VIP) through a cleavable linker. The cleavable linker can release the VIP and dye into the cell. In another example, the polynucleotides, modified nucleic acids, enhanced modified RNA or ribonucleic acids can be attached through the linker to an ADP-ribosylate, which is responsible for the actions of some bacterial toxins, such as cholera toxin, diphtheria toxin, and pertussis toxin. These toxin proteins are ADP-ribosyltransferases that modify target proteins in human cells. For example, cholera toxin ADP-ribosylates G proteins modifies human cells by causing massive fluid secretion from the lining of the small intestine, which results in life-threatening diarrhea.

[00658] In some embodiments, the payload may be a therapeutic agent such as a cytotoxin, radioactive ion, chemotherapeutic, or other therapeutic agent. A cytotoxin or cytotoxic agent includes any agent that may be detrimental to cells. Examples include, but are not limited to, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxyanthracenedione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol (see U.S. Pat. No. 5,208,020 incorporated herein in its entirety), rachelmycin (CC-1065, see U.S. Pat. Nos. 5,475,092, 5,585,499, and 5,846,545, all of which are incorporated herein by reference), and analogs or homologs thereof. Radioactive ions include, but are not limited to iodine (*e.g.*, iodine 125 or iodine 131), strontium 89, phosphorous, palladium, cesium, iridium, phosphate, cobalt, yttrium 90, samarium 153, and praseodymium. Other therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thiotepa chlorambucil, rachelmycin (CC-1065), melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics

(e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine, vinblastine, taxol and maytansinoids).

[00659] In some embodiments, the payload may be a detectable agent, such as various organic small molecules, inorganic compounds, nanoparticles, enzymes or enzyme substrates, fluorescent materials, luminescent materials (e.g., luminol), bioluminescent materials (e.g., luciferase, luciferin, and aequorin), chemiluminescent materials, radioactive materials (e.g., ^{18}F , ^{67}Ga , $^{81\text{m}}\text{Kr}$, ^{82}Rb , ^{111}In , ^{123}I , ^{133}Xe , ^{201}Tl , ^{125}I , ^{35}S , ^{14}C , ^3H , or $^{99\text{m}}\text{Tc}$ (e.g., as pertechnetate (technetate(VII), Tc(V))), and contrast agents (e.g., gold (e.g., gold nanoparticles), gadolinium (e.g., chelated Gd), iron oxides (e.g., superparamagnetic iron oxide (SPIO), monocrystalline iron oxide nanoparticles (MIONs), and ultrasmall superparamagnetic iron oxide (USPIO)), manganese chelates (e.g., Mn-DPDP), barium sulfate, iodinated contrast media (iohexol), microbubbles, or perfluorocarbons). Such optically-detectable labels include for example, without limitation, 4-acetamido-4'-isothiocyanatostilbene-2,2' disulfonic acid; acridine and derivatives (e.g., acridine and acridine isothiocyanate); 5-(2'-aminoethyl)aminonaphthalene-1 -sulfonic acid (EDANS); 4-amino-N-[3-vinylsulfonyl]phenyl]naphthalimide-3 ,5 disulfonate; N-(4-anilino-1-naphthyl)maleimide; anthranilamide; BODIPY; Brilliant Yellow; coumarin and derivatives (e.g., coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), and 7-amino-4-trifluoromethylcoumarin (Coumarin 151)); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5' 5"-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3 -(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2' -disulfonic acid; 5-[dimethylamino]-naphthalene- 1-sulfonyl chloride (DNS, dansylchloride); 4-dimethylaminophenylazophenyl-4' -isothiocyanate (DABITC); eosin and derivatives (e.g., eosin and eosin isothiocyanate); erythrosin and derivatives (e.g., erythrosin B and erythrosin isothiocyanate); ethidium; fluorescein and derivatives (e.g., 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF), 2',7'-dimethoxy-4'5' -dichloro-6-carboxyfluorescein, fluorescein, fluorescein isothiocyanate, X-rhodamine-5-(and-6)-isothiocyanate (QFITC or XRITC), and fluorescamine); 2-[2-[3-[[1,3-dihydro-1,1-dimethyl-3-(3-sulfopropyl)-

2H-benz[e]indol-2-ylidene]ethylidene]-2-[4-(ethoxycarbonyl)-1-piperazinyl]-1-cyclopenten-1-yl]ethenyl]-1,1-dimethyl-3-(3-sulforpropyl)-1H-benz[e]indolium hydroxide, inner salt, compound with n,n-diethylethanamine(1:1) (IR144); 5-chloro-2-[2-[3-[(5-chloro-3-ethyl-2(3H)-benzothiazol-ylidene)ethylidene]-2-(diphenylamino)-1-cyclopenten-1-yl]ethenyl]-3-ethyl benzothiazolium perchlorate (IR140); Malachite Green isothiocyanate; 4-methylumbelliferone orthocresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthaldialdehyde; pyrene and derivatives(e.g., pyrene, pyrene butyrate, and succinimidyl 1-pyrene); butyrate quantum dots; Reactive Red 4 (Cibacron™ Brilliant Red 3B-A); rhodamine and derivatives (e.g., 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red), N,N,N',N' tetramethyl-6-carboxyrhodamine (TAMRA) tetramethyl rhodamine, and tetramethyl rhodamine isothiocyanate (TRITC)); riboflavin; rosolic acid; terbium chelate derivatives; Cyanine-3 (Cy3); Cyanine-5 (Cy5); cyanine-5.5 (Cy5.5), Cyanine-7 (Cy7); IRD 700; IRD 800; Alexa 647; La Jolla Blue; phthalocyanine; and naphthalocyanine.

[00660] In some embodiments, the detectable agent may be a non-detectable precursor that becomes detectable upon activation (e.g., fluorogenic tetrazine-fluorophore constructs (e.g., tetrazine-BODIPY FL, tetrazine-Oregon Green 488, or tetrazine-BODIPY TMR-X) or enzyme activatable fluorogenic agents (e.g., PROSENSE® (VisEn Medical))). In vitro assays in which the enzyme labeled compositions can be used include, but are not limited to, enzyme linked immunosorbent assays (ELISAs), immunoprecipitation assays, immunofluorescence, enzyme immunoassays (EIA), radioimmunoassays (RIA), and Western blot analysis. *Combination*

[00661] The modified nucleic acids, enhanced modified RNA or ribonucleic acids may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. By "in combination with," it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other

desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic, or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body. As a non-limiting example, the modified nucleic acids, enhanced modified RNA or ribonucleic acids may be used in combination with a pharmaceutical agent for the treatment of cancer or to control hyperproliferative cells. In U.S. Pat. No. 7,964,571, herein incorporated by reference in its entirety, a combination therapy for the treatment of solid primary or metastasized tumor is described using a pharmaceutical composition including a DNA plasmid encoding for interleukin-12 with a lipopolymer and also administering at least one anticancer agent or chemotherapeutic. Further, the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention that encodes anti-proliferative molecules may be in a pharmaceutical composition with a lipopolymer (see e.g., U.S. Pub. No. 201 10218231, herein incorporated by reference in its entirety, claiming a pharmaceutical composition comprising a DNA plasmid encoding an anti-proliferative molecule and a lipopolymer) which may be administered with at least one chemotherapeutic or anticancer agent.

Payload Administration: Cell Penetrating Payload

[00662] In some embodiments, the polynucleotides, modified nucleotides and modified nucleic acid molecules, which are incorporated into a nucleic acid, e.g., RNA or mRNA, can also include a payload that can be a cell penetrating moiety or agent that enhances intracellular delivery of the compositions. For example, the compositions can include, but are not limited to, a cell-penetrating peptide sequence that facilitates delivery to the intracellular space, e.g., HIV-derived TAT peptide, penetratins, transportans, or hCT derived cell-penetrating peptides, see, e.g., Caron et al, (2001) Mol Ther. 3(3):3 10-8; Langel, Cell-Penetrating Peptides: Processes and Applications (CRC Press, Boca Raton FL 2002); El-Andaloussi et al, (2005) Curr Pharm Des. 11(28):3597-61 1; and Deshayes et al, (2005) Cell Mol Life Sci. 62(16): 1839-49; all of which are incorporated herein by reference. The compositions can also be formulated to include a cell

penetrating agent, *e.g.*, liposomes, which enhance delivery of the compositions to the intracellular space.

Payload Administration: Biological Target

[00663] The modified nucleotides and modified nucleic acid molecules described herein, which are incorporated into a nucleic acid, *e.g.*, RNA or mRNA, can be used to deliver a payload to any biological target for which a specific ligand exists or can be generated. The ligand can bind to the biological target either covalently or non-covalently.

[00664] Examples of biological targets include, but are not limited to, biopolymers, *e.g.*, antibodies, nucleic acids such as RNA and DNA, proteins, enzymes; examples of proteins include, but are not limited to, enzymes, receptors, and ion channels. In some embodiments the target may be a tissue- or a cell-type specific marker, *e.g.*, a protein that is expressed specifically on a selected tissue or cell type. In some embodiments, the target may be a receptor, such as, but not limited to, plasma membrane receptors and nuclear receptors; more specific examples include, but are not limited to, G-protein-coupled receptors, cell pore proteins, transporter proteins, surface-expressed antibodies, HLA proteins, MHC proteins and growth factor receptors.

Dosing

[00665] The present invention provides methods comprising administering modified mRNAs and their encoded proteins or complexes in accordance with the invention to a subject in need thereof. Nucleic acids, proteins or complexes, or pharmaceutical, imaging, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition (*e.g.*, a disease, disorder, and/or condition relating to working memory deficits). The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. Compositions in accordance with the invention are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention may be decided by the attending physician within

the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[00666] In certain embodiments, compositions in accordance with the present invention may be administered at dosage levels sufficient to deliver from about 0.0001 mg/kg to about 100 mg/kg, from about 0.001 mg/kg to about 0.05 mg/kg, from about 0.005 mg/kg to about 0.05 mg/kg, from about 0.001 mg/kg to about 0.005 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect. The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations).

[00667] According to the present invention, it has been discovered that administration of modified nucleic acids, enhanced modified RNA or ribonucleic acids in split-dose regimens produce higher levels of proteins in mammalian subjects. As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses, *e.g.*, two or more administrations of the single unit dose. As used herein, a "single unit dose" is a dose of any therapeutic administered in one dose/at one time/single route/single point of contact, *i.e.*, single administration event. As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose. In one embodiment, the modified nucleic acids, enhanced modified RNA or

ribonucleic acids of the present invention are administered to a subject in split doses. The modified nucleic acids, enhanced modified RNA or ribonucleic acids may be formulated in buffer only or in a formulation described herein.

Dosage Forms

[00668] A pharmaceutical composition described herein can be formulated into a dosage form described herein, such as a topical, intranasal, intratracheal, or injectable (e.g., intravenous, intraocular, intravitreal, intramuscular, intracardiac, intraperitoneal, subcutaneous).

Liquid dosage forms

[00669] Liquid dosage forms for parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art including, but not limited to, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. In certain embodiments for parenteral administration, compositions may be mixed with solubilizing agents such as CREMOPHOR[®], alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

Injectable

[00670] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art and may include suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed include, but are not limited to, are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono-

or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00671] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00672] In order to prolong the effect of an active ingredient, it may be desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of modified mRNA then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered modified mRNA may be accomplished by dissolving or suspending the modified mRNA in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the modified mRNA in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of modified mRNA to polymer and the nature of the particular polymer employed, the rate of modified mRNA release can be controlled. Examples of other biodegradable polymers include, but are not limited to, poly(orthoesters) and poly(anhydrides). Depot injectable formulations may be prepared by entrapping the modified mRNA in liposomes or microemulsions which are compatible with body tissues.

Pulmonary

[00673] Formulations described herein as being useful for pulmonary delivery may also be use for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration may be a coarse powder comprising the active ingredient and having an average particle from about 0.2 μm to 500 μm . Such a formulation may be administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00674] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and

may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, contain about 0.1% to 20% (w/w) active ingredient, where the balance may comprise an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

[00675] General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference in its entirety).

Coatings or Shells

[00676] Solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

Kits

[00677] The invention provides a variety of kits for conveniently and/or effectively carrying out methods of the present invention. Typically kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[00678] In one aspect, the present invention provides kits for protein production, comprising a first modified nucleic acids, enhanced modified RNA or ribonucleic acids comprising a translatable region. The kit may further comprise packaging and instructions and/or a delivery agent to form a formulation composition. The delivery agent may comprise a saline, a buffered solution, a lipidoid or any delivery agent disclosed herein.

[00679] In one embodiment, the buffer solution may include sodium chloride, calcium chloride, phosphate and/or EDTA. In another embodiment, the buffer solution may include, but is not limited to, saline, saline with 2mM calcium, 5% sucrose, 5% sucrose with 2mM calcium, 5% Mannitol, 5% Mannitol with 2mM calcium, Ringer's lactate, sodium chloride, sodium chloride with 2mM calcium. In a further embodiment, the buffer solutions may be precipitated or it may be lyophilized. The amount of each component may be varied to enable consistent, reproducible higher concentration saline or simple buffer formulations. The components may also be varied in order to increase the stability of modified RNA in the buffer solution over a period of time and/or under a variety of conditions.

[00680] In one aspect, the present invention provides kits for protein production, comprising: a modified nucleic acids, enhanced modified RNA or ribonucleic acids comprising a translatable region, provided in an amount effective to produce a desired amount of a protein encoded by the translatable region when introduced into a target cell; a second modified nucleic acids, enhanced modified RNA or ribonucleic acids comprising an inhibitory nucleic acid, provided in an amount effective to substantially inhibit the innate immune response of the cell; and packaging and instructions.

[00681] In one aspect, the present invention provides kits for protein production, comprising a modified nucleic acids, enhanced modified RNA or ribonucleic acids comprising a translatable region, wherein the nucleic acid exhibits reduced degradation by a cellular nuclease, and packaging and instructions.

[00682] In one aspect, the present invention provides kits for protein production, comprising a modified nucleic acids, enhanced modified RNA or ribonucleic acids comprising a translatable region, wherein the nucleic acid exhibits reduced degradation

by a cellular nuclease, and a mammalian cell suitable for translation of the translatable region of the first nucleic acid

Devices

[00683] The present invention provides for devices which may incorporate modified nucleic acids, enhanced modified RNA or ribonucleic acids that encode polypeptides of interest. These devices contain in a stable formulation the reagents to synthesize a nucleic acid in a formulation available to be immediately delivered to a subject in need thereof, such as a human patient. Non-limiting examples of such a polypeptide of interest include a growth factor and/or angiogenesis stimulator for wound healing, a peptide antibiotic to facilitate infection control, and an antigen to rapidly stimulate an immune response to a newly identified virus.

[00684] In some embodiments the device is self-contained, and is optionally capable of wireless remote access to obtain instructions for synthesis and/or analysis of the generated modified nucleic acids, enhanced modified RNA or ribonucleic acids. The device is capable of mobile synthesis of at least one modified nucleic acids, enhanced modified RNA or ribonucleic acids and preferably an unlimited number of different modified nucleic acids, enhanced modified RNA or ribonucleic acids. In certain embodiments, the device is capable of being transported by one or a small number of individuals. In other embodiments, the device is scaled to fit on a benchtop or desk. In other embodiments, the device is scaled to fit into a suitcase, backpack or similarly sized object. In another embodiment, the device may be a point of care or handheld device. In further embodiments, the device is scaled to fit into a vehicle, such as a car, truck or ambulance, or a military vehicle such as a tank or personnel carrier. The information necessary to generate a ribonucleic acid encoding polypeptide of interest is present within a computer readable medium present in the device.

[00685] In one embodiment, a device may be used to assess levels of a protein which has been administered in the form of a modified nucleic acids, enhanced modified RNA or ribonucleic acids. The device may comprise a blood, urine or other biofluidic test.

[00686] In some embodiments, the device is capable of communication (e.g., wireless communication) with a database of nucleic acid and polypeptide sequences. The device contains at least one sample block for insertion of one or more sample vessels. Such

sample vessels are capable of accepting in liquid or other form any number of materials such as template DNA, nucleotides, enzymes, buffers, and other reagents. The sample vessels are also capable of being heated and cooled by contact with the sample block. The sample block is generally in communication with a device base with one or more electronic control units for the at least one sample block. The sample block preferably contains a heating module, such heating molecule capable of heating and/or cooling the sample vessels and contents thereof to temperatures between about -20C and above +100C. The device base is in communication with a voltage supply such as a battery or external voltage supply. The device also contains means for storing and distributing the materials for RNA synthesis.

[00687] Optionally, the sample block contains a module for separating the synthesized nucleic acids. Alternatively, the device contains a separation module operably linked to the sample block. Preferably the device contains a means for analysis of the synthesized nucleic acid. Such analysis includes sequence identity (demonstrated such as by hybridization), absence of non-desired sequences, measurement of integrity of synthesized mRNA (such as by microfluidic viscometry combined with spectrophotometry), and concentration and/or potency of modified nucleic acids, enhanced modified RNA or ribonucleic acids (such as by spectrophotometry).

[00688] In certain embodiments, the device is combined with a means for detection of pathogens present in a biological material obtained from a subject, e.g., the IBIS PLEX-ID system (Abbott, Abbott Park, IL) for microbial identification.

[00689] Suitable devices for use in delivering intradermal pharmaceutical compositions described herein include short needle devices such as those described in U.S. Patents 4,886,499; 5,190,521; 5,328,483; 5,527,288; 4,270,537; 5,015,235; 5,141,496; and 5,417,662. Intradermal compositions may be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in PCT publication WO 99/34850 and functional equivalents thereof. Jet injection devices which deliver liquid compositions to the dermis via a liquid jet injector and/or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis are suitable. Jet injection devices are described, for example, in U.S. Patents 5,480,381; 5,599,302; 5,334,144; 5,993,412; 5,649,912; 5,569,189; 5,704,911; 5,383,851;

5,893,397; 5,466,220; 5,339,163; 5,312,335; 5,503,627; 5,064,413; 5,520,639; 4,596,556; 4,790,824; 4,941,880; 4,940,460; and PCT publications WO 97/37705 and WO 97/13537. Ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis are suitable.

[00690] Alternatively or additionally, conventional syringes may be used in the classical mantoux method of intradermal administration.

[00691] In some embodiments, the device may be a pump or comprise a catheter for administration of compounds or compositions of the invention across the blood brain barrier. Such devices include but are not limited to a pressurized olfactory delivery device, iontophoresis devices, multi-layered microfluidic devices, and the like. Such devices may be portable or stationary. They may be implantable or externally tethered to the body or combinations thereof.

[00692] Devices for administration may be employed to deliver the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention according to single, multi- or split-dosing regimens taught herein. Such devices are described below.

[00693] Method and devices known in the art for multi-administration to cells, organs and tissues are contemplated for use in conjunction with the methods and compositions disclosed herein as embodiments of the present invention. These include, for example, those methods and devices having multiple needles, hybrid devices employing for example lumens or catheters as well as devices utilizing heat, electric current or radiation driven mechanisms.

[00694] According to the present invention, these multi-administration devices may be utilized to deliver the single, multi- or split doses contemplated herein.

[00695] A method for delivering therapeutic agents to a solid tissue has been described by Bahrami et al. and is taught for example in US Patent Publication 201 10230839, the contents of which are incorporated herein by reference in their entirety. According to Bahrami, an array of needles is incorporated into a device which delivers a substantially equal amount of fluid at any location in said solid tissue along each needle's length.

[00696] A device for delivery of biological material across the biological tissue has been described by Kodgule et al. and is taught for example in US Patent Publication

201 10172610, the contents of which are incorporated herein by reference in their entirety. According to Kodgule, multiple hollow micro-needles made of one or more metals and having outer diameters from about 200 microns to about 350 microns and lengths of at least 100 microns are incorporated into the device which delivers peptides, proteins, carbohydrates, nucleic acid molecules, lipids and other pharmaceutically active ingredients or combinations thereof.

[00697] A delivery probe for delivering a therapeutic agent to a tissue has been described by Gunday et al. and is taught for example in US Patent Publication 201 10270184, the contents of which are incorporated herein by reference in their entirety. According to Gunday, multiple needles are incorporated into the device which moves the attached capsules between an activated position and an inactivated position to force the agent out of the capsules through the needles.

[00698] A multiple-injection medical apparatus has been described by Assaf and is taught for example in US Patent Publication 201 10218497, the contents of which are incorporated herein by reference in their entirety. According to Assaf, multiple needles are incorporated into the device which has a chamber connected to one or more of said needles and a means for continuously refilling the chamber with the medical fluid after each injection.

[00699] In one embodiment, the modified nucleic acids, enhanced modified RNA or ribonucleic acids are administered subcutaneously or intramuscularly via at least 3 needles to three different, optionally adjacent, sites simultaneously, or within a 60 minutes period (e.g., administration to 4, 5, 6, 7, 8, 9, or 10 sites simultaneously or within a 60 minute period). The split doses can be administered simultaneously to adjacent tissue using the devices described in U.S. Patent Publication Nos. 201 10230839 and 201 10218497, each of which is incorporated herein by reference.

[00700] An at least partially implantable system for injecting a substance into a patient's body, in particular a penis erection stimulation system has been described by Forsell and is taught for example in US Patent Publication 201 10196198, the contents of which are incorporated herein by reference in their entirety. According to Forsell, multiple needles are incorporated into the device which is implanted along with one or

more housings adjacent the patient's left and right corpora cavernosa. A reservoir and a pump are also implanted to supply drugs through the needles.

[00701] A method for the transdermal delivery of a therapeutic effective amount of iron has been described by Berenson and is taught for example in US Patent Publication 20100130910, the contents of which are incorporated herein by reference in their entirety. According to Berenson, multiple needles may be used to create multiple micro channels in stratum corneum to enhance transdermal delivery of the ionic iron on an iontophoretic patch.

[00702] A method for delivery of biological material across the biological tissue has been described by Kodgule et al and is taught for example in US Patent Publication 201 10196308, the contents of which are incorporated herein by reference in their entirety. According to Kodgule, multiple biodegradable microneedles containing a therapeutic active ingredient are incorporated in a device which delivers proteins, carbohydrates, nucleic acid molecules, lipids and other pharmaceutically active ingredients or combinations thereof.

[00703] A transdermal patch comprising a botulinum toxin composition has been described by Donovan and is taught for example in US Patent Publication 20080220020, the contents of which are incorporated herein by reference in their entirety. According to Donovan, multiple needles are incorporated into the patch which delivers botulinum toxin under stratum corneum through said needles which project through the stratum corneum of the skin without rupturing a blood vessel.

[00704] A small, disposable drug reservoir, or patch pump, which can hold approximately 0.2 to 15 mL of liquid formulations can be placed on the skin and deliver the formulation continuously subcutaneously using a small bore needle (e.g., 26 to 34 gauge). As non-limiting examples, the patch pump may be 50 mm by 76 mm by 20 mm spring loaded having a 30 to 34 gauge needle (BD™ Microinfuser, Franklin Lakes NJ), 41 mm by 62 mm by 17 mm with a 2 mL reservoir used for drug delivery such as insulin (OMNIPOD®, Insulet Corporation Bedford, MA), or 43-60 mm diameter, 10 mm thick with a 0.5 to 10 mL reservoir (PATCHPUMP®, SteadyMed Therapeutics, San Francisco, CA). Further, the patch pump may be battery powered and/or rechargeable.

[00705] A cryoprobe for administration of an active agent to a location of cryogenic treatment has been described by Toubia and is taught for example in US Patent Publication 20080140061, the contents of which are incorporated herein by reference in their entirety. According to Toubia, multiple needles are incorporated into the probe which receives the active agent into a chamber and administers the agent to the tissue.

[00706] A method for treating or preventing inflammation or promoting healthy joints has been described by Stock et al and is taught for example in US Patent Publication 20090155186, the contents of which are incorporated herein by reference in their entirety. According to Stock, multiple needles are incorporated in a device which administers compositions containing signal transduction modulator compounds.

[00707] A multi-site injection system has been described by Kimmell et al. and is taught for example in US Patent Publication 20100256594, the contents of which are incorporated herein by reference in their entirety. According to Kimmell, multiple needles are incorporated into a device which delivers a medication into a stratum corneum through the needles.

[00708] A method for delivering interferons to the intradermal compartment has been described by Dekker et al. and is taught for example in US Patent Publication 20050181033, the contents of which are incorporated herein by reference in their entirety. According to Dekker, multiple needles having an outlet with an exposed height between 0 and 1 mm are incorporated into a device which improves pharmacokinetics and bioavailability by delivering the substance at a depth between 0.3 mm and 2 mm.

[00709] A method for delivering genes, enzymes and biological agents to tissue cells has described by Desai and is taught for example in US Patent Publication 20030073908, the contents of which are incorporated herein by reference in their entirety. According to Desai, multiple needles are incorporated into a device which is inserted into a body and delivers a medication fluid through said needles.

[00710] A method for treating cardiac arrhythmias with fibroblast cells has been described by Lee et al and is taught for example in US Patent Publication 20040005295, the contents of which are incorporated herein by reference in their entirety. According to Lee, multiple needles are incorporated into the device which delivers fibroblast cells into the local region of the tissue.

[00711] A method using a magnetically controlled pump for treating a brain tumor has been described by Shachar et al. and is taught for example in US Patent 7,799,012 (method) and 7,799,016 (device), the contents of which are incorporated herein by reference in their entirety. According Shachar, multiple needles were incorporated into the pump which pushes a medicating agent through the needles at a controlled rate.

[00712] Methods of treating functional disorders of the bladder in mammalian females have been described by Versi et al. and are taught for example in US Patent 8,029,496, the contents of which are incorporated herein by reference in their entirety. According to Versi, an array of micro-needles is incorporated into a device which delivers a therapeutic agent through the needles directly into the trigone of the bladder.

[00713] A micro-needle transdermal transport device has been described by Angel et al and is taught for example in US Patent 7,364,568, the contents of which are incorporated herein by reference in their entirety. According to Angel, multiple needles are incorporated into the device which transports a substance into a body surface through the needles which are inserted into the surface from different directions. The micro-needle transdermal transport device may be a solid micro-needle system or a hollow micro-needle system. As a non-limiting example, the solid micro-needle system may have up to a 0.5 mg capacity, with 300-1500 solid micro-needles per cm² about 150-700 μm tall coated with a drug. The micro-needles penetrate the stratum corneum and remain in the skin for short duration (e.g., 20 seconds to 15 minutes). In another example, the hollow micro-needle system has up to a 3 mL capacity to deliver liquid formulations using 15-20 microneedles per cm² being approximately 950 μm tall. The micro-needles penetrate the skin to allow the liquid formulations to flow from the device into the skin. The hollow micro-needle system may be worn from 1 to 30 minutes depending on the formulation volume and viscosity.

[00714] A device for subcutaneous infusion has been described by Dalton et al and is taught for example in US Patent 7,150,726, the contents of which are incorporated herein by reference in their entirety. According to Dalton, multiple needles are incorporated into the device which delivers fluid through the needles into a subcutaneous tissue.

[00715] A device and a method for intradermal delivery of vaccines and gene therapeutic agents through microcannula have been described by Mikszta et al. and are

taught for example in US Patent 7,473,247, the contents of which are incorporated herein by reference in their entirety. According to Mitszta, at least one hollow micro-needle is incorporated into the device which delivers the vaccines to the subject's skin to a depth of between 0.025 mm and 2 mm.

[00716] A method of delivering insulin has been described by Pettis et al and is taught for example in US Patent 7,722,595, the contents of which are incorporated herein by reference in their entirety. According to Pettis, two needles are incorporated into a device wherein both needles insert essentially simultaneously into the skin with the first at a depth of less than 2.5 mm to deliver insulin to intradermal compartment and the second at a depth of greater than 2.5 mm and less than 5.0 mm to deliver insulin to subcutaneous compartment.

[00717] Cutaneous injection delivery under suction has been described by Kochamba et al. and is taught for example in US Patent 6,896,666, the contents of which are incorporated herein by reference in their entirety. According to Kochamba, multiple needles in relative adjacency with each other are incorporated into a device which injects a fluid below the cutaneous layer.

[00718] A device for withdrawing or delivering a substance through the skin has been described by Down et al and is taught for example in US Patent 6,607,513, the contents of which are incorporated herein by reference in their entirety. According to Down, multiple skin penetrating members which are incorporated into the device have lengths of about 100 microns to about 2000 microns and are about 30 to 50 gauge.

[00719] A device for delivering a substance to the skin has been described by Palmer et al and is taught for example in US Patent 6,537,242, the contents of which are incorporated herein by reference in their entirety. According to Palmer, an array of micro-needles is incorporated into the device which uses a stretching assembly to enhance the contact of the needles with the skin and provides a more uniform delivery of the substance.

[00720] A perfusion device for localized drug delivery has been described by Zamoyski and is taught for example in US Patent 6,468,247, the contents of which are incorporated herein by reference in their entirety. According to Zamoyski, multiple

hypodermic needles are incorporated into the device which injects the contents of the hypodermics into a tissue as said hypodermics are being retracted.

[00721] A method for enhanced transport of drugs and biological molecules across tissue by improving the interaction between micro-needles and human skin has been described by Prausnitz et al. and is taught for example in US Patent 6,743,211, the contents of which are incorporated herein by reference in their entirety. According to Prausnitz, multiple micro-needles are incorporated into a device which is able to present a more rigid and less deformable surface to which the micro-needles are applied.

[00722] A device for intraorgan administration of medicinal agents has been described by Ting et al and is taught for example in US Patent 6,077,251, the contents of which are incorporated herein by reference in their entirety. According to Ting, multiple needles having side openings for enhanced administration are incorporated into a device which by extending and retracting said needles from and into the needle chamber forces a medicinal agent from a reservoir into said needles and injects said medicinal agent into a target organ.

[00723] A multiple needle holder and a subcutaneous multiple channel infusion port has been described by Brown and is taught for example in US Patent 4,695,273, the contents of which are incorporated herein by reference in their entirety. According to Brown, multiple needles on the needle holder are inserted through the septum of the infusion port and communicate with isolated chambers in said infusion port.

[00724] A dual hypodermic syringe has been described by Horn and is taught for example in US Patent 3,552,394, the contents of which are incorporated herein by reference in their entirety. According to Horn, two needles incorporated into the device are spaced apart less than 68 mm and may be of different styles and lengths, thus enabling injections to be made to different depths.

[00725] A syringe with multiple needles and multiple fluid compartments has been described by Hershberg and is taught for example in US Patent 3,572,336, the contents of which are incorporated herein by reference in their entirety. According to Hershberg, multiple needles are incorporated into the syringe which has multiple fluid compartments and is capable of simultaneously administering incompatible drugs which are not able to be mixed for one injection.

[00726] A surgical instrument for intradermal injection of fluids has been described by Eliscu et al. and is taught for example in US Patent 2,588,623, the contents of which are incorporated herein by reference in their entirety. According to Eliscu, multiple needles are incorporated into the instrument which injects fluids intradermally with a wider disperse.

[00727] An apparatus for simultaneous delivery of a substance to multiple breast milk ducts has been described by Hung and is taught for example in EP 1818017, the contents of which are incorporated herein by reference in their entirety. According to Hung, multiple lumens are incorporated into the device which inserts through the orifices of the ductal networks and delivers a fluid to the ductal networks.

[00728] A catheter for introduction of medications to the tissue of a heart or other organs has been described by Tkebuchava and is taught for example in WO2006138109, the contents of which are incorporated herein by reference in their entirety. According to Tkebuchava, two curved needles are incorporated which enter the organ wall in a flattened trajectory.

[00729] Devices for delivering medical agents have been described by McKay et al. and are taught for example in WO2006118804, the content of which are incorporated herein by reference in their entirety. According to McKay, multiple needles with multiple orifices on each needle are incorporated into the devices to facilitate regional delivery to a tissue, such as the interior disc space of a spinal disc.

[00730] A method for directly delivering an immunomodulatory substance into an intradermal space within a mammalian skin has been described by Pettis and is taught for example in WO2004020014, the contents of which are incorporated herein by reference in their entirety. According to Pettis, multiple needles are incorporated into a device which delivers the substance through the needles to a depth between 0.3 mm and 2 mm.

[00731] Methods and devices for administration of substances into at least two compartments in skin for systemic absorption and improved pharmacokinetics have been described by Pettis et al. and are taught for example in WO2003094995, the contents of which are incorporated herein by reference in their entirety. According to Pettis, multiple needles having lengths between about 300 μm and about 5 mm are incorporated into a

device which delivers to intradermal and subcutaneous tissue compartments simultaneously.

[00732] A drug delivery device with needles and a roller has been described by Zimmerman et al. and is taught for example in WO2012006259, the contents of which are incorporated herein by reference in their entirety. According to Zimmerman, multiple hollow needles positioned in a roller are incorporated into the device which delivers the content in a reservoir through the needles as the roller rotates.

Methods and Devices utilizing catheters and/or lumens

[00733] Methods and devices using catheters and lumens may be employed to administer the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention on a single, multi- or split dosing schedule. Such methods and devices are described below.

[00734] A catheter-based delivery of skeletal myoblasts to the myocardium of damaged hearts has been described by Jacoby et al and is taught for example in US Patent Publication 20060263338, the contents of which are incorporated herein by reference in their entirety. According to Jacoby, multiple needles are incorporated into the device at least part of which is inserted into a blood vessel and delivers the cell composition through the needles into the localized region of the subject's heart.

[00735] An apparatus for treating asthma using neurotoxin has been described by Deem et al and is taught for example in US Patent Publication 20060225742, the contents of which are incorporated herein by reference in their entirety. According to Deem, multiple needles are incorporated into the device which delivers neurotoxin through the needles into the bronchial tissue.

[00736] A method for administering multiple-component therapies has been described by Nayak and is taught for example in US Patent 7,699,803, the contents of which are incorporated herein by reference in their entirety. According to Nayak, multiple injection cannulas may be incorporated into a device wherein depth slots may be included for controlling the depth at which the therapeutic substance is delivered within the tissue.

[00737] A surgical device for ablating a channel and delivering at least one therapeutic agent into a desired region of the tissue has been described by McIntyre et al and is taught for example in US Patent 8,012,096, the contents of which are incorporated herein

by reference in their entirety. According to McIntyre, multiple needles are incorporated into the device which dispenses a therapeutic agent into a region of tissue surrounding the channel and is particularly well suited for transmymocardial revascularization operations.

[00738] Methods of treating functional disorders of the bladder in mammalian females have been described by Versi et al and are taught for example in US Patent 8,029,496, the contents of which are incorporated herein by reference in their entirety. According to Versi, an array of micro-needles is incorporated into a device which delivers a therapeutic agent through the needles directly into the trigone of the bladder.

[00739] A device and a method for delivering fluid into a flexible biological barrier have been described by Yeshurun et al. and are taught for example in US Patent 7,998,119 (device) and 8,007,466 (method), the contents of which are incorporated herein by reference in their entirety. According to Yeshurun, the micro-needles on the device penetrate and extend into the flexible biological barrier and fluid is injected through the bore of the hollow micro-needles.

[00740] A method for epicardially injecting a substance into an area of tissue of a heart having an epicardial surface and disposed within a torso has been described by Bonner et al and is taught for example in US Patent 7,628,780, the contents of which are incorporated herein by reference in their entirety. According to Bonner, the devices have elongate shafts and distal injection heads for driving needles into tissue and injecting medical agents into the tissue through the needles.

[00741] A device for sealing a puncture has been described by Nielsen et al and is taught for example in US Patent 7,972,358, the contents of which are incorporated herein by reference in their entirety. According to Nielsen, multiple needles are incorporated into the device which delivers a closure agent into the tissue surrounding the puncture tract.

[00742] A method for myogenesis and angiogenesis has been described by Chiu et al. and is taught for example in US Patent 6,551,338, the contents of which are incorporated herein by reference in their entirety. According to Chiu, 5 to 15 needles having a maximum diameter of at least 1.25 mm and a length effective to provide a puncture depth of 6 to 20 mm are incorporated into a device which inserts into proximity with a

myocardium and supplies an exogeneous angiogenic or myogenic factor to said myocardium through the conduits which are in at least some of said needles.

[00743] A method for the treatment of prostate tissue has been described by Bolmsj et al. and is taught for example in US Patent 6,524,270, the contents of which are incorporated herein by reference in their entirety. According to Bolmsj, a device comprising a catheter which is inserted through the urethra has at least one hollow tip extendible into the surrounding prostate tissue. An astringent and analgesic medicine is administered through said tip into said prostate tissue.

[00744] A method for infusing fluids to an intraosseous site has been described by Findlay et al. and is taught for example in US Patent 6,761,726, the contents of which are incorporated herein by reference in their entirety. According to Findlay, multiple needles are incorporated into a device which is capable of penetrating a hard shell of material covered by a layer of soft material and delivers a fluid at a predetermined distance below said hard shell of material.

[00745] A device for injecting medications into a vessel wall has been described by Vigil et al. and is taught for example in US Patent 5,713,863, the contents of which are incorporated herein by reference in their entirety. According to Vigil, multiple injectors are mounted on each of the flexible tubes in the device which introduces a medication fluid through a multi-lumen catheter, into said flexible tubes and out of said injectors for infusion into the vessel wall.

[00746] A catheter for delivering therapeutic and/or diagnostic agents to the tissue surrounding a bodily passageway has been described by Faxon et al. and is taught for example in US Patent 5,464,395, the contents of which are incorporated herein by reference in their entirety. According to Faxon, at least one needle cannula is incorporated into the catheter which delivers the desired agents to the tissue through said needles which project outboard of the catheter.

[00747] Balloon catheters for delivering therapeutic agents have been described by Orr and are taught for example in WO2010024871, the contents of which are incorporated herein by reference in their entirety. According to Orr, multiple needles are incorporated into the devices which deliver the therapeutic agents to different depths within the tissue.

Methods and Devices utilizing electrical current

[00748] Methods and devices utilizing electric current may be employed to deliver the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention according to the single, multi- or split dosing regimens taught herein. Such methods and devices are described below.

[00749] An electro collagen induction therapy device has been described by Marquez and is taught for example in US Patent Publication 20090137945, the contents of which are incorporated herein by reference in their entirety. According to Marquez, multiple needles are incorporated into the device which repeatedly pierce the skin and draw in the skin a portion of the substance which is applied to the skin first.

[00750] An electrokinetic system has been described by Etheredge et al. and is taught for example in US Patent Publication 20070185432, the contents of which are incorporated herein by reference in their entirety. According to Etheredge, micro-needles are incorporated into a device which drives by an electrical current the medication through the needles into the targeted treatment site.

[00751] An iontophoresis device has been described by Matsumura et al. and is taught for example in US Patent 7,437,189, the contents of which are incorporated herein by reference in their entirety. According to Matsumura, multiple needles are incorporated into the device which is capable of delivering ionizable drug into a living body at higher speed or with higher efficiency.

[00752] Intradermal delivery of biologically active agents by needle-free injection and electroporation has been described by Hoffmann et al and is taught for example in US Patent 7,171,264, the contents of which are incorporated herein by reference in their entirety. According to Hoffmann, one or more needle-free injectors are incorporated into an electroporation device and the combination of needle-free injection and electroporation is sufficient to introduce the agent into cells in skin, muscle or mucosa.

[00753] A method for electroporation-mediated intracellular delivery has been described by Lundkvist et al. and is taught for example in US Patent 6,625,486, the contents of which are incorporated herein by reference in their entirety. According to Lundkvist, a pair of needle electrodes is incorporated into a catheter. Said catheter is positioned into a body lumen followed by extending said needle electrodes to penetrate into the tissue surrounding said lumen. Then the device introduces an agent through at

least one of said needle electrodes and applies electric field by said pair of needle electrodes to allow said agent pass through the cell membranes into the cells at the treatment site.

[00754] A delivery system for transdermal immunization has been described by Levin et al. and is taught for example in WO2006003659, the contents of which are incorporated herein by reference in their entirety. According to Levin, multiple electrodes are incorporated into the device which applies electrical energy between the electrodes to generate micro channels in the skin to facilitate transdermal delivery.

[00755] A method for delivering RF energy into skin has been described by Schomacker and is taught for example in WO201 1163264, the contents of which are incorporated herein by reference in their entirety. According to Schomacker, multiple needles are incorporated into a device which applies vacuum to draw skin into contact with a plate so that needles insert into skin through the holes on the plate and deliver RF energy.

Definitions

[00756] At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "C₁₋₆ alkyl" is specifically intended to individually disclose methyl, ethyl, C₃ alkyl, C₄ alkyl, C₅ alkyl, and C₆ alkyl.

[00757] *About:* As used herein, the term "about" means +/- 10% of the recited value.

[00758] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans at any stage of development. In some embodiments, "animal" refers to non-human animals at any stage of development. In certain embodiments, the non-human animal is a mammal {e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and worms. In some embodiments, the animal is a transgenic animal, genetically-engineered animal, or a clone.

[00759] *Approximately:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated

reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[00760] *Associated with:* As used herein, the terms "associated with," "conjugated," "linked," "attached," and "tethered," when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, *e.g.*, physiological conditions. An "association" need not be strictly through direct covalent chemical bonding. It may also suggest ionic or hydrogen bonding or a hybridization based connectivity sufficiently stable such that the "associated" entities remain physically associated.

[00761] *Auxotrophic:* As used herein, the term "auxotrophic" refers to mRNA that comprises at least one feature that triggers or induces the degradation or inactivation of the mRNA such that the protein expression is substantially prevented or reduced in a selected tissue or organ.

[00762] *Bifunctional:* As used herein, the term "bifunctional" refers to any substance, molecule or moiety which is capable of or maintains at least two functions. The functions may effect the same outcome or a different outcome. The structure that produces the function may be the same or different. For example, bifunctional modified RNAs of the present invention may encode a cytotoxic peptide (a first function) while those nucleosides which comprise the encoding RNA are, in and of themselves, cytotoxic (second function). In this example, delivery of the bifunctional modified RNA to a cancer cell would produce not only a peptide or protein molecule which may ameliorate or treat the cancer but would also deliver a cytotoxic payload of nucleosides to the cell should degradation, instead of translation of the modified RNA, occur.

[00763] *Biocompatible:* As used herein, the term "biocompatible" means compatible with living cells, tissues, organs or systems posing little to no risk of injury, toxicity or rejection by the immune system.

[00764] *Biodegradable:* As used herein, the term "biodegradable" means capable of being broken down into innocuous products by the action of living things.

[00765] *Biologically active:* As used herein, the phrase "biologically active" refers to a characteristic of any substance that has activity in a biological system and/or organism. For instance, a substance that, when administered to an organism, has a biological affect on that organism, is considered to be biologically active. In particular embodiments, a nucleic acid molecule of the present invention may be considered biologically active if even a portion of the nucleic acid molecule is biologically active or mimics an activity considered biologically relevant.

[00766] *Chemical terms:* The following provides the definition of various chemical terms from "acyl" to "thiol."

[00767] The term "acyl," as used herein, represents a hydrogen or an alkyl group (e.g., a haloalkyl group), as defined herein, that is attached to the parent molecular group through a carbonyl group, as defined herein, and is exemplified by formyl (i.e., a carboxyaldehyde group), acetyl, propionyl, butanoyl and the like. Exemplary unsubstituted acyl groups include from 1 to 7, from 1 to 11, or from 1 to 21 carbons. In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein.

[00768] The term "acylamino," as used herein, represents an acyl group, as defined herein, attached to the parent molecular group through an amino group, as defined herein (i.e., $-N(R^{N1})-C(=O)-R$, where R is H or an optionally substituted C_{1-6} , C_{1-10} , or C_{1-20} alkyl group and R^{N1} is as defined herein). Exemplary unsubstituted acylamino groups include from 1 to 41 carbons (e.g., from 1 to 7, from 1 to 13, from 1 to 21, from 2 to 7, from 2 to 13, from 2 to 21, or from 2 to 41 carbons). In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein, and/or the amino group is $-NH_2$ or $-NHR^{N1}$, wherein R^{N1} is, independently, OH, NO_2 , NH_2 , NR^{N2}_2 , SO_2OR^{N2} , SO_2R^{N2} , SOR^{N2} , alkyl, or aryl, and each R^{N2} can be H, alkyl, or aryl.

[00769] The term "acyloxy," as used herein, represents an acyl group, as defined herein, attached to the parent molecular group through an oxygen atom (i.e., -O-C(=O)-R, where R is H or an optionally substituted C₁₋₆, C₁₋₁₀, or C₁₋₂₀ alkyl group). Exemplary unsubstituted acyloxy groups include from 1 to 21 carbons (e.g., from 1 to 7 or from 1 to 11 carbons). In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein, and/or the amino group is -NH₂ or -NHR^{N1}, wherein R^{N1} is, independently, OH, NO₂, NH₂, NR^{N2}₂, SO₂OR^{N2}, SO₂R^{N2}, SOR^{N2}, alkyl, or aryl, and each R^{N2} can be H, alkyl, or aryl.

[00770] The term "alkaryl," as used herein, represents an aryl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkaryl groups are from 7 to 30 carbons (e.g., from 7 to 16 or from 7 to 20 carbons, such as C₁₋₆ alk-C₆₋₁₀ aryl, C₁₋₁₀ alk-C₆₋₁₀ aryl, or C₁₋₂₀ alk-C₆₋₁₀ aryl). In some embodiments, the alkylene and the aryl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective groups. Other groups preceded by the prefix "alk-" are defined in the same manner, where "alk" refers to a C₁₋₆ alkylene, unless otherwise noted, and the attached chemical structure is as defined herein.

[00771] The term "alkcycloalkyl" represents a cycloalkyl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein (e.g., an alkylene group of from 1 to 4, from 1 to 6, from 1 to 10, or from 1 to 20 carbons). In some embodiments, the alkylene and the cycloalkyl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00772] The term "alkenyl," as used herein, represents monovalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds and is exemplified by ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, and the like. Alkenyls include both cis and trans isomers. Alkenyl groups may be optionally substituted with 1, 2, 3, or 4 substituent groups that are selected, independently, from amino, aryl, cycloalkyl, or heterocyclyl (e.g., heteroaryl), as defined herein, or any of the exemplary alkyl substituent groups described herein.

[00773] The term "alkenyloxy" represents a chemical substituent of formula -OR, where R is a C₂₋₂₀ alkenyl group (e.g., C₂₋₆ or C₂₋₁₀ alkenyl), unless otherwise specified. Exemplary alkenyloxy groups include ethenyloxy, propenyloxy, and the like. In some embodiments, the alkenyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., a hydroxy group).

[00774] The term "alkheteroaryl" refers to a heteroaryl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkheteroaryl groups are from 2 to 32 carbons (e.g., from 2 to 22, from 2 to 18, from 2 to 17, from 2 to 16, from 3 to 15, from 2 to 14, from 2 to 13, or from 2 to 12 carbons, such as Ci₆ alk-Ci_{i2} heteroaryl, C₁₋₁₀ alk-Ci_{i2} heteroaryl, or Ci₂₀ alk-Ci_{i2} heteroaryl). In some embodiments, the alkylene and the heteroaryl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group. Alkheteroaryl groups are a subset of alkheterocyclyl groups.

[00775] The term "alkheterocyclyl" represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkheterocyclyl groups are from 2 to 32 carbons (e.g., from 2 to 22, from 2 to 18, from 2 to 17, from 2 to 16, from 3 to 15, from 2 to 14, from 2 to 13, or from 2 to 12 carbons, such as Ci₆ alk-Ci_{i2} heterocyclyl, C₁₋₁₀ alk-Ci_{i2} heterocyclyl, or Ci₂₀ alk-Ci_{i2} heterocyclyl). In some embodiments, the alkylene and the heterocyclyl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00776] The term "alkoxy" represents a chemical substituent of formula -OR, where R is a Ci₂₀ alkyl group (e.g., Ci₆ or C₁₋₁₀ alkyl), unless otherwise specified. Exemplary alkoxy groups include methoxy, ethoxy, propoxy (e.g., n-propoxy and isopropoxy), t-butoxy, and the like. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., hydroxy or alkoxy).

[00777] The term "alkoxyalkoxy" represents an alkoxy group that is substituted with an alkoxy group. Exemplary unsubstituted alkoxyalkoxy groups include between 2 to 40 carbons (e.g., from 2 to 12 or from 2 to 20 carbons, such as Ci₆ alkoxy-Ci₆ alkoxy, C₁₋₁₀ alkoxy-Ci₁₀ alkoxy, or Ci₂₀ alkoxy-Ci₂₀ alkoxy). In some embodiments, the each

alkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00778] The term "alkoxyalkyl" represents an alkyl group that is substituted with an alkoxy group. Exemplary unsubstituted alkoxyalkyl groups include between 2 to 40 carbons (e.g., from 2 to 12 or from 2 to 20 carbons, such as **Ci₆ alkoxy-Ci₆ alkyl**, **C₁₋₁₀ alkoxy-Ci₁₀ alkyl**, or **Ci₂₀ alkoxy-Ci₂₀ alkyl**). In some embodiments, the alkyl and the alkoxy each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00779] The term "alkoxycarbonyl," as used herein, represents an alkoxy, as defined herein, attached to the parent molecular group through a carbonyl atom (e.g., **-C(0)-OR**, where R is H or an optionally substituted **C₁₋₆**, **C₁₋₁₀**, or **Ci₂₀** alkyl group). Exemplary unsubstituted alkoxycarbonyl include from 1 to 21 carbons (e.g., from 1 to 11 or from 1 to 7 carbons). In some embodiments, the alkoxy group is further substituted with 1, 2, 3, or 4 substituents as described herein.

[00780] The term "alkoxycarbonylalkoxy," as used herein, represents an alkoxy group, as defined herein, that is substituted with an alkoxycarbonyl group, as defined herein (e.g., **-O-alkyl -C(0)-OR**, where R is an optionally substituted **C₁₋₆**, **C₁₋₁₀**, or **Ci₂₀** alkyl group). Exemplary unsubstituted alkoxycarbonylalkoxy include from 3 to 41 carbons (e.g., from 3 to 10, from 3 to 13, from 3 to 17, from 3 to 21, or from 3 to 31 carbons, such as **Ci₆ alkoxycarbonyl-Ci₆ alkoxy**, **C₁₋₁₀ alkoxycarbonyl-Ci₁₀ alkoxy**, or **Ci₂₀ alkoxycarbonyl-Ci₂₀ alkoxy**). In some embodiments, each alkoxy group is further independently substituted with 1, 2, 3, or 4 substituents, as described herein (e.g., a hydroxy group).

[00781] The term "alkoxycarbonylalkyl," as used herein, represents an alkyl group, as defined herein, that is substituted with an alkoxycarbonyl group, as defined herein (e.g., **-alkyl-C(0)-OR**, where R is an optionally substituted **Ci₂₀**, **C₁₋₁₀**, or **Ci₆** alkyl group). Exemplary unsubstituted alkoxycarbonylalkyl include from 3 to 41 carbons (e.g., from 3 to 10, from 3 to 13, from 3 to 17, from 3 to 21, or from 3 to 31 carbons, such as **Ci₆ alkoxycarbonyl-Ci₆ alkyl**, **C₁₋₁₀ alkoxycarbonyl-Ci₁₀ alkyl**, or **Ci₂₀ alkoxycarbonyl-Ci₂₀ alkyl**). In some embodiments, each alkyl and alkoxy group is further independently substituted with 1, 2, 3, or 4 substituents as described herein (e.g., a hydroxy group).

[00782] The term "alkyl," as used herein, is inclusive of both straight chain and branched chain saturated groups from 1 to 20 carbons (e.g., from 1 to 10 or from 1 to 6), unless otherwise specified. Alkyl groups are exemplified by methyl, ethyl, n- and iso-propyl, n-, sec-, iso- and tert-butyl, neopentyl, and the like, and may be optionally substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four substituents independently selected from the group consisting of: (1) C_{1-6} alkoxy; (2) C_{1-6} alkylsulfinyl; (3) amino, as defined herein (e.g., unsubstituted amino (i.e., $-NH_2$) or a substituted amino (i.e., $-N(R^{N1})_2$, where R^{N1} is as defined for amino); (4) C_{6-10} aryl- C_{1-6} alkoxy; (5) azido; (6) halo; (7) (C_{2-9} heterocyclyl)oxy; (8) hydroxy; (9) nitro; (10) oxo (e.g., carboxyaldehyde or acyl); (11) C_{1-7} spirocyclyl; (12) thioalkoxy; (13) thiol; (14) $-C(=O)R^A$, where R^A is selected from the group consisting of (a) C_{1-20} alkyl (e.g., C_{1-6} alkyl), (b) C_{2-20} alkenyl (e.g., C_{2-6} alkenyl), (c) C_{6-10} aryl, (d) hydrogen, (e) C_{1-6} alk- C_{6-10} aryl, (f) amino- C_{1-20} alkyl, (g) polyethylene glycol of $-(CH_2)_{s2}(OCH_2CH_2)_{s3}(CH_2)_{s3}OR'$, wherein $s1$ is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of $s2$ and $s3$, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{1-20} alkyl, and (h) amino-polyethylene glycol of $-NR^{N1}(CH_2)_{s2}(CH_2CH_2O)_{s3}(CH_2)_{s3}NR^{N1}$, wherein $s1$ is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of $s2$ and $s3$, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{1-6} alkyl; (15) $-C(=O)NR^{B'}R^{C'}$, where each of $R^{B'}$ and $R^{C'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) C_{1-6} alkyl, (c) C_{6-10} aryl, and (d) C_{1-6} alk- C_{6-10} aryl; (16) $-SO_2R^D$, where R^D is selected from the group consisting of (a) C_{1-6} alkyl, (b) C_{6-10} aryl, (c) C_{1-6} alk- C_{6-10} aryl, and (d) hydroxy; (17) $-SO_2NR^{E'}R^{F'}$, where each of $R^{E'}$ and $R^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) C_{1-6} alkyl, (c) C_{6-10} aryl and (d) C_{1-6} alk- C_{6-10} aryl; (18) $-C(=O)R^G$, where R^G is selected from the group consisting of (a) C_{1-20} alkyl (e.g., C_{1-6} alkyl), (b) C_{2-20} alkenyl (e.g., C_{2-6} alkenyl), (c) C_{6-10} aryl, (d) hydrogen, (e) C_{1-6} alk- C_{6-10} aryl, (f) amino- C_{1-20} alkyl, (g) polyethylene glycol of $-(CH_2)_{s2}(OCH_2CH_2)_{s3}(CH_2)_{s3}OR'$, wherein $s1$ is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of $s2$ and $s3$, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{1-20} alkyl, and

(h) amino-polyethylene glycol of $-NR^{N1}(CH_2)_{s2}(CH_2CH_2O)_s(CH_2)_{s3}NR^{N1}$, wherein s_i is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s_2 and s_3 , independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{1-6} alkyl; (19) $-NR^{H'}C(0)R^{I'}$, wherein $R^{H'}$ is selected from the group consisting of (a1) hydrogen and (b1) C_{1-6} alkyl, and $R^{I'}$ is selected from the group consisting of (a2) C_{1-20} alkyl (e.g., C_{1-6} alkyl), (b2) C_{2-20} alkenyl (e.g., C_{2-6} alkenyl), (c2) C_{6-10} aryl, (d2) hydrogen, (e2) C_{1-6} alk- C_{6-10} aryl, (f2) amino- C_{1-20} alkyl, (g2) polyethylene glycol of $-(CH_2)_{s2}(OCH_2CH_2)_s(CH_2)_{s3}OR'$, wherein s_i is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s_2 and s_3 , independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{1-20} alkyl, and (h2) amino-polyethylene glycol of $-NR^{N1}(CH_2)_{s2}(CH_2CH_2O)_s(CH_2)_{s3}NR^{N1}$, wherein s_i is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s_2 and s_3 , independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{1-6} alkyl; (20) $-NR^{J'}C(0)OR^{K'}$, wherein $R^{J'}$ is selected from the group consisting of (a1) hydrogen and (b1) C_{1-6} alkyl, and $R^{K'}$ is selected from the group consisting of (a2) C_{1-20} alkyl (e.g., C_{1-6} alkyl), (b2) C_{2-20} alkenyl (e.g., C_{2-6} alkenyl), (c2) C_{6-10} aryl, (d2) hydrogen, (e2) C_{1-6} alk- C_{6-10} aryl, (f2) amino- C_{1-20} alkyl, (g2) polyethylene glycol of $-(CH_2)_{s2}(OCH_2CH_2)_s(CH_2)_{s3}OR'$, wherein s_i is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s_2 and s_3 , independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{1-20} alkyl, and (h2) amino-polyethylene glycol of $-NR^{N1}(CH_2)_{s2}(CH_2CH_2O)_s(CH_2)_{s3}NR^{N1}$, wherein s_i is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s_2 and s_3 , independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{1-6} alkyl; and (21) amidine. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a C_i -alkaryl can be further substituted with an oxo group to afford the respective aryloyl substituent.

[00783] The term "alkylene" and the prefix "alk-," as used herein, represent a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, and is exemplified by methylene, ethylene, isopropylene, and the like. The term " C_{x-y} alkylene" and the prefix " C_{x-y} alk-" represent alkylene groups having between x and y carbons. Exemplary values for x are 1, 2, 3, 4, 5, and 6, and exemplary values for y are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 (e.g., C_{1-6} , C_{1-10} , C_{2-20} , C_{2-6} , C_{2-10} , or C_{2-20} alkylene). In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for an alkyl group.

[00784] The term "alkylsulfonyl," as used herein, represents an alkyl group attached to the parent molecular group through an -S(O)- group. Exemplary unsubstituted alkylsulfonyl groups are from 1 to 6, from 1 to 10, or from 1 to 20 carbons. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00785] The term "alkylsulfinylalkyl," as used herein, represents an alkyl group, as defined herein, substituted by an alkylsulfonyl group. Exemplary unsubstituted alkylsulfinylalkyl groups are from 2 to 12, from 2 to 20, or from 2 to 40 carbons. In some embodiments, each alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00786] The term "alkynyl," as used herein, represents monovalent straight or branched chain groups from 2 to 20 carbon atoms (e.g., from 2 to 4, from 2 to 6, or from 2 to 10 carbons) containing a carbon-carbon triple bond and is exemplified by ethynyl, 1-propynyl, and the like. Alkynyl groups may be optionally substituted with 1, 2, 3, or 4 substituent groups that are selected, independently, from aryl, cycloalkyl, or heterocyclyl (e.g., heteroaryl), as defined herein, or any of the exemplary alkyl substituent groups described herein.

[00787] The term "alkynyloxy" represents a chemical substituent of formula -OR, where R is a C_{2-20} alkynyl group (e.g., C_{2-6} or C_{2-10} alkynyl), unless otherwise specified. Exemplary alkynyloxy groups include ethynyloxy, propynyloxy, and the like. In some embodiments, the alkynyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., a hydroxy group).

[00788] The term "amidine," as used herein, represents a $-C(=NH)NH_2$ group.

[00789] The term "amino," as used herein, represents $-N(R^{N1})_2$, wherein each R^{N1} is, independently, H, OH, NO_2 , $N(R^{N2})_2$, SO_2OR^{N2} , SO_2R^{N2} , SOR^{N2} , an *N*-protecting group, alkyl, alkenyl, alkynyl, alkoxy, aryl, alkaryl, cycloalkyl, alkycycloalkyl, carboxyalkyl, sulfoalkyl, heterocyclyl (e.g., heteroaryl), or alkheterocyclyl (e.g., alkheteroaryl), wherein each of these recited R^{N1} groups can be optionally substituted, as defined herein for each group; or two R^{N1} combine to form a heterocyclyl or an *N*-protecting group, and wherein each R^{N2} is, independently, H, alkyl, or aryl. The amino groups of the invention can be an unsubstituted amino (i.e., $-NH_2$) or a substituted amino (i.e., $-N(R^{N1})_2$). In a preferred embodiment, amino is $-NH_2$ or $-NHR^{N1}$, wherein R^{N1} is, independently, OH, NO_2 , NH_2 , NR^{N2}_2 , SO_2OR^{N2} , SO_2R^{N2} , SOR^{N2} , alkyl, carboxyalkyl, sulfoalkyl, or aryl, and each R^{N2} can be H, C_{1-20} alkyl (e.g., C_{1-6} alkyl), or C_{6-10} aryl.

[00790] The term "amino acid," as described herein, refers to a molecule having a side chain, an amino group, and an acid group (e.g., a carboxy group of $-CO_2H$ or a sulfo group of $-SO_3H$), wherein the amino acid is attached to the parent molecular group by the side chain, amino group, or acid group (e.g., the side chain). In some embodiments, the amino acid is attached to the parent molecular group by a carbonyl group, where the side chain or amino group is attached to the carbonyl group. Exemplary side chains include an optionally substituted alkyl, aryl, heterocyclyl, alkaryl, alkheterocyclyl, aminoalkyl, carbamoylalkyl, and carboxyalkyl. Exemplary amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, hydroxynorvaline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, pyrrollysine, selenocysteine, serine, taurine, threonine, tryptophan, tyrosine, and valine. Amino acid groups may be optionally substituted with one, two, three, or, in the case of amino acid groups of two carbons or more, four substituents independently selected from the group consisting of: (1) C_{1-6} alkoxy; (2) C_{1-6} alkylsulfonyl; (3) amino, as defined herein (e.g., unsubstituted amino (i.e., $-NH_2$) or a substituted amino (i.e., $-N(R^{N1})_2$, where R^{N1} is as defined for amino); (4) C_{6-10} aryl- C_{1-6} alkoxy; (5) azido; (6) halo; (7) $(C_{2-9}$ heterocyclyl)oxy; (8) hydroxy; (9) nitro; (10) oxo (e.g., carboxyaldehyde or acyl); (11) C_{1-7} spirocyclyl; (12) thioalkoxy; (13) thiol; (14) $-CO_2R^A$, where R^A is selected from the group consisting of (a) C_{1-20} alkyl (e.g., C_{1-6}

alkyl), (b) C₂₋₂₀ alkenyl (e.g., C₂₋₆ alkenyl), (c) C₆₋₁₀ aryl, (d) hydrogen, (e) C_{i-6} alk-C₆₋₁₀ aryl, (f) amino-C_{i-20} alkyl, (g) polyethylene glycol of $-(CH_2)_{s2}(OCH_2CH_2)_{s1}(CH_2)_{s3}OR'$, wherein s₁ is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s₂ and s₃, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{i-20} alkyl, and (h) amino-polyethylene glycol of $-NR^{N1}(CH_2)_{s2}(CH_2CH_2O)_s(CH_2)_{s3}NR^{N1}$, wherein s₁ is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s₂ and s₃, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{i-6} alkyl; (15) $-C(0)NR^{B'}R^{C'}$, where each of R^{B'} and R^{C'} is, independently, selected from the group consisting of (a) hydrogen, (b) C_{i-6} alkyl, (c) C_{6-io} aryl, and (d) C_{i-6} alk-C_{6-io} aryl; (16) $-SO_2R^D$, where R^D is selected from the group consisting of (a) C_{i-6} alkyl, (b) C_{6-io} aryl, (c) C_{i-6} alk-C_{6-io} aryl, and (d) hydroxy; (17) $-SO_2NR^{E'}R^{F'}$, where each of R^{E'} and R^{F'} is, independently, selected from the group consisting of (a) hydrogen, (b) C_{i-6} alkyl, (c) C_{6-io} aryl and (d) C_{i-6} alk-C₆₋₁₀ aryl; (18) $-C(0)R^{G'}$, where R^{G'} is selected from the group consisting of (a) C_{i-20} alkyl (e.g., C_{i-6} alkyl), (b) C₂₋₂₀ alkenyl (e.g., C₂₋₆ alkenyl), (c) C₆₋₁₀ aryl, (d) hydrogen, (e) C_{i-6} alk-C_{6-io} aryl, (f) amino-C_{i-20} alkyl, (g) polyethylene glycol of $-(CH_2)_{s2}(OCH_2CH_2)_s(CH_2)_{s3}OR'$, wherein s₁ is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s₂ and s₃, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{i-20} alkyl, and (h) amino-polyethylene glycol of $-NR^{N1}(CH_2)_{s2}(CH_2CH_2O)_s(CH_2)_{s3}NR^{N1}$, wherein s₁ is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s₂ and s₃, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{i-6} alkyl; (19) $-NR^{H'}C(0)R^{I'}$, wherein R^{H'} is selected from the group consisting of (a1) hydrogen and (b1) C_{i-6} alkyl, and R^{I'} is selected from the group consisting of (a2) C_{i-20} alkyl (e.g., C_{i-6} alkyl), (b2) C₂₋₂₀ alkenyl (e.g., C₂₋₆ alkenyl), (c2) C_{6-io} aryl, (d2) hydrogen, (e2) C_{i-6} alk-C_{6-io} aryl, (f2) amino-C_{i-20} alkyl, (g2) polyethylene glycol of $-(CH_2)_{s2}(OCH_2CH_2)_s(CH_2)_{s3}OR'$, wherein s₁ is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s₂ and s₃, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and

R' is **H** or **Ci₂₀** alkyl, and (h2) amino-polyethylene glycol of -

NR^{N1}(CH₂)_{s2}(CH₂CH₂O)_si(CH₂)_{s3}NR^{N1}, wherein **si** is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of **s2** and **s3**, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each **R^{N1}** is, independently, hydrogen or optionally substituted **Ci₆** alkyl; (20) **-NR^{J'}C(OR^{K'})**, wherein **R^{J'}** is selected from the group consisting of (a1) hydrogen and (b1) **Ci₆** alkyl, and **R^{K'}** is selected from the group consisting of (a2) **Ci₂₀** alkyl (e.g., **Ci₆** alkyl), (b2) **C₂₋₂₀** alkenyl (e.g., **C₂₋₆** alkenyl), (c2) **C₆₋₁₀** aryl, (d2) hydrogen, (e2) **Ci₆ alk-C₆₋₁₀** aryl, (f2) amino-**Ci₂₀** alkyl, (g2) polyethylene glycol of **-(CH₂)_{s2}(OCH₂CH₂)_si(CH₂)_{s3}OR'**, wherein **si** is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of **s2** and **s3**, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and **R'** is **H** or **Ci₂₀** alkyl, and (h2) amino-polyethylene glycol of **-NR^{N1}(CH₂)_{s2}(CH₂CH₂O)_si(CH₂)_{s3}NR^{N1}**, wherein **si** is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of **s2** and **s3**, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each **R^{N1}** is, independently, hydrogen or optionally substituted **Ci₆** alkyl; and (21) amidine. In some embodiments, each of these groups can be further substituted as described herein.

[00791] The term "aminoalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by an amino group, as defined herein. The alkyl and amino each can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the respective group (e.g., **CC'₂R^{A'}**, where **R^{A'}** is selected from the group consisting of (a) **Ci₆** alkyl, (b) **C₆₋₁₀** aryl, (c) hydrogen, and (d) **Ci₆ alk-C₆₋₁₀** aryl, e.g., carboxy).

[00792] The term "aminoalkyl," as used herein, represents an alkyl group, as defined herein, substituted by an amino group, as defined herein. The alkyl and amino each can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the respective group (e.g., **CC'₂R^{A'}**, where **R^{A'}** is selected from the group consisting of (a) **Ci₆** alkyl, (b) **C₆₋₁₀** aryl, (c) hydrogen, and (d) **Ci₆ alk-C₆₋₁₀** aryl, e.g., carboxy).

[00793] The term "aryl," as used herein, represents a mono-, bicyclic, or multicyclic carbocyclic ring system having one or two aromatic rings and is exemplified by phenyl, naphthyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, anthracenyl, phenanthrenyl, fluorenyl, indanyl, indenyl, and the like, and may be optionally substituted with 1, 2, 3, 4,

or 5 substituents independently selected from the group consisting of: (1) Ci_{-7} acyl (e.g., carboxyaldehyde); (2) Ci_{-2o} alkyl (e.g., Ci_{-6} alkyl, Ci_{-6} alkoxy- Ci_{-6} alkyl, Ci_{-6} alkylsulfanyl- Ci_{-6} alkyl, amino- Ci_{-6} alkyl, azido- Ci_{-6} alkyl, (carboxyaldehyde)- Ci_{-6} alkyl, halo- Ci_{-6} alkyl (e.g., perfluoroalkyl), hydroxy- Ci_{-6} alkyl, nitro- Ci_{-6} alkyl, or Ci_{-6} thioalkoxy- Ci_{-6} alkyl); (3) Ci_{-2o} alkoxy (e.g., Ci_{-6} alkoxy, such as perfluoroalkoxy); (4) Ci_{-6} alkylsulfanyl; (5) C_{6-10} aryl; (6) amino; (7) Ci_{-6} alk- C_{6-io} aryl; (8) azido; (9) C_{3-8} cycloalkyl; (10) Ci_{-6} alk- C_{3-8} cycloalkyl; (11) halo; (12) C_{1-12} heterocyclyl (e.g., C_{1-12} heteroaryl); (13) (C_{1-12} heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) Ci_{-2o} thioalkoxy (e.g., Ci_{-6} thioalkoxy); (17) $-(CH_2)_qCO_2R^{A'}$, where q is an integer from zero to four, and $R^{A'}$ is selected from the group consisting of (a) Ci_{-6} alkyl, (b) C_{6-io} aryl, (c) hydrogen, and (d) Ci_{-6} alk- C_{6-io} aryl; (18) $-(CH_2)_qCONR^{B'}R^{C'}$, where q is an integer from zero to four and where $R^{B'}$ and $R^{C'}$ are independently selected from the group consisting of (a) hydrogen, (b) Ci_{-6} alkyl, (c) C_{6-io} aryl, and (d) Ci_{-6} alk- C_{6-io} aryl; (19) $-(CH_2)_qSO_2R^{D'}$, where q is an integer from zero to four and where $R^{D'}$ is selected from the group consisting of (a) alkyl, (b) C_{6-io} aryl, and (c) alk- C_{6-io} aryl; (20) $-(CH_2)_qSO_2NR^{E'}R^{F'}$, where q is an integer from zero to four and where each of $R^{E'}$ and $R^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) Ci_{-6} alkyl, (c) C_{6-1o} aryl, and (d) Ci_{-6} alk- C_{6-io} aryl; (21) thiol; (22) C_{6-io} aryloxy; (23) C_{3-8} cycloalkoxy; (24) C_{6-io} aryl- Ci_{-6} alkoxy; (25) Ci_{-6} alk- Ci_{i_2} heterocyclyl (e.g., Ci_{-6} alk- Ci_{i_2} heteroaryl); (26) C_{2-20} alkenyl; and (27) C_{2-2o} alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a Ci -alkaryl or a Ci -alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00794] The term "arylalkoxy," as used herein, represents an alkaryl group, as defined herein, attached to the parent molecular group through an oxygen atom. Exemplary unsubstituted alkoxyalkyl groups include from 7 to 30 carbons (e.g., from 7 to 16 or from 7 to 20 carbons, such as C_{6-io} aryl- Ci_{-6} alkoxy, C_{6-io} aryl- Ci_{i_0} alkoxy, or C_{6-io} aryl- Ci_{-20} alkoxy). In some embodiments, the arylalkoxy group can be substituted with 1, 2, 3, or 4 substituents as defined herein

[00795] The term "aryloxy" represents a chemical substituent of formula -OR', where R' is an aryl group of 6 to 18 carbons, unless otherwise specified. In some embodiments, the aryl group can be substituted with 1, 2, 3, or 4 substituents as defined herein.

[00796] The term "aryloyl," as used herein, represents an aryl group, as defined herein, that is attached to the parent molecular group through a carbonyl group. Exemplary unsubstituted aryloyl groups are of 7 to 11 carbons. In some embodiments, the aryl group can be substituted with 1, 2, 3, or 4 substituents as defined herein.

[00797] The term "azido" represents an -N₃ group, which can also be represented as -N=N=N.

[00798] The term "bicyclic," as used herein, refer to a structure having two rings, which may be aromatic or non-aromatic. Bicyclic structures include spirocyclyl groups, as defined herein, and two rings that share one or more bridges, where such bridges can include one atom or a chain including two, three, or more atoms. Exemplary bicyclic groups include a bicyclic carbocyclyl group, where the first and second rings are carbocyclyl groups, as defined herein; a bicyclic aryl groups, where the first and second rings are aryl groups, as defined herein; bicyclic heterocyclyl groups, where the first ring is a heterocyclyl group and the second ring is a carbocyclyl (e.g., aryl) or heterocyclyl (e.g., heteroaryl) group; and bicyclic heteroaryl groups, where the first ring is a heteroaryl group and the second ring is a carbocyclyl (e.g., aryl) or heterocyclyl (e.g., heteroaryl) group. In some embodiments, the bicyclic group can be substituted with 1, 2, 3, or 4 substituents as defined herein for cycloalkyl, heterocyclyl, and aryl groups.

[00799] The terms "carbocyclic" and "carbocyclyl," as used herein, refer to an optionally substituted C₃₋₁₂ monocyclic, bicyclic, or tricyclic structure in which the rings, which may be aromatic or non-aromatic, are formed by carbon atoms. Carbocyclic structures include cycloalkyl, cycloalkenyl, and aryl groups.

[00800] The term "carbamoyl," as used herein, represents -C(=O)-N(R^{N1})₂, where the meaning of each R^{N1} is found in the definition of "amino" provided herein.

[00801] The term "carbamoylalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a carbamoyl group, as defined herein. The alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00802] The term "carbamyl," as used herein, refers to a carbamate group having the structure

$\text{-NR}^{\text{N}1}\text{C(=O)OR}$ or $\text{-OC(=O)N(R}^{\text{N}1})_2$, where the meaning of each $\text{R}^{\text{N}1}$ is found in the definition of "amino" provided herein, and R is alkyl, cycloalkyl, alkylcycloalkyl, aryl, alkaryl, heterocyclyl (e.g., heteroaryl), or alkheterocyclyl (e.g., alkheteroaryl), as defined herein.

[00803] The term "carbonyl," as used herein, represents a C(O) group, which can also be represented as C=O .

[00804] The term "carboxyaldehyde" represents an acyl group having the structure -CHO .

[00805] The term "carboxy," as used herein, means $\text{-CO}_2\text{H}$.

[00806] The term "carboxyalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by a carboxy group, as defined herein. The alkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the alkyl group.

[00807] The term "carboxyalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a carboxy group, as defined herein. The alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00808] The term "cyano," as used herein, represents an -CN group.

[00809] The term "cycloalkoxy" represents a chemical substituent of formula -OR , where R is a C_{3-8} cycloalkyl group, as defined herein, unless otherwise specified. The cycloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein. Exemplary unsubstituted cycloalkoxy groups are from 3 to 8 carbons. In some embodiment, the cycloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00810] The term "cycloalkyl," as used herein represents a monovalent saturated or unsaturated non-aromatic cyclic hydrocarbon group from three to eight carbons, unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, bicyclo[2.2.1.]heptyl, and the like. When the cycloalkyl group includes one carbon-carbon double bond, the cycloalkyl group can be referred to as a "cycloalkenyl" group. Exemplary cycloalkenyl groups include cyclopentenyl,

cyclohexenyl, and the like. The cycloalkyl groups of this invention can be optionally substituted with: (1) Ci_{1-7} acyl (e.g., carboxy aldehyde); (2) Ci_{1-20} alkyl (e.g., Ci_{1-6} alkyl, Ci_{1-6} alkoxy- Ci_{1-6} alkyl, Ci_{1-6} alkylsulfinyl- Ci_{1-6} alkyl, amino- Ci_{1-6} alkyl, azido- Ci_{1-6} alkyl, (carboxyaldehyde)- Ci_{1-6} alkyl, halo- Ci_{1-6} alkyl (e.g., perfluoroalkyl), hydroxy- Ci_{1-6} alkyl, nitro- Ci_{1-6} alkyl, or Ci_{1-6} thioalkoxy- Ci_{1-6} alkyl); (3) Ci_{1-20} alkoxy (e.g., Ci_{1-6} alkoxy, such as perfluoroalkoxy); (4) Ci_{1-6} alkylsulfinyl; (5) C_{6-10} aryl; (6) amino; (7) Ci_{1-6} alk- C_{6-10} aryl; (8) azido; (9) C_{3-8} cycloalkyl; (10) Ci_{1-6} alk- C_{3-8} cycloalkyl; (11) halo; (12) C_{1-12} heterocyclyl (e.g., C_{1-12} heteroaryl); (13) (C_{1-12} heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) Ci_{1-20} thioalkoxy (e.g., Ci_{1-6} thioalkoxy); (17) $-(CH_2)_q C(=O)R^{A'}$, where q is an integer from zero to four, and $R^{A'}$ is selected from the group consisting of (a) Ci_{1-6} alkyl, (b) C_{6-10} aryl, (c) hydrogen, and (d) Ci_{1-6} alk- C_{6-10} aryl; (18) $-(CH_2)_q CONR^{B'}R^{C'}$, where q is an integer from zero to four and where $R^{B'}$ and $R^{C'}$ are independently selected from the group consisting of (a) hydrogen, (b) C_{6-10} alkyl, (c) C_{6-10} aryl, and (d) Ci_{1-6} alk- C_{6-10} aryl; (19) $-(CH_2)_q SO_2 R^{D'}$, where q is an integer from zero to four and where $R^{D'}$ is selected from the group consisting of (a) C_{6-10} alkyl, (b) C_{6-10} aryl, and (c) Ci_{1-6} alk- C_{6-10} aryl; (20) $-(CH_2)_q SO_2 NR^{E'}R^{F'}$, where q is an integer from zero to four and where each of $R^{E'}$ and $R^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) C_{6-10} alkyl, (c) C_{6-10} aryl, and (d) Ci_{1-6} alk- C_{6-10} aryl; (21) thiol; (22) C_{6-10} aryloxy; (23) C_{3-8} cycloalkoxy; (24) C_{6-10} aryl- Ci_{1-6} alkoxy; (25) Ci_{1-6} alk- Ci_{1-12} heterocyclyl (e.g., Ci_{1-6} alk- Ci_{1-12} heteroaryl); (26) oxo; (27) C_{2-20} alkenyl; and (28) C_{2-20} alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a Ci -alkaryl or a Ci -alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00811] The term "diastereomer," as used herein means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

[00812] The term "effective amount" of an agent, as used herein, is that amount sufficient to effect beneficial or desired results, for example, clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that treats cancer, an effective amount of an agent is, for example, an amount sufficient to achieve treatment, as defined herein, of cancer, as compared to the response obtained without administration of the agent.

[00813] The term "enantiomer," as used herein, means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e., at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

[00814] The term "halo," as used herein, represents a halogen selected from bromine, chlorine, iodine, or fluorine.

[00815] The term "haloalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by a halogen group (i.e., F, Cl, Br, or I). A haloalkoxy may be substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four halogens. Haloalkoxy groups include perfluoroalkoxys (e.g., $-\text{OCF}_3$), $-\text{OCHF}_2$, $-\text{OCH}_2\text{F}$, $-\text{OCCl}_3$, $-\text{OCH}_2\text{CH}_2\text{Br}$, $-\text{OCH}_2\text{CH}(\text{CH}_2\text{CH}_2\text{Br})\text{CH}_3$, and $-\text{OCHICH}_3$. In some embodiments, the haloalkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups.

[00816] The term "haloalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a halogen group (i.e., F, Cl, Br, or I). A haloalkyl may be substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four halogens. Haloalkyl groups include perfluoroalkyls (e.g., $-\text{CF}_3$), $-\text{CHF}_2$, $-\text{CH}_2\text{F}$, $-\text{CCl}_3$, $-\text{CH}_2\text{CH}_2\text{Br}$, $-\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_2\text{Br})\text{CH}_3$, and $-\text{CHICH}_3$. In some embodiments, the haloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups.

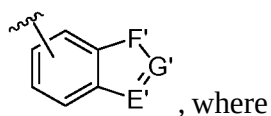
[00817] The term "heteroalkylene," as used herein, refers to an alkylene group, as defined herein, in which one or two of the constituent carbon atoms have each been replaced by nitrogen, oxygen, or sulfur. In some embodiments, the heteroalkylene group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkylene groups.

[00818] The term "heteroaryl," as used herein, represents that subset of heterocyclyls, as defined herein, which are aromatic: i.e., they contain $4n+2$ pi electrons within the mono- or multicyclic ring system. Exemplary unsubstituted heteroaryl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. In some

embodiment, the heteroaryl is substituted with 1, 2, 3, or 4 substituents groups as defined for a heterocyclyl group.

[00819] The term "heterocyclyl," as used herein represents a 5-, 6- or 7-membered ring, unless otherwise specified, containing one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The 5-membered ring has zero to two double bonds, and the 6- and 7-membered rings have zero to three double bonds. Exemplary unsubstituted heterocyclyl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heterocyclyl" also represents a heterocyclic compound having a bridged multicyclic structure in which one or more carbons and/or heteroatoms bridges two non-adjacent members of a monocyclic ring, e.g., a quinuclidinyl group. The term "heterocyclyl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heterocyclic rings is fused to one, two, or three carbocyclic rings, e.g., an aryl ring, a cyclohexane ring, a cyclohexene ring, a cyclopentane ring, a cyclopentene ring, or another monocyclic heterocyclic ring, such as indolyl, quinolyl, isoquinolyl, tetrahydroquinolyl, benzofuryl, benzothienyl and the like. Examples of fused heterocyclyls include tropanes and 1,2,3,5,8,8a-hexahydroindolizine. Heterocyclics include pyrrolyl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, piperidinyl, homopiperidinyl, pyrazinyl, piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiomorpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, indolyl, indazolyl, quinolyl, isoquinolyl, quinoxalinyl, dihydroquinoxalinyl, quinazolinyl, cinnolinyl, phthalazinyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, benzothiadiazolyl, furyl, thienyl, thiazolidinyl, isothiazolyl, triazolyl, tetrazolyl, oxadiazolyl (e.g., 1,2,3-oxadiazolyl), purinyl, thiadiazolyl (e.g., 1,2,3-thiadiazolyl), tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, dihydrothienyl, dihydroindolyl, dihydroquinolyl, tetrahydroquinolyl, tetrahydroisoquinolyl, dihydroisoquinolyl, pyranyl, dihydropyranyl, dithiazolyl, benzofuranyl, isobenzofuranyl, benzothienyl, and the like, including dihydro and tetrahydro forms thereof, where one or more double bonds are reduced and replaced with hydrogens. Still other exemplary heterocyclyls include: 2,3,4,5-tetrahydro-2-oxo-oxazolyl; 2,3-dihydro-2-oxo-1H-imidazolyl; 2,3,4,5-tetrahydro-5-oxo-1H-pyrazolyl (e.g.,

2,3,4,5-tetrahydro-2-phenyl-5-oxo-1H-pyrazolyl); 2,3,4,5-tetrahydro-2,4-dioxo-1H-imidazolyl (e.g., 2,3,4,5-tetrahydro-2,4-dioxo-5-methyl-5-phenyl-1H-imidazolyl); 2,3-dihydro-2-thioxo-1,3,4-oxadiazolyl (e.g., 2,3-dihydro-2-thioxo-5-phenyl-1,3,4-oxadiazolyl); 4,5-dihydro-5-oxo-1H-triazolyl (e.g., 4,5-dihydro-3-methyl-4-amino 5-oxo-1H-triazolyl); 1,2,3,4-tetrahydro-2,4-dioxopyridinyl (e.g., 1,2,3,4-tetrahydro-2,4-dioxo-3,3-diethylpyridinyl); 2,6-dioxo-piperidinyl (e.g., 2,6-dioxo-3-ethyl-3-phenylpiperidinyl); 1,6-dihydro-6-oxopyridiminy; 1,6-dihydro-4-oxopyrimidinyl (e.g., 2-(methylthio)-1,6-dihydro-4-oxo-5-methylpyrimidin-1-yl); 1,2,3,4-tetrahydro-2,4-dioxopyrimidinyl (e.g., 1,2,3,4-tetrahydro-2,4-dioxo-3-ethylpyrimidinyl); 1,6-dihydro-6-oxo-pyridazinyl (e.g., 1,6-dihydro-6-oxo-3-ethylpyridazinyl); 1,6-dihydro-6-oxo-1,2,4-triazinyl (e.g., 1,6-dihydro-5-isopropyl-6-oxo-1,2,4-triazinyl); 2,3-dihydro-2-oxo-1H-indolyl (e.g., 3,3-dimethyl-2,3-dihydro-2-oxo-1H-indolyl and 2,3-dihydro-2-oxo-3,3'-spiropropane-1H-indol-1-yl); 1,3-dihydro-1-oxo-2H-iso-indolyl; 1,3-dihydro-1,3-dioxo-2H-iso-indolyl; 1H-benzopyrazolyl (e.g., 1-(ethoxycarbonyl)-1H-benzopyrazolyl); 2,3-dihydro-2-oxo-1H-benzimidazolyl (e.g., 3-ethyl-2,3-dihydro-2-oxo-1H-benzimidazolyl); 2,3-dihydro-2-oxo-benzoxazolyl (e.g., 5-chloro-2,3-dihydro-2-oxo-benzoxazolyl); 2,3-dihydro-2-oxo-benzoxazolyl; 2-oxo-2H-benzopyranyl; 1,4-benzodioxanyl; 1,3-benzodioxanyl; 2,3-dihydro-3-oxo,4H-1,3-benzothiazinyl; 3,4-dihydro-4-oxo-3H-quinazolinyl (e.g., 2-methyl-3,4-dihydro-4-oxo-3H-quinazolinyl); 1,2,3,4-tetrahydro-2,4-dioxo-3H-quinazolyl (e.g., 1-ethyl-1,2,3,4-tetrahydro-2,4-dioxo-3H-quinazolyl); 1,2,3,6-tetrahydro-2,6-dioxo-7H-purinyl (e.g., 1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7H-purinyl); 1,2,3,6-tetrahydro-2,6-dioxo-1H-purinyl (e.g., 1,2,3,6-tetrahydro-3,7-dimethyl-2,6-dioxo-1H-purinyl); 2-oxobenz[c,i]indolyl; 1,1-dioxo-2H-naphth[1,8-c,i]isothiazolyl; and 1,8-naphthylenedicarboxamido. Additional heterocyclics include 3,3a,4,5,6,6a-hexahydro-pyrrolo[3,4-b]pyrrol-(2H)-yl, and 2,5-diazabicyclo[2.2.1]heptan-2-yl, homopiperazinyl (or diazepanyl), tetrahydropyranyl, dithiazolyl, benzofuranyl, benzothienyl, oxepanyl, thiepanyl, azocanyl, oxecanyl, and thiocanyl. Heterocyclic groups also include groups of the formula



[00820] E' is selected from the group consisting of -N- and -CH-; F' is selected from the group consisting of -N=CH-, -NH-CH₂-, -NH-C(O)-, -NH-, -CH=N-, -CH₂-NH-, -C(O)-NH-, -CH=CH-, -CH₂-, -CH₂CH₂-, -CH₂O-, -OCH₂-, -O-, and -S-; and G' is selected from the group consisting of -CH- and -N-. Any of the heterocyclyl groups mentioned herein may be optionally substituted with one, two, three, four or five substituents independently selected from the group consisting of: (1) Ci₇ acyl (e.g., carboxyaldehyde); (2) Ci₂₀ alkyl (e.g., Ci₆ alkyl, Ci₆ alkoxy-Ci₆ alkyl, Ci₆ alkylsulfmlyl-Ci₆ alkyl, amino-Ci₆ alkyl, azido-Ci₆ alkyl, (carboxyaldehyde)-Ci₆ alkyl, halo-Ci₆ alkyl (e.g., perfluoroalkyl), hydroxy-Ci₆ alkyl, nitro-Ci₆ alkyl, or Ci₆ thioalkoxy-Ci₆ alkyl); (3) Ci₂₀ alkoxy (e.g., Ci₆ alkoxy, such as perfluoroalkoxy); (4) Ci₆ alkylsulfinyl; (5) C₆₋₁₀ aryl; (6) amino; (7) Ci₆ alk-C₆₋₁₀ aryl; (8) azido; (9) C₃₋₈ cycloalkyl; (10) Ci₆ alk-C₃₋₈ cycloalkyl; (11) halo; (12) C₁₋₁₂ heterocyclyl (e.g., C₂₋₁₂ heteroaryl); (13) (C₁₋₁₂ heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) Ci₂₀ thioalkoxy (e.g., Ci₆ thioalkoxy); (17) -(CH₂)_qC(=O)R^{A'}, where q is an integer from zero to four, and R^{A'} is selected from the group consisting of (a) Ci₆ alkyl, (b) C₆₋₁₀ aryl, (c) hydrogen, and (d) Ci₆ alk-C₆₋₁₀ aryl; (18) -(CH₂)_qCONR^{B'}R^{C'}, where q is an integer from zero to four and where R^{B'} and R^{C'} are independently selected from the group consisting of (a) hydrogen, (b) Ci₆ alkyl, (c) C₆₋₁₀ aryl, and (d) Ci₆ alk-C₆₋₁₀ aryl; (19) -(CH₂)_qS(=O)₂R^{D'}, where q is an integer from zero to four and where R^{D'} is selected from the group consisting of (a) Ci₆ alkyl, (b) C₆₋₁₀ aryl, and (c) Ci₆ alk-C₆₋₁₀ aryl; (20) -(CH₂)_qS(=O)₂NR^{E'}R^{F'}, where q is an integer from zero to four and where each of R^{E'} and R^{F'} is, independently, selected from the group consisting of (a) hydrogen, (b) Ci₆ alkyl, (c) C₆₋₁₀ aryl, and (d) Ci₆ alk-C₆₋₁₀ aryl; (21) thiol; (22) C₆₋₁₀ aryloxy; (23) C₃₋₈ cycloalkoxy; (24) arylalkoxy; (25) Ci₆ alk-Ci₁₋₁₂ heterocyclyl (e.g., Ci₆ alk-Ci₁₋₁₂ heteroaryl); (26) oxo; (27) (C₁₋₁₂ heterocyclyl)imino; (28) C₂₋₂₀ alkenyl; and (29) C₂₋₂₀ alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a Ci-alkaryl or a Ci-alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00821] The term "(heterocyclyl)imino," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an imino group.

In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00822] The term "(heterocyclyl)oxy," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an oxygen atom. In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00823] The term "(heterocyclyl)oyl," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through a carbonyl group. In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00824] The term "hydrocarbon," as used herein, represents a group consisting only of carbon and hydrogen atoms.

[00825] The term "hydroxy," as used herein, represents an -OH group.

[00826] The term "hydroxyalkenyl," as used herein, represents an alkenyl group, as defined herein, substituted by one to three hydroxy groups, with the proviso that no more than one hydroxy group may be attached to a single carbon atom of the alkyl group, and is exemplified by dihydroxypropenyl, hydroxyisopentenyl, and the like.

[00827] The term "hydroxyalkyl," as used herein, represents an alkyl group, as defined herein, substituted by one to three hydroxy groups, with the proviso that no more than one hydroxy group may be attached to a single carbon atom of the alkyl group, and is exemplified by hydroxymethyl, dihydroxypropyl, and the like.

[00828] The term "isomer," as used herein, means any tautomer, stereoisomer, enantiomer, or diastereomer of any compound of the invention. It is recognized that the compounds of the invention can have one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (-)) or cis/trans isomers).

According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all of the corresponding stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically

be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

[00829] The term "*N*-protected amino," as used herein, refers to an amino group, as defined herein, to which is attached one or two *N*-protecting groups, as defined herein.

[00830] The term "*N*-protecting group," as used herein, represents those groups intended to protect an amino group against undesirable reactions during synthetic procedures. Commonly used *N*-protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis," 3rd Edition (John Wiley & Sons, New York, 1999), which is incorporated herein by reference. *N*-protecting groups include acyl, aryloyl, or carbamyl groups such as formyl, acetyl, propionyl, pivaloyl, *t*-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, *o*-nitrophenoxyacetyl, *a*-chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and chiral auxiliaries such as protected or unprotected D, L or D, L-amino acids such as alanine, leucine, phenylalanine, and the like; sulfonyl-containing groups such as benzenesulfonyl, *p*-toluenesulfonyl, and the like; carbamate forming groups such as benzyloxycarbonyl, *p*-chlorobenzyloxycarbonyl, *p*-methoxybenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(*p*-biphenyl)-1-methylethoxycarbonyl, α,α -dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxy carbonyl, *t*-butyloxycarbonyl, diisopropylmethoxy carbonyl, isopropylloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxy carbonyl, fluorenyl-9-methoxycarbonyl, cyclopentylloxycarbonyl, adamantylloxycarbonyl, cyclohexylloxycarbonyl, phenylthiocarbonyl, and the like, alkaryl groups such as benzyl, triphenylmethyl, benzyloxymethyl, and the like and silyl groups, such as trimethylsilyl, and the like. Preferred *N*-protecting groups are formyl, acetyl,

benzoyl, pivaloyl, t-butylacetyl, alanyl, phenylsulfonyl, benzyl, t-butyloxycarbonyl (Boc), and benzyloxycarbonyl (Cbz).

[00831] The term "nitro," as used herein, represents an -NO₂ group.

[00832] The term "oxo" as used herein, represents =O.

[00833] The term "perfluoroalkyl," as used herein, represents an alkyl group, as defined herein, where each hydrogen radical bound to the alkyl group has been replaced by a fluoride radical. Perfluoroalkyl groups are exemplified by trifluoromethyl, pentafluoroethyl, and the like.

[00834] The term "perfluoroalkoxy," as used herein, represents an alkoxy group, as defined herein, where each hydrogen radical bound to the alkoxy group has been replaced by a fluoride radical. Perfluoroalkoxy groups are exemplified by trifluoromethoxy, pentafluoroethoxy, and the like.

[00835] The term "spirocyclyl," as used herein, represents a C₂₋₇ alkylene diradical, both ends of which are bonded to the same carbon atom of the parent group to form a spirocyclic group, and also a C₁₋₆ heteroalkylene diradical, both ends of which are bonded to the same atom. The heteroalkylene radical forming the spirocyclyl group can contain one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the spirocyclyl group includes one to seven carbons, excluding the carbon atom to which the diradical is attached. The spirocyclyl groups of the invention may be optionally substituted with 1, 2, 3, or 4 substituents provided herein as optional substituents for cycloalkyl and/or heterocyclyl groups.

[00836] The term "stereoisomer," as used herein, refers to all possible different isomeric as well as conformational forms which a compound may possess (e.g., a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers and/or conformers of the basic molecular structure. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

[00837] The term "sulfoalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a sulfo group of $-SO_3H$. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00838] The term "sulfonyl," as used herein, represents an $-S(O)_2-$ group.

[00839] The term "thioalkaryl," as used herein, represents a chemical substituent of formula $-SR$, where R is an alkaryl group. In some embodiments, the alkaryl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00840] The term "thioalkheterocyclyl," as used herein, represents a chemical substituent of formula $-SR$, where R is an alkheterocyclyl group. In some embodiments, the alkheterocyclyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00841] The term "thioalkoxy," as used herein, represents a chemical substituent of formula $-SR$, where R is an alkyl group, as defined herein. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00842] The term "thiol" represents an $-SH$ group.

[00843] *Compound:* As used herein, the term "compound," as used herein, is meant to include all stereoisomers, geometric isomers, tautomers, and isotopes of the structures depicted.

[00844] The compounds described herein can be asymmetric (*e.g.*, having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

[00845] Compounds of the present disclosure also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond together with the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Example prototropic tautomers include ketone - enol pairs, amide - imidic acid pairs, lactam - lactim pairs, amide - imidic acid pairs, enamine - imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, for example, 1H- and 3H-imidazole, 1H-, 2H- and 4H- 1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution.

[00846] Compounds of the present disclosure also include all of the isotopes of the atoms occurring in the intermediate or final compounds. "Isotopes" refers to atoms having the same atomic number but different mass numbers resulting from a different number of neutrons in the nuclei. For example, isotopes of hydrogen include tritium and deuterium.

[00847] The compounds and salts of the present disclosure can be prepared in combination with solvent or water molecules to form solvates and hydrates by routine methods.

[00848] *Conserved:* As used herein, the term "conserved" refers to nucleotides or amino acid residues of a polynucleotide sequence or polypeptide sequence, respectively, that are those that occur unaltered in the same position of two or more sequences being compared. Nucleotides or amino acids that are relatively conserved are those that are conserved amongst more related sequences than nucleotides or amino acids appearing elsewhere in the sequences.

[00849] In some embodiments, two or more sequences are said to be "completely conserved" if they are 100% identical to one another. In some embodiments, two or more sequences are said to be "highly conserved" if they are at least 70%> identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some embodiments, two or more sequences are said to be "highly conserved" if they are about 70% identical, about 80%> identical, about 90%> identical, about 95%, about 98%>, or about 99% identical to one another. In some embodiments, two or more sequences are

said to be "conserved" if they are at least 30% identical, at least 40% identical, at least 50% identical, at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some embodiments, two or more sequences are said to be "conserved" if they are about 30%> identical, about 40%> identical, about 50%> identical, about 60%> identical, about 70%> identical, about 80%> identical, about 90%> identical, about 95% identical, about 98%> identical, or about 99% identical to one another. Conservation of sequence may apply to the entire length of an oligonucleotide or polypeptide or may apply to a portion, region or feature thereof. Conservation of sequence may apply to the entire length of an oligonucleotide or polypeptide or may apply to a portion, region or feature thereof.

[00850] *Delivery:* As used herein, "delivery" refers to the act or manner of delivering a compound, substance, entity, moiety, cargo or payload.

[00851] *Delivery Agent:* As used herein, "delivery agent" refers to any substance which facilitates, at least in part, the *in vivo* delivery of a modified nucleic acid to targeted cells.

[00852] *Detectable label:* As used herein, "detectable label" refers to one or more markers, signals, or moieties which are attached, incorporated or associated with another entity that is readily detected by methods known in the art including radiography, fluorescence, chemiluminescence, enzymatic activity, absorbance and the like. Detectable labels include radioisotopes, fluorophores, chromophores, enzymes, dyes, metal ions, ligands such as biotin, avidin, streptavidin and haptens, quantum dots, and the like. Detectable labels may be located at any position in the peptides or proteins disclosed herein. They may be within the amino acids, the peptides, or proteins, or located at the N- or C- termini.

[00853] *Device:* As used herein, the term "device" means a piece of equipment designed to serve a special purpose. The device may comprise many features such as, but not limited to, components, electrical (e.g., wiring and circuits), storage modules and analysis modules.

[00854] *Disease:* As used herein, the term "disease" refers to an abnormal condition affecting the body of an organism often showing specific bodily symptoms.

[00855] *Disorder*. As used herein, the term "disorder," refers to a disruption of or an interference with normal functions or established systems of the body.

[00856] *Digest*: As used herein, the term "digest" means to break apart into smaller pieces or components. When referring to polypeptides or proteins, digestion results in the production of peptides.

[00857] *Encoded protein cleavage signal*: As used herein, "encoded protein cleavage signal" refers to the nucleotide sequence which encodes a protein cleavage signal.

[00858] *Engineered*: As used herein, embodiments of the invention are "engineered" when they are designed to have a feature or property, whether structural or chemical, that varies from a starting point, wild type or native molecule.

[00859] *Exosome*: As used herein, "exosome" is a vesicle secreted by mammalian cells or a complex involved in RNA degradation.

[00860] *Expression*: As used herein, "expression" of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (*e.g.*, by transcription); (2) processing of an RNA transcript (*e.g.*, by splicing, editing, 5' cap formation, and/or 3' end processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

[00861] *Feature*: As used herein, a "feature" refers to a characteristic, a property, or a distinctive element.

[00862] *Formulation*: As used herein, a "formulation" includes at least a modified nucleic acid and a delivery agent.

[00863] *Fragment*: A "fragment," as used herein, refers to a portion. For example, fragments of proteins may comprise polypeptides obtained by digesting full-length protein isolated from cultured cells.

[00864] *Functional*: As used herein, a "functional" biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[00865] *Heterologous*: As used herein, the term "heterologous" in reference to an untranslated region such as a 5'UTR or 3'UTR means a region of nucleic acid, particularly untranslated nucleic acid which is not naturally found with the coding region encoded on the same or instant polynucleotide, primary construct or mmRNA.

Homologous UTRs for example would represent those UTRs which are naturally found associated with the coding region of the mRNA, such as the wild type UTR.

[00866] *Homology:* As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, *e.g.* between nucleic acid molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% similar. The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences).

[00867] In accordance with the invention, two polynucleotide sequences are considered to be homologous if the polypeptides they encode are at least about 50% identical, at least about 60% identical, at least about 70% identical, at least about 80% identical, or at least about 90% identical for at least one stretch of at least about 20 amino acids.

[00868] In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a stretch of at least 4-5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in length, homology is determined by the ability to encode a stretch of at least 4-5 uniquely specified amino acids. In accordance with the invention, two protein sequences are considered to be homologous if the proteins are at least about 50% identical, at least about 60% identical, at least about 70% identical, at least about 80% identical, or at least about 90% identical for at least one stretch of at least about 20 amino acids.

[00869] *Identity:* As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, *e.g.*, between oligonucleotide molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of

the percent identity of two polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using methods such as those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; each of which is incorporated herein by reference. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4:1 1-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988); incorporated herein by reference. Techniques for determining

identity are codified in publicly available computer programs. Exemplary computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux, J., *et al.*, *Nucleic Acids Research*, 12(1), 387 (1984)), BLASTP, BLASTN, and FASTA Atschul, S. F. *et al.*, *J. Molec. Biol.*, 215, 403 (1990)).

[00870] *Inhibit expression of a gene:* As used herein, the phrase "inhibit expression of a gene" means to cause a reduction in the amount of an expression product of the gene. The expression product can be an RNA transcribed from the gene (*e.g.*, an mRNA) or a polypeptide translated from an mRNA transcribed from the gene. Typically a reduction in the level of an mRNA results in a reduction in the level of a polypeptide translated therefrom. The level of expression may be determined using standard techniques for measuring mRNA or protein.

[00871] *In vitro:* As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, in a Petri dish, *etc.*, rather than within an organism (*e.g.*, animal, plant, or microbe).

[00872] *In vivo:* As used herein, the term "*in vivo*" refers to events that occur within an organism (*e.g.*, animal, plant, or microbe or cell or tissue thereof).

[00873] *Isolated:* As used herein, the term "isolated" refers to a substance or entity that has been separated from at least some of the components with which it was associated (whether in nature or in an experimental setting). Isolated substances may have varying levels of purity in reference to the substances from which they have been associated. Isolated substances and/or entities may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated agents are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. *Substantially isolated:* By "substantially isolated" is meant that the compound is substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the compound of the present disclosure. Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at

least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of the compound of the present disclosure, or salt thereof. Methods for isolating compounds and their salts are routine in the art.

[00874] *Linker:* As used herein, a linker refers to a group of atoms, e.g., 10-1,000 atoms, and can be comprised of the atoms or groups such as, but not limited to, carbon, amino, alkylamino, oxygen, sulfur, sulfoxide, sulfonyl, carbonyl, and imine. The linker can be attached to a modified nucleoside or nucleotide on the nucleobase or sugar moiety at a first end, and to a payload, e.g., a detectable or therapeutic agent, at a second end. The linker may be of sufficient length as to not interfere with incorporation into a nucleic acid sequence. The linker can be used for any useful purpose, such as to form modified mRNA multimers (e.g., through linkage of two or more modified nucleic acids) or modified mRNA conjugates, as well as to administer a payload, as described herein. Examples of chemical groups that can be incorporated into the linker include, but are not limited to, alkyl, alkenyl, alkynyl, amido, amino, ether, thioether, ester, alkylene, heteroalkylene, aryl, or heterocyclyl, each of which can be optionally substituted, as described herein. Examples of linkers include, but are not limited to, unsaturated alkanes, polyethylene glycols (e.g., ethylene or propylene glycol monomeric units, e.g., diethylene glycol, dipropylene glycol, triethylene glycol, tripropylene glycol, tetraethylene glycol, or tetraethylene glycol), and dextran polymers. Other examples include, but are not limited to, cleavable moieties within the linker, such as, for example, a disulfide bond (-S-S-) or an azo bond (-N=N-), which can be cleaved using a reducing agent or photolysis. Non-limiting examples of a selectively cleavable bond include an amido bond can be cleaved for example by the use of tris(2-carboxyethyl)phosphine (TCEP), or other reducing agents, and/or photolysis, as well as an ester bond can be cleaved for example by acidic or basic hydrolysis.

[00875] *Modified:* As used herein "modified" refers to a changed state or structure of a molecule of the invention. Molecules may be modified in many ways including chemically, structurally, and functionally. In one embodiment, the mRNA molecules of the present invention are modified by the introduction of non-natural nucleosides and/or nucleotides.

[00876] *Naturally occurring:* As used herein, "naturally occurring" means existing in nature without artificial aid.

[00877] *Open reading frame:* As used herein, "open reading frame" or "ORF" refers to a sequence which does not contain a stop codon in a given reading frame.

[00878] *Operably linked:* As used herein, the phrase "operably linked" refers to a functional connection between two or more molecules, constructs, transcripts, entities, moieties or the like.

[00879] *Patient:* As used herein, "patient" refers to a subject who may seek or be in need of treatment, requires treatment, is receiving treatment, will receive treatment, or a subject who is under care by a trained professional for a particular disease or condition.

[00880] *Optionally substituted:* Herein a phrase of the form "optionally substituted X" (*e.g.*, optionally substituted alkyl) is intended to be equivalent to "X, wherein X is optionally substituted" (*e.g.*, "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" (*e.g.* alkyl) *per se* is optional. *Peptide:* As used herein, "peptide" is less than or equal to 50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[00881] *Peptide:* As used herein, "peptide" is less than or equal to 50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[00882] *Pharmaceutical composition:* The phrase "pharmaceutical composition" refers to a composition that alters the etiology of a disease, disorder and/or condition.

[00883] *Pharmaceutically acceptable:* The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00884] *Pharmaceutically acceptable excipients:* The phrase "pharmaceutically acceptable excipient," as used herein, refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a patient. Excipients may include, for example: antiadherents,

antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluent), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspending or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

[00885] *Pharmaceutically acceptable salts:* The present disclosure also includes pharmaceutically acceptable salts of the compounds described herein. As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form (e.g., by reacting the free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and

the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present disclosure can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and *Journal of Pharmaceutical Science*, 66, 2 (1977), each of which is incorporated herein by reference in its entirety.

[00886] *Pharmacokinetic*: As used herein, "pharmacokinetic" refers to any one or more properties of a molecule or compound as it relates to the determination of the fate of substances administered to a living organism. Pharmacokinetics is divided into several areas including the extent and rate of absorption, distribution, metabolism and excretion. This is commonly referred to as ADME where: (A) Absorption is the process of a substance entering the blood circulation; (D) Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body; (M) Metabolism (or Biotransformation) is the irreversible transformation of parent compounds into daughter metabolites; and (E) Excretion (or Elimination) refers to the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in body tissue.

[00887] *Pharmaceutically acceptable solvate*: The term "pharmaceutically acceptable solvate," as used herein, means a compound of the invention wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. For example, solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes

organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), *N*-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), *N,N'*-dimethylformamide (DMF), *N,N'*-dimethylacetamide (DMAC), 1,3-dimethyl-2-imidazolidinone (DMEU), 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a "hydrate."

[00888] *Physicochemical*: As used herein, "physicochemical" means of or relating to a physical and/or chemical property.

[00889] *Preventing*: As used herein, the term "preventing" refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition.

[00890] *Prodrug*: The present disclosure also includes prodrugs of the compounds described herein. As used herein, "prodrugs" refer to any substance, molecule or entity which is in a form predicate for that substance, molecule or entity to act as a therapeutic upon chemical or physical alteration. Prodrugs may be covalently bonded or sequestered in some way and which release or are converted into the active drug moiety prior to, upon or after administered to a mammalian subject. Prodrugs can be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compounds. Prodrugs include compounds wherein hydroxyl, amino, sulfhydryl, or carboxyl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, sulfhydryl, or carboxyl group respectively. Preparation and use of prodrugs is discussed in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in

Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference in their entirety.

[00891] *Protein cleavage site*: As used herein, "protein cleavage site" refers to a site where controlled cleavage of the amino acid chain can be accomplished by chemical, enzymatic or photochemical means.

[00892] *Protein cleavage signal*: As used herein "protein cleavage signal" refers to at least one amino acid that flags or marks a polypeptide for cleavage.

[00893] *Protein of interest*: As used herein, the terms "proteins of interest" or "desired proteins" include those provided herein and fragments, mutants, variants, and alterations thereof.

[00894] *Proximal*: As used herein, the term "proximal" means situated nearer to the center or to a point or region of interest.

[00895] *Pseudouridine*: As used herein, pseudouridine refers to the C-glycoside isomer of the nucleoside uridine. A "pseudouridine analog" is any modification, variant, isoform or derivative of pseudouridine. For example, pseudouridine analogs include but are not limited to 1-carboxymethyl-pseudouridine, 1-propynyl-pseudouridine, 1-taurinomethyl-pseudouridine, 1-taurinomethyl-4-thio-pseudouridine, 1-methyl-pseudouridine (m^1), 1-methyl-4-thio-pseudouridine ($m^1\psi$), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydropseudouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\psi$), and 2'-O-methyl-pseudouridine (ψm).

[00896] *Purified*: As used herein, "purify," "purified," "purification" means to make substantially pure or clear from unwanted components, material defilement, admixture or imperfection.

[00897] *Reducing the effect*: As used herein, the phrase "reducing the effect" when referring to symptoms, means reducing, eliminating or alleviating the symptom in the subject. It does not necessarily mean that the symptom will, in fact, be completely eliminated, reduced or alleviated.

[00898] *Sample:* As used herein, the term "sample" or "biological sample" refers to a subset of its tissues, cells or component parts (e.g. body fluids, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). A sample further may include a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. A sample further refers to a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecule.

[00899] *Sample:* As used herein, the term "sample" or "biological sample" refers to a subset of its tissues, cells or component parts (e.g. body fluids, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). A sample further may include a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. A sample further refers to a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecule.

[00900] *Seed:* As used herein with respect to micro RNA (miRNA), a miRNA "seed" is a sequence with nucleotide identity at positions 2-8 of the mature miRNA. In one embodiment, a miRNA seed comprises positions 2-7 of the mature miRNA.

[00901] *Side effect:* As used herein, the phrase "side effect" refers to a secondary effect of treatment.

[00902] *Signal Peptide Sequences:* As used herein, the phrase "signal peptide sequences" refers to a sequence which can direct the transport or localization of a protein.

[00903] *Similarity:* As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, e.g. between polynucleotide molecules (e.g. DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed

in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art.

[00904] *Split dose:* As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses.

[00905] *Stable:* As used herein "stable" refers to a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and preferably capable of formulation into an efficacious therapeutic agent.

[00906] *Stabilized:* As used herein, the term "stabilize", "stabilized," "stabilized region" means to make or become stable.

[00907] *Subject:* As used herein, the term "subject" or "patient" refers to any organism to which a composition in accordance with the invention may be administered, *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants.

[00908] *Substantially:* As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[00909] *Substantially equal:* As used herein as it relates to time differences between doses, the term means plus/minus 2%.

[00910] *Substantially simultaneously:* As used herein and as it relates to plurality of doses, the term means within 15 seconds.

[00911] *Suffering from:* An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of a disease, disorder, and/or condition.

[00912] *Susceptible to:* An individual who is "susceptible to" a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition but harbors a propensity to develop a disease or its

symptoms. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition (for example, cancer) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein and/or nucleic acid associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; and (6) exposure to and/or infection with a microbe associated with development of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[00913] *Symptom:* As used herein, the term "symptom" is a signal of a disease, disorder and/or condition. For example, symptoms may be felt or noticed by the subject who has them but may not be easily accessed by looking at a subject's outward appearance or behaviors. Examples of symptoms include, but are not limited to, weakness, aches and pains, fever, fatigue, weight loss, blood clots, increased blood calcium levels, low white blood cell count, short of breath, dizziness, headaches, hyperpigmentation, jaundice, erythema, pruritis, excessive hair growth, change in bowel habits, change in bladder function, long-lasting sores, white patches inside the mouth, white spots on the tongue, unusual bleeding or discharge, thickening or lump on parts of the body, indigestion, trouble swallowing, changes in warts or moles, change in new skin and nagging cough or hoarseness.

[00914] *Synthetic:* The term "synthetic" means produced, prepared, and/or manufactured by the hand of man. Synthesis of polynucleotides or polypeptides or other molecules of the present invention may be chemical or enzymatic.

[00915] *Targeted Cells:* As used herein, "targeted cells" refers to any one or more cells of interest. The cells may be found *in vitro*, *in vivo*, *in situ* or in the tissue or organ of an organism. The organism may be an animal, preferably a mammal, more preferably a human and most preferably a patient.

[00916] *Terminal region:* As used herein, the term "terminal region" refers to a region on the 5' or 3' end of a region of linked nucleosides encoding a polypeptide of interest or coding region.

[00917] *Terminally optimized:* The term "terminally optimized" when referring to nucleic acids means the terminal regions of the nucleic acid are improved over the native terminal regions.

[00918] *Therapeutic Agent:* The term "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

[00919] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered (*e.g.*, nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.*) that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition.

[00920] *Therapeutically effective outcome:* As used herein, "therapeutically effective amount" means an amount of an agent to be delivered (*e.g.*, nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.*) that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition.

[00921] *Total daily dose:* As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose.

[00922] *Transcriptionfactor:* As used herein, the term "transcription factor" refers to a DNA-binding protein that regulates transcription of DNA into RNA, for example, by activation or repression of transcription. Some transcription factors effect regulation of transcription alone, while others act in concert with other proteins. Some transcription factor can both activate and repress transcription under certain conditions. In general, transcription factors bind a specific target sequence or sequences highly similar to a specific consensus sequence in a regulatory region of a target gene. Transcription factors may regulate transcription of a target gene alone or in a complex with other molecules.

[00923] *Treating:* As used herein, the term "treating" refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. For example, "treating" cancer may refer to inhibiting survival, growth, and/or spread of a tumor. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[00924] *Unmodified:* As used herein, "unmodified" refers to any substance, compound or molecule prior to being changed in any way. Unmodified may, but does not always, refer to the wild type or native form of a biomolecule. Molecules may undergo a series of modifications whereby each modified molecule may serve as the "unmodified" starting molecule for a subsequent modification.

Equivalents and Scope

[00925] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00926] In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[00927] It is also noted that the term "comprising" is intended to be open and permits the inclusion of additional elements or steps.

[00928] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00929] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention (*e.g.*, any nucleic acid or protein encoded thereby; any method of production; any method of use; *etc.*) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

EXAMPLES

Example 1. Modified mRNA Production

[00930] Modified mRNAs according to the invention are made using standard laboratory methods and materials.

[00931] The open reading frame with various upstream or downstream regions (β -globin, tags, *etc.*) is ordered from DNA2.0 (Menlo Park, CA) and typically contains a multiple cloning site with XbaI recognition. Upon receipt of the construct, it is reconstituted and transformed into chemically competent *E. coli*. For the present invention, NEB DH5-alpha Competent *E. coli* are used. A typical clone map is shown in Figure 3. Transformations are performed according to NEB instructions using 100 ng of plasmid. The protocol is as follows:

1. Thaw a tube of NEB 5-alpha Competent *E. coli* cells on ice for 10 minutes.
2. Add 1-5 μ l containing 1 pg-100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.

4. Heat shock at 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting.
10. Spread 50-100 µl of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24-36 hours or 25°C for 48 hours.

[00932] A single colony is then used to inoculate 5 ml of LB growth media using the appropriate antibiotic and then allowed to grow (250 RPM, 37° C) for 5 hours. This is then used to inoculate a 200 ml culture medium and allowed to grow overnight under the same conditions.

[00933] To isolate the plasmid (up to 850 µg), a maxi prep is performed using the Invitrogen PureLink™ HiPure Maxiprep Kit (Carlsbad, CA), following the manufacturer's instructions.

[00934] In order to generate cDNA for *In Vitro* Transcription (IVT), the plasmid is first linearized using a restriction enzyme such as XbaI. A typical restriction digest with XbaI will comprise the following: Plasmid 1.0 µg; 10x Buffer 1.0 µl; XbaI 1.5 µl; dH₂O

Up to 10 µl; incubated at 37° C for 1 hr. If performing at lab scale (< 5µg), the reaction is cleaned up using Invitrogen's PureLink™ PCR Micro Kit (Carlsbad, CA) per manufacturer's instructions. Larger scale purifications may need to be done with a product that has a larger load capacity such as Invitrogen's standard PureLink PCR Kit (Carlsbad, CA). Following the cleanup, the linearized vector is quantified using the NanoDrop and analyzed to confirm linearization using agarose gel electrophoresis.

[00935] As a non-limiting example, G-CSF may represent the polypeptide of interest. Sequences used in the steps outlined in Examples 1-5 are shown in Table 16. It should be noted that the start codon (ATG) has been underlined in each sequence of Table 16.

Table 16. G-CSF Sequences

SEQ ID NO	Description

4255	cDNA sequence: <u>ATGGCTGGACCTGCCACCCAGAGCCCCATGAAGCTGATGGCCCTGCAG</u> CTGCTGCTGTGGCACAGTGCAGTCTGGACAGTGCAGGAAGCCACCCCC CTGGGCCCTGCCAGCTCCCTGCCCCAGAGCTTCCTGCTCAAGTGCTTAG AGCAAGTGAGGAAGATCCAGGGCGATGGCGCAGCGCTCCAGGAGAAG CTGTGTGCCACCTACAAGCTGTGCCACCCCGAGGAGCTGGTGCTGCTCG GAACTCTCTGGGCATCCCCTGGGCTCCCCTGAGCAGCTGCCCCAGCCA GGCCCTGCAGCTGGCAGGCTGCTTGAGCCAACTCCATAGCGGCCTTTTC CTTACCAGGGGCTCCTGCAGGCCCTGGAAGGGATCTCCCCCGAGTTG GGTCCACCTTGGACACACTGCAGCTGGACGTCGCCGACTTTGCCACCA CCATCTGGCAGCAGATGGAAGAACTGGGAATGGCCCCTGCCCTGCAGC CCACCCAGGGTGCCATGCCGGCCTTCGCCTCTGCTTTCCAGCGCCGGGC AGGAGGGGTCTGGTTGCCTCCCATCTGCAGAGCTTCCTGGAGGTGTCTG TACCGCGTTCTACGCCACCTTGCCCAGCCCTGA
4256	cDNA having T7 polymerase site, Afel and Xba restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC <u>ATGGCTGGACCTGCCACCCAGAGCCCCATGAAGCTGATGGCCCTGCAG</u> CTGCTGCTGTGGCACAGTGCAGTCTGGACAGTGCAGGAAGCCACCCCC CTGGGCCCTGCCAGCTCCCTGCCCCAGAGCTTCCTGCTCAAGTGCTTAG AGCAAGTGAGGAAGATCCAGGGCGATGGCGCAGCGCTCCAGGAGAAG CTGTGTGCCACCTACAAGCTGTGCCACCCCGAGGAGCTGGTGCTGCTCG GAACTCTCTGGGCATCCCCTGGGCTCCCCTGAGCAGCTGCCCCAGCCA GGCCCTGCAGCTGGCAGGCTGCTTGAGCCAACTCCATAGCGGCCTTTTC CTTACCAGGGGCTCCTGCAGGCCCTGGAAGGGATCTCCCCCGAGTTG GGTCCACCTTGGACACACTGCAGCTGGACGTCGCCGACTTTGCCACCA CCATCTGGCAGCAGATGGAAGAACTGGGAATGGCCCCTGCCCTGCAGC CCACCCAGGGTGCCATGCCGGCCTTCGCCTCTGCTTTCCAGCGCCGGGC AGGAGGGGTCTGGTTGCCTCCCATCTGCAGAGCTTCCTGGAGGTGTCTG TACCGCGTTCTACGCCACCTTGCCCAGCCCTGA AGCGCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTCTCC CTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCG GCCGCTCGAGCATGCATCTAGA
4257	Optimized sequence; containing T7 polymerase site, Afel and Xba restriction site TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC <u>ATGGCCCGTCCCGCGACCCAAAGCCCCATGAACTTATGGCCCTGCAG</u> TTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAGCGACTCCTC TCGGACCTGCCTCATCGTTGCCGCACTCATTCTTTTGAAGTGTCTGGA GCAGGTGCGAAAGATTGAGGGCGATGGAGCCGCACTCCAAGAGAAGC TCTGCGCGACATACAACTTTGCCATCCCGAGGAGCTCGTACTGCTCGG GCACAGCTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTGCGAG GCTTTGCAGTTGGCAGGGTGCCTTTCCAGCTCCACTCCGTTTGTCTT GTATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATTGGG CCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTTGCAACAAC CATCTGGCAGCAGATGGAGGAACTGGGGATGGCACCCGCGCTGCAGCC

	CACGCAGGGGGCAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGC GGGTGGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCG TACCGGGTGCTGAGACATCTTGCGCAGCCGTGA AGCGCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTCTCC CTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCG GCCGCTCGAGCATGCATCTAGA
4258	mRNA sequence (transcribed) GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCAC C <u>AUG</u> GCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCCCUGCA GUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAAGAAGCGACUC CUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUCCUUUUGAAGUGU CUGGAGCAGGUGCGAAAGAUUCAGGGCGAUGGAGCCGCACUCCAAGA GAAGCUCUGCGGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUAC UGCUCGGGCACAGCUUGGGGAUUCCCUUGGGCUCCUCUCUGUCCUGU CCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCCGAGCUCCACUC CGGUUUGUUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGGAAUC UCGCCAGAAUUGGGCCCGACGCUGGACACGUUGCAGCUCGACGUGGC GGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUG GCACCCGCGCUGCAGCCCACGCAGGGGGCAAUGCCGGCCUUUGCGUC CGCGUUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUC AAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCA GCCGUGA AGCGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUC UCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGA AG

Example 2: PCR for cDNA Production

[00936] PCR procedures for the preparation of cDNA is performed using 2x KAPA HiFi™ HotStart ReadyMix by Kapa Biosystems (Woburn, MA). This system includes 2x KAPA ReadyMix 12.5 μl; Forward Primer (10 uM)0.75 μl; Reverse Primer (10 uM)0.75 μl; Template cDNA 100 ng; and dH₂O diluted to 25.0 μl. The reaction conditions are at 95° C for 5 min. and 25 cycles of 98° C20 sec, then 58° C 15 sec, then 72° C45 sec, then 72° C 5 min. then 4° C to termination.

[00937] The reverse primer of the instant invention incorporates a poly-Ti₂o for a poly-Ai₂o in the mRNA. Other reverse primers with longer or shorter poly(T) tracts can be used to adjust the length of the poly(A) tail in the mRNA.

[00938] The reaction is cleaned up using Invitrogen's PureLink™ PCR Micro Kit (Carlsbad, CA) per manufacturer's instructions (up to 5 μg). Larger reactions will require

a cleanup using a product with a larger capacity. Following the cleanup, the cDNA is quantified using the NanoDrop and analyzed by agarose gel electrophoresis to confirm the cDNA is the expected size. The cDNA is then submitted for sequencing analysis before proceeding to the *in vitro* transcription reaction.

Example 3. In vitro transcription

[00939] The *in vitro* transcription reaction generates mRNA containing modified nucleotides or modified RNA. The input nucleotide triphosphate (NTP) mix is made in-house using natural and un-natural NTPs.

[00940] A typical *in vitro* transcription reaction includes the following:

1. Template cDNA 1.0 µg
2. IOx transcription buffer (400 mM Tris-HCl pH 8.0, 190 mM MgCl₂, 50 mM DTT, 10 mM Spermidine) 2.0 µl
3. Custom NTPs (25mM each) 7.2 µl
4. RNase Inhibitor 20 U
5. T7 RNA polymerase 3000 U
6. dH₂O Up to 20.0 µl. and
7. Incubation at 37° C for 3 hr-5 hrs.

[00941] The crude IVT mix may be stored at 4° C overnight for cleanup the next day. 1 U of RNase-free DNase is then used to digest the original template. After 15 minutes of incubation at 37° C, the mRNA is purified using Ambion's MEGAClear™ Kit (Austin, TX) following the manufacturer's instructions. This kit can purify up to 500 µg of RNA. Following the cleanup, the RNA is quantified using the NanoDrop and analyzed by agarose gel electrophoresis to confirm the RNA is the proper size and that no degradation of the RNA has occurred.

Example 4. Enzymatic Capping of mRNA

[00942] Capping of the mRNA is performed as follows where the mixture includes: IVT RNA 60 µg-180 µg and dH₂O up to 72 µl. The mixture is incubated at 65° C for 5 minutes to denature RNA, then transfer immediately to ice.

[00943] The protocol then involves the mixing of IOx Capping Buffer (0.5 M Tris-HCl (pH 8.0), 60 mM KCl, 12.5 mM MgCl₂)(10.0 µl); 20 mM GTP (5.0 µl); 20 mM S-Adenosyl Methionine (2.5 µl); RNase Inhibitor (100 U); 2'-O-Methyltransferase (400

U); Vaccinia capping enzyme (Guanylyl transferase) (40 U); dH₂O (Up to 28 µl); and incubation at 37° C for 30 minutes for 60 µg RNA or up to 2 hours for 180 µg of RNA.

[00944] The mRNA is then purified using Ambion's MEGAClear™ Kit (Austin, TX) following the manufacturer's instructions. Following the cleanup, the RNA is quantified using the NanoDrop (ThermoFisher, Waltham, MA) and analyzed by agarose gel electrophoresis to confirm the RNA is the proper size and that no degradation of the RNA has occurred. The RNA product may also be sequenced by running a reverse-transcription-PCR to generate the cDNA for sequencing.

Example 5. PolyA tailing reaction

[00945] Without a poly-T in the cDNA, a poly-A tailing reaction must be performed before cleaning the final product. This is done by mixing Capped IVT RNA(100 µl); RNase Inhibitor (20 U); IOx Tailing Buffer (0.5 M Tris-HCl (pH 8.0), 2.5 M NaCl, 100 mM MgCl₂)(12.0 µl); 20 mM ATP (6.0 µl); Poly-A Polymerase (20 U); dH₂O up to 123.5 µl and incubation at 37° C for 30 min. If the poly-A tail is already in the transcript, then the tailing reaction may be skipped and proceed directly to cleanup with Ambion's MEGAClear™ kit (up to 500 µg). Poly-A Polymerase is preferably a recombinant enzyme expressed in yeast.

[00946] For studies performed and described herein, the poly-A tail is encoded in the IVT template to comprise 160 nucleotides in length. However, it should be understood that the processivity or integrity of the polyA tailing reaction may not always result in exactly 160 nucleotides. Hence polyA tails of approximately 160 nucleotides, e.g., about 150-165, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164 or 165 are within the scope of the invention.

Example 6. Natural 5' Caps and 5' Cap Analogues

[00947] 5'-capping of modified RNA may be completed concomitantly during the *in vitro*-transcription reaction using the following chemical RNA cap analogs to generate the 5'-guanosine cap structure according to manufacturer protocols: 3'-O-Me-m⁷G(5')ppp(5')G; G(5')ppp(5')A; G(5')ppp(5')G; m⁷G(5')ppp(5')A; m⁷G(5')ppp(5')G (New England BioLabs, Ipswich, MA). 5'-capping of modified RNA may be completed post-transcriptionally using a Vaccinia Virus Capping Enzyme to generate the "Cap 0" structure: m⁷G(5')ppp(5')G (New England BioLabs, Ipswich, MA). Cap 1 structure may

be generated using both Vaccinia Virus Capping Enzyme and a 2'-O methyl-transferase to generate: m7G(5')ppp(5')G-2'-O-methyl. Cap 2 structure may be generated from the Cap 1 structure followed by the 2'-O-methylation of the 5'-antepenultimate nucleotide using a 2'-O methyl-transferase. Cap 3 structure may be generated from the Cap 2 structure followed by the 2'-O-methylation of the 5'-preantepenultimate nucleotide using a 2'-O methyl-transferase. Enzymes are preferably derived from a recombinant source.

[00948] When transfected into mammalian cells, the modified mRNAs may have a stability of between 12-18 hours or more than 18 hours, e.g., 24, 36, 48, 60, 72 or greater than 72 hours.

Example 7. Chemical Cap vs. Enzymatically-Derived Cap Protein Expression Assay

[00949] Synthetic mRNAs encoding human G-CSF containing the ARCA cap analog or the Cap1 structure can be transfected into human primary keratinocytes at equal concentrations. 6, 12, 24 and 36 hours post-transfection the amount of G-CSF secreted into the culture medium can be assayed by ELISA. Synthetic mRNAs that secrete higher levels of G-CSF into the medium would correspond to a synthetic mRNA with a higher translationally-competent Cap structure.

Example 8. Chemical Cap vs. Enzymatically-Derived Cap Purity Analysis

[00950] Synthetic mRNAs encoding human G-CSF containing the ARCA cap analog or the Cap1 structure crude synthesis products can be compared for purity using denaturing Agarose-Urea gel electrophoresis or HPLC analysis. Synthetic mRNAs with a single, consolidated band by electrophoresis correspond to the higher purity product compared to a synthetic mRNA with multiple bands or streaking bands. Synthetic mRNAs with a single HPLC peak would also correspond to a higher purity product. The capping reaction with a higher efficiency would provide a more pure mRNA population.

Example 9. Chemical Cap vs. Enzymatically-Derived Cap cytokine Analysis

[00951] Synthetic mRNAs encoding human G-CSF containing the ARCA cap analog or the Cap1 structure can be transfected into human primary keratinocytes at multiple concentrations. 6, 12, 24 and 36 hours post-transfection the amount of pro-inflammatory cytokines such as TNF-alpha and IFN-beta secreted into the culture medium can be assayed by ELISA. Synthetic mRNAs that secrete higher levels of pro-inflammatory

cytokines into the medium would correspond to a synthetic mRNA containing an immune-activating cap structure.

Example 10. Chemical Cap vs. Enzymatically-Derived Cap capping reaction efficiency

[00952] Synthetic mRNAs encoding human G-CSF containing the ARCA cap analog or the Cap1 structure can be analyzed for capping reaction efficiency by LC-MS after capped mRNA nuclease treatment. Nuclease treatment of capped mRNAs would yield a mixture of free nucleotides and the capped 5'-5-triphosphate cap structure detectable by LC-MS. The amount of capped product on the LC-MS spectra can be expressed as a percent of total mRNA from the reaction and would correspond to capping reaction efficiency. The Cap structure with a higher capping reaction efficiency would have a higher amount of capped product by LC-MS.

Example 11. Agarose Gel Electrophoresis of modified RNA or RT PCR products

[00953] Individual modRNAs (200-400 ng in a 20 μ l volume) or reverse transcribed PCR products (200-400 ng) are loaded into a well on a non-denaturing 1.2% Agarose E-Gel (Invitrogen, Carlsbad, CA) and run for 12-15 minutes according to the manufacturer protocol.

Example 12. Nanodrop modified RNA quantification and UV spectral data:

[00954] Modified RNAs in TE buffer (1 μ l) are used for Nanodrop UV absorbance readings to quantitate the yield of each modified RNA from an *in vitro* transcription reaction.

Example 13. In vitro Transcription of Modified RNA Containing Varying Poly-A Tail Lengths

[00955] Modified mRNAs were made using standard laboratory methods and materials for *in vitro* transcription with the exception that the nucleotide mix contains modified nucleotides. Modified mRNAs of the present example included 5-methylcytosine and pseudouridine. The open reading frame (ORF) of the gene of interest is flanked by a 5' untranslated region (UTR) containing a strong Kozak translational initiation signal and an alpha-globin 3' UTR terminating with an oligo(dT) sequence for templated addition of a polyA tail for modified RNAs not incorporating Adenosine analogs. Adenosine-containing modRNAs are synthesized without an oligo (dT)

sequence to allow for post-transcription poly (A) polymerase poly-(A) tailing. Poly-a tail lengths of Onts, 80nts, 120nts, 160nts were generated for human G-CSF. G-CSF sequences include the cDNA sequence (SEQ ID NO: 4257), the mRNA sequence (SEQ ID NO: 4258) and the protein sequence (SEQ ID NO: 4259). Detection of G-CSF may be performed by the primer probe sets for cDNA including the forward primer TTG GAC CCT CGT ACA GAA GCT AAT ACG (SEQ ID NO: 4260), a reverse primer for template Poly(A) tailing T(i₂₀)CT TCC TAC TCA GGC TTT ATT CAA AGA CCA (SEQ ID NO: 4261) and a reverse primer for post-transcriptional Poly(A) polymerase tailing CTT CCT ACT CAG GCT TTA TTC AAA GAC CA (SED ID NO: 4262). Detection may also be performed by G-CSF modified nucleic acid molecule reverse-transcriptase polymerase chain reaction (RT-PCR) forward primer TGG CCG GTC CCG CGA CCC AA (SEQ ID NO: 4263) and reverse primer GCT TCA CGG CTG CGC AAG AT (SEQ ID NO: 4264).

[00956] Synthesized reverse primers were designed and ordered from IDT. The reverse primers incorporate a poly-T40, poly-T80, poly-T120, poly-T160 for a poly-A40, poly-A80, poly-A120, and poly-A160 respectively. The Human Embryonic Kidney (HEK) 293 were grown in Eagles' Minimal Essential Medium (EMEM) and 10% Fetal Bovine Serum (FBS) until they reached a confluence of 80-90%. Approximately 80,000 cells were transfected with 100ng and 500ng of modified RNA complexed with RNAiMax from Invitrogen (Carlsbad, CA) in a 24-well plate. The RNA:RNAiMax complex was formed by first incubating the RNAiMax with EMEM in a 5X volumetric dilution for 10 minutes at room temperature.

[00957] The RNA vial was then mixed with the RNAiMAX vial and incubated for 20-30 at room temperature before being added to the cells in a drop-wise fashion. Recombinant Human G-CSF was added at 2ng/mL to the control cell culture wells. The concentration of secreted Human G-CSF was measured at 12 hours post-transfection. Figure 4 shows the histogram for the Enzyme-linked immunosorbent assay (ELISA) for Human G-CSF from HEK293 cells transfected with human G-CSF modified RNA that had varying poly-A tail lengths: Onts, 80nts, 120nts, 160nts. We observed increased protein expression with the 160nts poly-A tail.

[00958] From the data it can be determined that longer poly-A tails produce more protein and that this activity is dose dependent.

Example 14. Expression of modified nucleic acid with microRNA binding site

[00959] Human embryonic kidney epithelial cells (HEK293A) and primary human hepatocytes (Hepatocytes) were seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). G-CSF mRNA having an alpha-globin 3'UTR (G-CSF alpha) (cDNA sequence is shown in SEQ ID NO: 4265; mRNA sequence is shown in SEQ ID NO: 4266; polyA tail of approximately 160 nucleotides not shown in sequence; 5'Cap, CapI; fully modified with 5-methylcytidine and pseudouridine) G-CSF mRNA having an alpha-globin 3'UTR and a miR-122 binding site (G-CSF miR-122) (cDNA sequence is shown in SEQ ID NO: 4267; mRNA sequence is shown in SEQ ID NO: 4268; polyA tail of approximately 160 nucleotides not shown in sequence; 5'Cap, CapI; fully modified with 5-methylcytidine and pseudouridine) or G-CSF mRNA having an alpha-globin 3'UTR with four miR-122 binding sites with the seed deleted (G-CSF no seed) (cDNA sequence is shown in SEQ ID NO: 4269; mRNA sequence is shown in SEQ ID NO: 4270; polyA tail of approximately 160 nucleotides not shown in sequence; 5'Cap, CapI; fully modified with 5-methylcytidine and pseudouridine) was tested at a concentration of 250 ng per well in 24 well plates. The expression of G-CSF was measured by ELISA and the results are shown in Table 17

Table 17. miR-122 Binding Sites

	HEK293A	Hepatocytes
	Protein Expression (ng/mL)	Protein Expression (ng/mL)
G-CSF alpha	99.85	8.18
G-CSF miR-122	87.67	0
G-CSF no seed	200.2	8.05

[00960] Since HEK293 cells do not express miR-122 there was no down-regulation of G-CSF protein from the sequence containing miR-122. Whereas, the human hepatocytes express high levels of miR-122 and there was a drastic down-regulation of G-CSF protein observed when the G-CSF sequence contained the miR-122 target sequence. Consequently, the mRNA functioned as an auxotrophic mRNA.

Example 15. Directed SAR of Pseudouridine and N1-methyl PseudoUridine

[00961] With the recent focus on the pyrimidine nucleoside pseudouridine, a series of structure-activity studies were designed to investigate mRNA containing modifications to pseudouridine or N1-methyl-pseudouridine.

[00962] The study was designed to explore the effect of chain length, increased lipophilicity, presence of ring structures, and alteration of hydrophobic or hydrophilic interactions when modifications were made at the N1 position, C6 position, the 2-position, the 4-position and on the phosphate backbone. Stability is also investigated.

[00963] To this end, modifications involving alkylation, cycloalkylation, alkyl-cycloalkylation, arylation, alkyl-arylation, alkylation moieties with amino groups, alkylation moieties with carboxylic acid groups, and alkylation moieties containing amino acid charged moieties are investigated. The degree of alkylation is generally C₁-C₆. Examples of the chemistry modifications include those listed in Table 18 and 19.

Table 18. Pseudouridine and N1-methyl Pseudo Uridine SAR

Chemistry Modification	Compound #	Naturally occurring
N1-Modifications		
N1-Ethyl-pseudo-UTP	1	N
N1-Propyl-pseudo-UTP	2	N
N1- <i>iso</i> -propyl-pseudo-UTP	3	N
N1-(2,2,2-Trifluoroethyl)-pseudo-UTP	4	N
N1-Cyclopropyl-pseudo-UTP	5	N
N1-Cyclopropylmethyl-pseudo-UTP	6	N
N1-Phenyl-pseudo-UTP	7	N
N1-Benzyl-pseudo-UTP	8	N
N1-Aminomethyl-pseudo-UTP	9	N
Pseudo-UTP-N1-2-ethanoic acid	10	N
N1-(3-Amino-3-carboxypropyl)pseudo-UTP	11	N
N1-Methyl-3-(3-amino-3-carboxypropyl)pseudo-UTP	12	Y
C-6 Modifications		
6-Methyl-pseudo-UTP	13	N
6-Trifluoromethyl-pseudo-UTP	14	N
6-Methoxy-pseudo-UTP	15	N
6-Phenyl-pseudo-UTP	16	N
6-Iodo-pseudo-UTP	17	N
6-Bromo-pseudo-UTP	18	N

6-Chloro-pseudo-UTP	19	N
6-Fluoro-pseudo-UTP	20	N
2- or 4-position Modifications		
4-Thio-pseudo-UTP	21	N
2-Thio-pseudo-UTP	22	N
Phosphate backbone Modifications		
Alpha-thio-pseudo-UTP	23	N
N1-Me-alpha-thio-pseudo-UTP	24	N

Table 19. Pseudouridine and N1-methyl Pseudo Uridine SAR

Chemistry Modification	Compound #	Naturally occurring
N1-Methyl-pseudo-UTP	1	Y
N1-Butyl-pseudo-UTP	2	N
N1- <i>tert</i> -Butyl-pseudo-UTP	3	N
N1-Pentyl-pseudo-UTP	4	N
N1-Hexyl-pseudo-UTP	5	N
N1-Trifluoromethyl-pseudo-UTP	6	Y
N1-Cyclobutyl-pseudo-UTP	7	N
N1-Cyclopentyl-pseudo-UTP	8	N
N1-Cyclohexyl-pseudo-UTP	9	N
N1-Cycloheptyl-pseudo-UTP	10	N
N1-Cyclooctyl-pseudo-UTP	11	N
N1-Cyclobutylmethyl-pseudo-UTP	12	N
N1-Cyclopentylmethyl-pseudo-UTP	13	N
N1-Cyclohexylmethyl-pseudo-UTP	14	N
N1-Cycloheptylmethyl-pseudo-UTP	15	N
N1-Cyclooctylmethyl-pseudo-UTP	16	N
N1-p-tolyl-pseudo-UTP	17	N
N1-(2,4,6-Trimethyl-phenyl)pseudo-UTP	18	N
N1-(4-Methoxy-phenyl)pseudo-UTP	19	N
N1-(4-Amino-phenyl)pseudo-UTP	20	N
N1-(4-Nitro-phenyl)pseudo-UTP	21	N
Pseudo-UTP-N1-/?-benzoic acid	22	N
N1-(4-Methyl-benzyl)pseudo-UTP	24	N
N1-(2,4,6-Trimethyl-benzyl)pseudo-UTP	23	N
N1-(4-Methoxy-benzyl)pseudo-UTP	25	N
N1-(4-Amino-benzyl)pseudo-UTP	26	N
N1-(4-Nitro-benzyl)pseudo-UTP	27	N
Pseudo-UTP-N1-methyl-p-benzoic acid	28	N
N1-(2-Amino-ethyl)pseudo-UTP	29	N
N1-(3-Amino-propyl)pseudo-UTP	30	N

N 1-(4-Amino-butyl)pseudo-UTP	31	N
N 1-(5-Amino-pentyl)pseudo-UTP	32	N
N 1-(6-Amino-hexyl)pseudo-UTP	33	N
Pseudo-UTP-N 1-3-propionic acid	34	N
Pseudo-UTP-N 1-4-butanoic acid	35	N
Pseudo-UTP-N 1-5-pentanoic acid	36	N
Pseudo-UTP-N 1-6-hexanoic acid	37	N
Pseudo-UTP-N 1-7-heptanoic acid	38	N
N 1-(2-Amino-2-carboxyethyl)pseudo-UTP	39	N
N 1-(4-Amino-4-carboxybutyl)pseudo-UTP	40	N
N3-Alkyl-pseudo-UTP	41	N
6-Ethyl-pseudo-UTP	42	N
6-Propyl-pseudo-UTP	43	N
6-iso-Propyl-pseudo-UTP	44	N
6-Butyl-pseudo-UTP	45	N
6-tert-Butyl-pseudo-UTP	46	N
6-(2,2,2-Trifluoroethyl)-pseudo-UTP	47	N
6-Ethoxy-pseudo-UTP	48	N
6-Trifluoromethoxy-pseudo-UTP	49	N
6-Phenyl-pseudo-UTP	50	N
6-(Substituted-Phenyl)-pseudo-UTP	51	N
6-Cyano-pseudo-UTP	52	N
6-Azido-pseudo-UTP	53	N
6-Amino-pseudo-UTP	54	N
6-Ethylcarboxylate-pseudo-UTP	54b	N
6-Hydroxy-pseudo-UTP	55	N
6-Methylamino-pseudo-UTP	55b	N
6-Dimethylamino-pseudo-UTP	57	N
6-Hydroxyamino-pseudo-UTP	59	N
6-Formyl-pseudo-UTP	60	N
6-(4-Morpholino)-pseudo-UTP	61	N
6-(4-Thiomorpholino)-pseudo-UTP	62	N
N 1-Me-4-thio-pseudo-UTP	63	N
N 1-Me-2-thio-pseudo-UTP	64	N
1,6-Dimethyl-pseudo-UTP	65	N
1-Methyl-6-trifluoromethyl-pseudo-UTP	66	N
1-Methyl-6-ethyl-pseudo-UTP	67	N
1-Methyl-6-propyl-pseudo-UTP	68	N
1-Methyl-6-iso-propyl-pseudo-UTP	69	N
1-Methyl-6-butyl-pseudo-UTP	70	N
1-Methyl-6-tert-butyl-pseudo-UTP	71	N
1-Methyl-6-(2,2,2-Trifluoroethyl)pseudo-UTP	72	N
1-Methyl-6-iodo-pseudo-UTP	73	N

1-Methyl-6-bromo-pseudo-UTP	74	N
1-Methyl-6-chloro-pseudo-UTP	75	N
1-Methyl-6-fluoro-pseudo-UTP	76	N
1-Methyl-6-methoxy-pseudo-UTP	77	N
1-Methyl-6-ethoxy-pseudo-UTP	78	N
1-Methyl-6-trifluoromethoxy-pseudo-UTP	79	N
1-Methyl-6-phenyl-pseudo-UTP	80	N
1-Methyl-6-(substituted phenyl)pseudo-UTP	81	N
1-Methyl-6-cyano-pseudo-UTP	82	N
1-Methyl-6-azido-pseudo-UTP	83	N
1-Methyl-6-amino-pseudo-UTP	84	N
1-Methyl-6-ethylcarboxylate-pseudo-UTP	85	N
1-Methyl-6-hydroxy-pseudo-UTP	86	N
1-Methyl-6-methylamino-pseudo-UTP	87	N
1-Methyl-6-dimethylamino-pseudo-UTP	88	N
1-Methyl-6-hydroxyamino-pseudo-UTP	89	N
1-Methyl-6-formyl-pseudo-UTP	90	N
1-Methyl-6-(4-morpholino)-pseudo-UTP	91	N
1-Methyl-6-(4-thiomorpholino)-pseudo-UTP	92	N
1-Alkyl-6-vinyl-pseudo-UTP	93	N
1-Alkyl-6-allyl-pseudo-UTP	94	N
1-Alkyl-6-homoallyl-pseudo-UTP	95	N
1-Alkyl-6-ethynyl-pseudo-UTP	96	N
1-Alkyl-6-(2-propynyl)-pseudo-UTP	97	N
1-Alkyl-6-(1-propynyl)-pseudo-UTP	98	N

Example 16. Incorporation of naturally and non-naturally occurring nucleosides

[00964] Naturally and non-naturally occurring nucleosides are incorporated into mRNA encoding a polypeptide of interest. Examples of these are given in Tables 20 and 21. Certain commercially available nucleoside triphosphates (NTPs) are investigated in the polynucleotides of the invention. A selection of these are given in Table 20. The resultant mRNA are then examined for their ability to produce protein, induce cytokines, and/or produce a therapeutic outcome.

Table 20. Naturally and non-naturally occurring nucleosides

Chemistry Modification	Compound #	Naturally occurring
N4-Methyl-Cytosine	1	Y
N4,N4-Dimethyl-2'-OMe-Cytosine	2	Y
5-Oxyacetic acid-methyl ester-Uridine	3	Y

N3-Methyl-pseudo-Uridine	4	Y
5-Hydroxymethyl-1-Cytosine	5	Y
5-Trifluoromethyl-Cytosine	6	N
5-Trifluoromethyl-1-Uridine	7	N
5-Methyl-amino-methyl-Uridine	8	Y
5-Carboxy-methyl-amino-methyl-Uridine	9	Y
5-Carboxymethylaminomethyl-2'-OMe-Uridine	10	Y
5-Carboxymethylaminomethyl-2-thio-Uridine	11	Y
5-Methylaminomethyl-2-thio-Uridine	12	Y
5-Methoxy-carbonyl-methyl-Uridine	13	Y
5-Methoxy-carbonyl-methyl-2'-OMe-Uridine	14	Y
5-Oxyacetic acid- Uridine	15	Y
3-(3-Amino-3-carboxypropyl)-Uridine	16	Y
5-(carboxyhydroxymethyl)uridine methyl ester	17	Y
5-(carboxyhydroxymethyl)uridine	18	Y

Table 21. Non-naturally occurring nucleoside triphosphates

Chemistry Modification	Compound #	Naturally occurring
N1-Me-GTP	1	N
2'-OMe-2-Amino-ATP	2	N
2'-OMe-pseudo-UTP	3	Y
2'-OMe-6-Me-UTP	4	N
2'-Azido-2'-deoxy-ATP	5	N
2'-Azido-2'-deoxy-GTP	6	N
2'-Azido-2'-deoxy-UTP	7	N
2'-Azido-2'-deoxy-CTP	8	N
2'-Amino-2'-deoxy-ATP	9	N
2'-Amino-2'-deoxy-GTP	10	N
2'-Amino-2'-deoxy-UTP	11	N
2'-Amino-2'-deoxy-CTP	12	N
2-Amino-ATP	13	N
8-Aza-ATP	14	N
Xanthosine-5'-TP	15	N
5-Bromo-CTP	16	N
2'-F-5-Methyl-2'-deoxy-UTP	17	N
5-Aminoallyl-CTP	18	N

2-Amino-riboside-TP	19	N

Example 17. Incorporation of modifications to the nucleobase and carbohydrate (sugar)

[00965] Naturally and non-naturally occurring nucleosides are incorporated into mRNA encoding a polypeptide of interest. Commercially available nucleosides and NTPs having modifications to both the nucleobase and carbohydrate (sugar) are examined for their ability to be incorporated into mRNA and to produce protein, induce cytokines, and/or produce a therapeutic outcome. Examples of these nucleosides are given in Tables 22 and 23.

Table 22. Combination modifications

Chemistry Modification	Compound #
5-iodo-2'-fluoro-deoxyuridine	1
5-iodo-cytidine	6
2'-bromo-deoxyuridine	7
8-bromo-adenosine	8
8-bromo-guanosine	9
2,2'-anhydro-cytidine hydrochloride	10
2,2'-anhydro-uridine	11
2'-Azido-deoxyuridine	12
2-amino-adenosine	13
N4-Benzoyl-cytidine	14
N4-Amino-cytidine	15
2'-O-Methyl-N4-Acetyl-cytidine	16
2'Fluoro-N4-Acetyl-cytidine	17
2'Fluor-N4-Bz-cytidine	18
2'O-methyl-N4-Bz-cytidine	19
2'O-methyl-N6-Bz-deoxyadenosine	20
2'Fluoro-N6-Bz-deoxyadenosine	21
N2-isobutyl-guanosine	22
2'Fluro-N2-isobutyl-guanosine	23
2'O-methyl-N2-isobutyl-guanosine	24

Table 23. Naturally occuring combinations

Name	Compound	Naturally

	#	occurring
5-Methoxycarbonylmethyl-2-thiouridine TP	1	Y
5-Methylaminomethyl-2-thiouridine TP	2	Y
5-Crbamoylmethyluridine TP	3	Y
5-Carbamoylmethyl-2'-0-methyluridine TP	4	Y
1-Methyl-3-(3-amino-3-carboxypropyl) pseudouridine TP	5	Y
5-Methylaminomethyl-2-selenouridine TP	6	Y
5-Carboxymethyluridine TP	7	Y
5-Methyldihydrouridine TP	8	Y
lysidine TP	9	Y
5-Taurinomethyluridine TP	10	Y
5-Taurinomethyl-2-thiouridine TP	11	Y
5-(zso-Pentenylaminomethyl)uridine TP	12	Y
5-(zso-Pentenylaminomethyl)- 2-thiouridine TP	13	Y
5-(zso-Pentenylaminomethyl)-2'-0- methyluridine TP	14	Y
N4-Acetyl-2'-0-methylcytidine TP	15	Y
N4,2'-0-Dimethylcytidine TP	16	Y
5-Formyl-2'-0-methylcytidine TP	17	Y
2'-0-Methylpseudouridine TP	18	Y
2-Thio-2'-0-methyluridine TP	19	Y
3,2'-0-Dimethyluridine TP	20	Y

[00966] In the tables "UTP" stands for uridine triphosphate, "GTP" stands for guanosine triphosphate, "ATP" stands for adenosine triphosphate, "CTP" stands for cytosine triphosphate, "TP" stands for triphosphate and "Bz" stands for benzyl.

Example 18. Signal Sequence Exchange Study

[00967] Several variants of mmRNAs encoding human Granulocyte colony stimulating factor (G-CSF) (mRNA sequence shown in SEQ ID NO: 4258; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Cap1) were synthesized using modified nucleotides pseudouridine and 5-methylcytidine (pseudo-U/5mC). These variants included the G-CSF constructs encoding either the wild-type N terminal secretory signal peptide sequence (MAGPATQSPMKLMALQLLLWHSALWTVQEA; SEQ ID NO: 4271), no secretory signal peptide sequence, or secretory signal peptide

sequences taken from other mRNAs. These included sequences where the wild type G-CSF signal peptide sequence was replaced with the signal peptide sequence of either: human α -1-anti trypsin (AAT) (MMPSSVSWGILLLAGLCCLVPVSLA; SEQ ID NO: 4272), human Factor IX (FIX) (MQRVNMIMAEPSLITICLLGYLLSAECTVFLDHENANKILNRPKR; SEQ ID NO: 4273), human Prolactin (Prolac) (MKGSLLLLLVSNLLLCQSVAP; SEQ ID NO: 4274), or human Albumin (Alb) (MKWVTFISLLFLFSSAYSRGVFRR; SEQ ID NO: 4275).

[00968] 250ng of modified mRNA encoding each G-CSF variant was transfected into HEK293A (293A in the table), mouse myoblast (MM in the table) (C2C12, CRL-1772, ATCC) and rat myoblast (RM in the table) (L6 line, CRL-1458, ATCC) cell lines in a 24 well plate using 1 ul of Lipofectamine 2000 (Life Technologies), each well containing 300,000 cells. The supernatants were harvested after 24 hrs and the secreted G-CSF protein was analyzed by ELISA using the Human G-CSF ELISA kit (Life Technologies). The data shown in Table 24 reveal that cells transfected with G-CSF mmRNA encoding the Albumin signal peptide secrete at least 12 fold more G-CSF protein than its wild type counterpart.

Table 24. Signal Peptide Exchange

Signal peptides	293A (pg/ml)	MM (pg/ml)	RM (pg/ml)
G-CSF Natural	9650	3450	6050
α -1-anti trypsin	9950	5000	8475
Factor IX	11675	6175	11675
Prolactin	7875	1525	9800
Albumin	122050	81050	173300
No Signal peptide	0	0	0

Example 19. 3' Untranslated Regions

[00969] A 3' UTR may be provided a flanking region. Multiple 3' UTRs may be included in the flanking region and may be the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical modifications, before and/or after codon optimization.

[00970] Shown in Table 7 is a listing of 3'-untranslated regions of the invention. Variants of 3' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G.

Example 20. Alteration of Polynucleotide trafficking: NLS and NES

[00971] Two nuclear export signals (NES) which may be incorporated into the polynucleotides of the present invention includes those reported by Muller, et al (Traffic, 2009, 10: 514-527) and are associated with signaling via the gene COMMD1. These are NES1, PVAIIIELEL (SEQ ID NO 4276) and NES2, VNQILKTLSE (SEQ ID NO 4277).

[00972] Nuclear localization signals may also be used. One such sequence is PKKKRKV (SEQ ID NO: 4278).

[00973] Cell lines or mice are administered one or more polynucleotides having a NLS or NES encoded therein. Upon administration the polynucleotide is trafficked to an alternate location, e.g., into the nucleus using the NLS. The polypeptide having the NLS would be trafficked to the nucleus where it would deliver either a survival or death signal to the nuclear microenvironment. Polypeptides which may be localized to the nucleus include those with altered binding properties for DNA which will function to alter the expression profile of the cell in a therapeutically beneficial manner for the cell, tissue or organism.

[00974] In one experiment, the polynucleotide encodes a COMMD1 mutl/mut 2 + NLS (e.g., both NES signals disrupted plus a NLS added) following the methods of Muller et al, (Traffic 2009; 10: 514-527) and van de Sluis et al, (J Clin Invest. 2010; 120 (6):2119-2130). The signal sequence may encode a polypeptide or a scrambled sequence which is not translatable. The signal sequence encoded would interact with HIF1 -alpha to alter the transcriptome of the cancer cells.

[00975] The experiment is repeated under normal and hypoxic conditions.

[00976] Once identified the HIF1 -alpha dependent polynucleotide is tested in cancer cell lines clonal survival or a marker of apoptosis is measured and compared to control or mock treated cells.

Example 21. miRNA Binding Sites (BS) Useful as Sensor Sequences in Polynucleotides

[00977] miRNA-binding sites are used in the 3'UTR of mRNA therapeutics to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells (normal and/or cancerous).

[00978] A strong apoptotic signal (i.e., AIFsh - Apoptosis Inducing Factor short isoform) is encoded as the polypeptide or "signal" and is encoded along with a series of 3'UTR miR binding sites, such as that for mir-122a, that would make the polynucleotide relatively much more stable in cancerous cells than in normal cells.

[00979] Experiments comparing cancer vs. normal hepatic cell lines where the cancer cell lines have a specific miR signature are performed in vitro. SNU449 or HEP3B (human derived HCC cell lines) are used because both have been shown to have "undetectable miR-122a", whereas normal hepatocytes should have very high miR-122a levels. First a cancer cell is selected which is sensitive to AIFsh polynucleotide (i.e., it results in apoptosis).

[00980] Three miR-122a binding sites are encoded into the 3'UTR of an mRNA sequence for AIFsh and the study arms include 2 cell lines (normal hepatocyte, SNU449 or HEP3B) x 5 treatments (vehicle alone, polynucleotide untranslatable, polynucleotide AIFsh (no miR BS in 3'UTR), 3'UTR[miR122a BS x3]- polynucleotide untranslatable, 3'UTR[miR122a BS x3]- polynucleotide AIFsh).

[00981] The expected result would be significant apoptosis in the face of polynucleotide AIFsh in both normal and cancer (HEP3B or SNU449) cell lines in the absence of any 3'UTR-miR122a BS. However, a significant difference in the relative apoptosis of normal vs. cancer cell lines in the face of 3'UTR [miR122a BS x3]- polynucleotide AIFsh.

[00982] Reversibility of the effect is shown with the co-administration of miR122a to the cancer cell line (e.g., through some transduction of the miR122a activity back into the cancer cell line).

[00983] In vivo animal studies are then performed using any of the models disclosed herein or a commercially available orthotopic HCC model.

Example 22. Cell lines for the study of Polynucleotides

[00984] Polynucleotides of the present invention and formulations comprising the polynucleotides of the present invention or described in International application No

PCT/US20 12/696 10, herein incorporated by reference in its entirety, may be investigated in any number of cancer or normal cell lines. Cell lines useful in the present invention include those from ATCC (Manassas, VA) and are listed in Table 25.

Table 25. Cell lines

ATCC Number	Hybridoma or Cell line Description	Name
CCL-171	Homo sapiens (human) Source: Organ: lung Disease: normal Cell Type: fibroblast	MRC-5
CCL-185	Homo sapiens (human) Source: Organ: lung Disease: carcinoma	A549
CCL-248	Homo sapiens (human) Source: Organ: colon Disease: colorectal carcinoma Derived from metastatic site: lung	T84
CCL-256	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H2126 [H2126]
CCL-257	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; classic small cell lung cancer	NCI-H1688 [H1688]
CCL-75	Homo sapiens (human) Source: Organ: lung Disease: normal Cell Type: fibroblast	WI-38
CCL-75.1	Homo sapiens (human) Source: Organ: lung Cell Type: fibroblastSV40 transformed	WI-38 VA-13 subline 2RA
CCL-95.1	Homo sapiens (human) Source: Organ: lung Cell Type: SV40 transformed	WI-26 VA4
CRL-10741	Homo sapiens (human) Source: Organ: liver Disease: hepatocellular carcinoma	C3A [HepG2/C3A, derivative of Hep G2 (ATCC HB- 8065)]
CRL-11233	Homo sapiens (human) Source: Organ: liver Tissue: left lobe Cell Type: epithelialimmortalized with SV40 large T antigen	THLE-3
CRL-11351	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer; multidrug resistant Cell Type: epithelial	H69AR
CRL-1848	Homo sapiens (human) Source: Organ: lung Disease: mucoepidermoid pulmonary carcinoma	NCI-H292 [H292]
CRL-1918	Homo sapiens (human) Source: Organ: pancreas Disease: ductal adenocarcinoma; cystic fibrosis Derived from metastatic site: liver metastasis	CFPAC-1

CRL-1973	Homo sapiens (human) Source: Organ: testis Disease: malignant pluripotent embryonal carcinoma Derived from metastatic site: lung	NTERA-2 cl.D1 [NT2/D1]
CRL-2049	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	DMS 79
CRL-2062	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	DMS 53
CRL-2064	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: liver	DMS 153
CRL-2066	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	DMS 114
CRL-2081	Homo sapiens (human) Source: Disease: biphasic mesothelioma Derived from metastatic site: lung	MSTO-211H
CRL-2170	Homo sapiens (human) Source: Organ: lung Disease: alveolar cell carcinoma	SW 1573 [SW-1573, SW1573]
CRL-2177	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	SW 1271 [SW-1271, SW1271]
CRL-2195	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Cell Type: large cell, variant;	SHP-77
CRL-2233	Homo sapiens (human) Source: Organ: liver Disease: hepatocellular carcinoma	SNU-398
CRL-2234	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade II-III/IV Disease: hepatocellular carcinoma	SNU-449
CRL-2235	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade III/IV Disease: hepatocellular carcinoma	SNU-182
CRL-2236	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade II-IV/V Disease: hepatocellular carcinoma	SNU-475
CRL-2237	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade IV/V Disease: pleomorphic hepatocellular carcinoma	SNU-387
CRL-2238	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade III/IV Disease: pleomorphic hepatocellular carcinoma	SNU-423
CRL-2503	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: normal	NL20
CRL-2504	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: normal	NL20-TA [NL20T-A]
CRL-2706	Homo sapiens (human) Source: Organ: liver Tissue: left lobe	THLE-2

	Cell Type: epithelialSV40 transformed	
CRL-2741	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Cell Type: epithelialHPV- 16 E6/E7 transformed	HBE135-E6E7
CRL-2868	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma Cell Type: epithelial	HCC827
CRL-2871	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma Derived from metastatic site: pleural effusion Cell Type: epithelial	HCC4006
CRL-5800	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H23 [H23]
CRL-5803	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1299
CRL-5804	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H187 [H187]
CRL-5807	Homo sapiens (human) Source: Organ: lung Tissue: bronchiole; alveolus Disease: bronchioalveolar carcinoma; non-small cell lung cancer	NCI-H358 [H-358, H358]
CRL-5808	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H378 [H378]
CRL-5810	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 2 Disease: adenocarcinoma; non-small cell lung cancer	NCI-H522 [H522]
CRL-581 1	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; variant small cell lung cancer Derived from metastatic site: bone marrow	NCI-H526 [H526]
CRL-5815	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: carcinoid	NCI-H727 [H727]
CRL-5816	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 2 Disease: carcinoma; non-small cell lung cancer	NCI-H810 [H810]
CRL-5817	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung	NCI-H889 [H889]

	cancer Derived from metastatic site: lymph node	
CRL-5818	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1 155 [HI 155]
CRL-5819	Homo sapiens (human) Source: Organ: lung Disease: papillary adenocarcinoma Derived from metastatic site: lymph node	NCI-H1404 [HI 404]
CRL-5822	Homo sapiens (human) Source: Organ: stomach Disease: gastric carcinoma Derived from metastatic site: liver	NCI-N87 [N87]
CRL-5823	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; variant small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H196 [HI 96]
CRL-5824	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H21 1 [H21 1]
CRL-5825	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H220 [H220]
CRL-5828	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: brain	NCI-H250 [H250]
CRL-5831	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; variant small cell lung cancer Derived from metastatic site: lymph node	NCI-H524 [H524]
CRL-5834	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenosquamous carcinoma; non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H647 [H647]
CRL-5835	Homo sapiens (human) Source: Organ: lung Disease: bronchioalveolar carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H650 [H650]
CRL-5836	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: bone marrow	NCI-H71 1 [H71 1]

CRL-5837	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: bone marrow	NCI-H719 [H719]
CRL-5840	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H740 [H740]
CRL-5841	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H748 [H748]
CRL-5842	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: soft tissue	NCI-H774 [H774]
CRL-5844	Homo sapiens (human) Source: Organ: lung Tumor stage: 3B Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H838 [H838]
CRL-5845	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; variant small cell lung cancer Derived from metastatic site: lymph node	NCI-H841 [H841]
CRL-5846	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H847 [H847]
CRL-5849	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H865 [H865]
CRL-5850	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H920 [H920]
CRL-5853	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H1048 [H1048]
CRL-5855	Homo sapiens (human) Source: Organ: lung	NCI-H1092

	Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: bone marrow	[HI 092]
CRL-5856	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H1 105 [HI 105]
CRL-5858	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H1 184 [HI 184]
CRL-5859	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H1238 [H1238]
CRL-5864	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: cervix	NCI-H1341 [H1341]
CRL-5867	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1385 [H1385]
CRL-5869	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer	NCI-H1417 [H1417]
CRL-5870	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1435 [H1435]
CRL-5871	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H1436 [H1436]
CRL-5872	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H1437 [H1437]
CRL-5874	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H1522 [HI 522]
CRL-5875	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1563 [H1563]
CRL-5876	Homo sapiens (human) Source: Organ: lung	NCI-H1568

	Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	[H1568]
CRL-5877	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma Derived from metastatic site: soft tissue	NCI-H1573 [H1573]
CRL-5878	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: non-small cell lung cancer Cell Type: large cell;	NCI-H1581 [H1581]
CRL-5879	Homo sapiens (human) Source: Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H1618 [H1618]
CRL-5881	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3B Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1623 [H1623]
CRL-5883	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3B Disease: adenocarcinoma; bronchoalveolar carcinoma Derived from metastatic site: pleural effusion	NCI-H1650 [H-1650, H1650]
CRL-5884	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1651 [H1651]
CRL-5885	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; bronchoalveolar carcinoma Derived from metastatic site: pleural effusion	NCI-H1666 [H-1666, H1666]
CRL-5886	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer	NCI-H1672 [H1672]
CRL-5887	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3B Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1693 [H1693]
CRL-5888	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: ascites	NCI-H1694 [H1694]
CRL-5889	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: non-small cell lung cancer	NCI-H1703 [H1703]

	Cell Type: squamous cell;	
CRL-5891	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1734 [H-1734, H1734]
CRL-5892	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: liver	NCI-H1755 [H1755]
CRL-5892	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: liver	NCI-H1755 [H1755]
CRL-5893	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node Cell Type: neuroendocrine;	NCI-H1770 [HI 770]
CRL-5896	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1793 [H1793]
CRL-5898	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer	NCI-H1836 [H1836]
CRL-5899	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1838 [H1838]
CRL-5900	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: non-small cell lung cancer Derived from metastatic site: pleural effusion Cell Type: squamous cell;	NCI-H1869 [HI 869]
CRL-5902	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H1876 [HI 876]
CRL-5903	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H1882 [H1882]
CRL-5904	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: poorly differentiated carcinoma; non-small cell lung cancer Derived from metastatic site: brain Cell Type: large cell;	NCI-H1915 [H1915]

CRL-5906	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H1930 [H1930]
CRL-5907	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3B Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: soft tissue	NCI-H1944 [H1944]
CRL-5908	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1975 [H-1975, H1975]
CRL-5909	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1993 [H1993]
CRL-5912	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H2023 [H2023]
CRL-5913	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H2029 [H2029]
CRL-5914	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H2030 [H2030]
CRL-5917	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: mixed; small cell lung cancer; adenocarcinoma; squamous cell carcinoma	NCI-H2066 [H2066]
CRL-5918	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2073 [H2073]
CRL-5920	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H2081 [H2081]
CRL-5921	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2085 [H2085]
CRL-5922	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1	NCI-H2087 [H2087]

	Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	
CRL-5923	Homo sapiens (human) Source: Organ: lung Tissue: neuroendocrine Tumor Stage: stage 4 Disease: non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H2106 [H2106]
CRL-5924	Homo sapiens (human) Source: Organ: lung Disease: non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H21 10 [H21 10]
CRL-5926	Homo sapiens (human) Source: Organ: lung Disease: non-small cell lung cancer	NCI-H2135 [H2135]
CRL-5927	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H2141 [H2141]
CRL-5929	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H2171 [H2171]
CRL-5930	Homo sapiens (human) Source: Organ: lung Disease: non-small cell lung cancer	NCI-H2172 [H2172]
CRL-5931	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H2195 [H2195]
CRL-5932	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H2196 [H2196]
CRL-5933	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H2198 [H2198]
CRL-5934	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer	NCI-H2227 [H2227]
CRL-5935	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2228 [H2228]
CRL-5938	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: mixed; small cell lung cancer; adenocarcinoma; squamous cell carcinoma	NCI-H2286 [H2286]
CRL-5939	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2291 [H2291]

	Derived from metastatic site: lymph node	
CRL-5940	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H2330 [H2330]
CRL-5941	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2342 [H2342]
CRL-5942	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2347 [H2347]
CRL-5944	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: ascites	NCI-H2405 [H2405]
CRL-5945	Homo sapiens (human) Source: Organ: lung Disease: non-small cell lung cancer	NCI-H2444 [H2444]
CRL-5975	Homo sapiens (human) Source: Organ: lung Disease: carcinoid	UMC-1 1
CRL-5976	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H64 [H64]
CRL-5978	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: liver	NCI-H735 [H735]
CRL-5978	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: liver	NCI-H735 [H735]
CRL-5982	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; small cell lung cancer	NCI-H1963 [H1963]
CRL-5983	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H2107 [H2107]
CRL-5984	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H2108 [H2108]
CRL-5985	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung	NCI-H2122 [H2122]

	cancer Derived from metastatic site: pleural effusion	
CRL-7343	Homo sapiens (human) Source: Organ: lung Disease: cancer	Hs 573.T
CRL-7344	Homo sapiens (human) Source: Organ: lung	Hs 573.Lu
CRL-8024	Homo sapiens (human) Source: Organ: liver Disease: hepatoma Cell Type: Alexander cells;	PLC/PRF/5
CRL-9609	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: normal Cell Type: epithelialvirus transformed	BEAS-2B
HB-8065	Homo sapiens (human) Source: Organ: liver Disease: hepatocellular carcinoma	Hep G2
HTB-105	Homo sapiens (human) Source: Organ: testes Disease: embryonal carcinoma, malignant Derived from metastatic site: lung	Tera- 1
HTB-106	Homo sapiens (human) Source: Disease: malignant embryonal carcinoma Derived from metastatic site: lung	Tera-2
HTB-1 19	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	NCI-H69 [H69]
HTB-120	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H128 [H128]
HTB-168	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: bronchogenic carcinoma	ChaGo-K-1
HTB-171	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H446 [H446]
HTB-172	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H209 [H209]
HTB-173	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H146 [HI 46]
HTB-174	Homo sapiens (human) Source: Organ: lung Disease: papillary adenocarcinoma	NCI-H441 [H441]
HTB-175	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H82 [H82]
HTB-177	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; large cell lung cancer Derived from metastatic site: pleural effusion	NCI-H460 [H460]
HTB-178	Homo sapiens (human) Source: Organ: lung Disease: adenosquamous carcinoma	NCI-H596 [H596]
HTB-179	Homo sapiens (human) Source: Organ: lung	NCI-H676B

	Disease: adenocarcinoma Derived from metastatic site: pleural effusion	[H676B]
HTB-180	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H345 [H345]
HTB-181	Homo sapiens (human) Source: Organ: lung Disease: papillary adenocarcinoma Derived from metastatic site: lymph node	NCI-H820 [H820]
HTB-182	Homo sapiens (human) Source: Organ: lung Disease: squamous cell carcinoma	NCI-H520 [H520]
HTB-183	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; large cell lung cancer Derived from metastatic site: lymph node	NCI-H661 [H661]
HTB-184	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer; extrapulmonary origin Derived from metastatic site: adrenal gland	NCI-H510A [H510A, NCI- IK 10]
HTB-52	Homo sapiens (human) Source: Organ: liver Tissue: ascites Disease: adenocarcinoma	SK-HEP-1
HTB-53	Homo sapiens (human) Source: Organ: lung Disease: carcinoma	A-427
HTB-54	Homo sapiens (human) Source: Organ: lung Tumor Stage: grade III Disease: epidermoid carcinoma Derived from metastatic site: pleura	Calu-1
HTB-55	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma Derived from metastatic site: pleural effusion	Calu-3
HTB-56	Homo sapiens (human) Source: Organ: unknown, probably lung Disease: anaplastic carcinoma	Calu-6
HTB-57	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma	SK-LU-1
HTB-58	Homo sapiens (human) Source: Organ: lung Disease: squamous cell carcinoma Derived from metastatic site: pleural effusion	SK-MES-1
HTB-59	Homo sapiens (human) Source: Organ: lung Tumor Stage: grade IV Disease: squamous cell carcinoma	SW 900 [SW-900, SW900]
HTB-64	Homo sapiens (human) Source: Disease: malignant melanoma Derived from metastatic site: lung	Malme-3M
HTB-79	Homo sapiens (human) Source: Organ: pancreas Disease: adenocarcinoma Derived from metastatic site: liver	Capan- 1

Example 23. RNA binding proteins

[00985] RNA binding proteins may be provided as proteins and/or as nucleic acids encoding such proteins. RNA binding proteins play a multitude of roles in regulating RNA stability and protein translation. In some embodiments, RNA binding proteins are provided in protein and/or nucleic acid form with elements of the present invention. Such RNA binding proteins include, but are not limited to those listed (along with the ENSG number, identifying the corresponding gene as well as one or more ENST number, identifying transcriptional variants of each) in Table 26.

Table 26. RNA binding proteins

Protein No.	RNA binding protein	ENSG	ENST	ENST SEQ ID NO	ENSP	ENSP SEQ ID NO
1	AU RNA binding protein/enoyl-CoA hydratase	148090	422391	4279	402026	4632
2	AU RNA binding protein/enoyl-CoA hydratase	148090	303617	4280	307334	4633
3	AU RNA binding protein/enoyl-CoA hydratase	148090	375731	4281	364883	4634
4	cold inducible RNA binding protein	99622	320936	4282	322887	4635
5	cold inducible RNA binding protein	99622	444172	4283	407512	4636
6	cold inducible RNA binding protein	99622	413636	4284	412831	4637
7	cold shock domain containing C2, RNA binding	172346	306149	4285	302485	4638
8	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	138668	543098	4286	439380	4639
9	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	138668	313899	4287	313199	4640
10	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	138668	541060	4288	437416	4641
11	heterogeneous nuclear ribonucleoprotein D (AU-rich	138668	503822	4289	422615	4642

	element RNA binding protein 1, 37kDa)					
12	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	138668	507010	4290	421952	4643
13	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	138668	353341	4291	313327	4644
14	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	138668	514671	4292	426446	4645
15	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	138668	352301	4293	305860	4646
16	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	138668	307213	4294	307544	4647
17	insulin-like growth factor 2 mRNA binding protein 1	159217	290341	4295	290341	4648
18	insulin-like growth factor 2 mRNA binding protein 2	73792	382199	4296	371634	4649
19	insulin-like growth factor 2 mRNA binding protein 2	73792	421047	4297	413787	4650
20	insulin-like growth factor 2 mRNA binding protein 2	73792	346192	4298	320204	4651
21	insulin-like growth factor 2 mRNA binding protein 3	136231	258729	4299	258729	4652
22	KH domain containing, RNA binding, signal transduction associated 1	121774	327300	4300	313829	4653
23	KH domain containing, RNA binding, signal transduction associated 1	121774	492989	4301	417731	4654
24	KH domain containing, RNA binding, signal transduction associated 1	121774	355201	4302	347336	4655
25	KH domain containing, RNA binding, signal transduction associated 2	112232	281156	4303	281156	4656
26	KH domain containing, RNA binding, signal transduction associated 2	112232	539571	4304	443437	4657

27	KH domain containing, RNA binding, signal transduction associated 3	131773	355849	4305	348108	4658
28	QKI, KH domain containing, RNA binding	112531	361752	4306	355094	4659
29	QKI, KH domain containing, RNA binding	112531	275262	4307	275262	4660
30	QKI, KH domain containing, RNA binding	112531	392127	4308	375973	4661
31	QKI, KH domain containing, RNA binding	112531	361 195	4309	354867	4662
32	QKI, KH domain containing, RNA binding	112531	453779	4310	408775	4663
33	RALY RNA binding protein-like	184672	522613	431 1	427787	4664
34	RALY RNA binding protein-like	184672	523850	4312	428807	4665
35	RALY RNA binding protein-like	184672	521695	4313	428667	4666
36	RALY RNA binding protein-like	184672	521268	4314	430367	4667
37	RALY RNA binding protein-like	184672	517988	4315	42871 1	4668
38	RALY RNA binding protein-like	184672	522455	4316	430394	4669
39	RD RNA binding protein	204356	375425	4317	364574	4670
40	RD RNA binding protein	204356	44481 1	4318	388400	4671
41	RD RNA binding protein	204356	441998	4319	397914	4672
42	RD RNA binding protein	204356	375429	4320	364578	4673
43	RD RNA binding protein	204356	426722	4321	394340	4674
44	RD RNA binding protein	204356	454913	4322	409389	4675
45	RD RNA binding protein	206268	4 11745	4323	410872	4676
46	RD RNA binding protein	206268	456281	4324	396971	4677

47	RD RNA binding protein	206268	440478	4325	407528	4678
48	RD RNA binding protein	206268	383174	4326	372660	4679
49	RD RNA binding protein	206268	551833	4327	447903	4680
50	RD RNA binding protein	206268	458622	4328	409139	4681
51	RD RNA binding protein	206357	548056	4329	449897	4682
52	RD RNA binding protein	206357	434518	4330	409269	4683
53	RD RNA binding protein	206357	449057	4331	393793	4684
54	RD RNA binding protein	206357	383343	4332	372834	4685
55	RD RNA binding protein	206357	420039	4333	411487	4686
56	RD RNA binding protein	206357	425810	4334	403630	4687
57	RD RNA binding protein	229363	549252	4335	450250	4688
58	RD RNA binding protein	229363	448628	4336	394879	4689
59	RD RNA binding protein	229363	424762	4337	415567	4690
60	RD RNA binding protein	229363	418423	4338	395175	4691
61	RD RNA binding protein	229363	418059	4339	401342	4692
62	RD RNA binding protein	229363	453084	4340	393794	4693
63	RD RNA binding protein	231044	443464	4341	393103	4694
64	RD RNA binding protein	231044	548988	4342	449910	4695
65	RD RNA binding protein	231044	429857	4343	403623	4696
66	RD RNA binding protein	231044	437732	4344	397565	4697

67	RD RNA binding protein	231044	424967	4345	411724	4698
68	RD RNA binding protein	231044	420837	4346	414014	4699
69	RD RNA binding protein	233801	456263	4347	407630	4700
70	RD RNA binding protein	233801	452147	4348	401745	4701
71	RD RNA binding protein	233801	457397	4349	393005	4702
72	RD RNA binding protein	233801	552869	4350	447844	4703
73	RD RNA binding protein	233801	425721	4351	390689	4704
74	RD RNA binding protein	233801	435435	4352	396604	4705
75	RNA binding motif (RNP 1, RRM) protein 3	102317	430348	4353	412764	4706
76	RNA binding motif (RNP 1, RRM) protein 3	102317	376759	4354	365950	4707
77	RNA binding motif (RNP 1, RRM) protein 3	102317	376755	4355	365946	4708
78	RNA binding motif (RNP 1, RRM) protein 3	102317	354480	4356	346473	4709
79	RNA binding motif protein 10	182872	377604	4357	366829	4710
80	RNA binding motif protein 10	182872	329236	4358	328848	471 1
81	RNA binding motif protein 10	182872	345781	4359	329659	4712
82	RNA binding motif protein 11	185272	400577	4360	383421	4713
83	RNA binding motif protein 12	244462	359646	4361	352668	4714
84	RNA binding motif protein 12	244462	374104	4362	363217	4715
85	RNA binding motif protein 12	244462	374114	4363	363228	4716
86	RNA binding motif protein 12	244462	431 148	4364	392642	4717

87	RNA binding motif protein 12	244462	424458	4365	4 11036	4718
88	RNA binding motif protein 12	244462	435161	4366	4 11692	4719
89	RNA binding motif protein 12	244462	349942	4367	339879	4720
90	RNA binding motif protein 12B	183808	518597	4368	428269	4721
91	RNA binding motif protein 12B	183808	399300	4369	382239	4722
92	RNA binding motif protein 12B	183808	520560	4370	429807	4723
93	RNA binding motif protein 12B	183808	521947	4371	430466	4724
94	RNA binding motif protein 12B	183808	517700	4372	427729	4725
95	RNA binding motif protein 12B	183808	519109	4373	430474	4726
96	RNA binding motif protein 14	239306	310137	4374	3 11747	4727
97	RNA binding motif protein 15	162775	369784	4375	358799	4728
98	RNA binding motif protein 15B	179837	323686	4376	313890	4729
99	RNA binding motif protein 15B	179837	536338	4377	444388	4730
100	RNA binding motif protein 15B	179837	541 145	4378	443941	4731
101	RNA binding motif protein 15B	179837	540284	4379	437933	4732
102	RNA binding motif protein 17	134453	447032	4380	406024	4733
103	RNA binding motif protein 17	134453	437845	4381	395448	4734
104	RNA binding motif protein 17	134453	372795	4382	361881	4735
105	RNA binding motif protein 17	134453	418631	4383	402303	4736
106	RNA binding motif protein 17	134453	432931	4384	408214	4737

107	RNA binding motif protein 17	134453	379888	4385	369218	4738
108	RNA binding motif protein 17	134453	446108	4386	388638	4739
109	RNA binding motif protein 18	119446	417201	4387	409315	4740
110	RNA binding motif protein 19	122965	545145	4388	442053	4741
111	RNA binding motif protein 19	122965	261741	4389	261741	4742
112	RNA binding motif protein 19	122965	392561	4390	376344	4743
113	RNA binding motif protein 20	203867	539821	4391	446400	4744
114	RNA binding motif protein 20	203867	369519	4392	358532	4745
115	RNA binding motif protein 22	86589	447771	4393	412118	4746
116	RNA binding motif protein 22	86589	199814	4394	199814	4747
117	RNA binding motif protein 22	86589	540000	4395	441594	4748
118	RNA binding motif protein 23	100461	399922	4396	382806	4749
119	RNA binding motif protein 23	100461	359890	4397	352956	4750
120	RNA binding motif protein 23	100461	346528	4398	339220	4751
121	RNA binding motif protein 23	100461	554618	4399	451448	4752
122	RNA binding motif protein 23	100461	553876	4400	450672	4753
123	RNA binding motif protein 23	100461	557571	4401	452382	4754
124	RNA binding motif protein 23	100461	557549	4402	450558	4755
125	RNA binding motif protein 23	100461	338980	4403	345496	4756
126	RNA binding motif protein 23	100461	554256	4404	452583	4757

127	RNA binding motif protein 23	100461	557464	4405	451403	4758
128	RNA binding motif protein 23	100461	555691	4406	452538	4759
129	RNA binding motif protein 23	100461	556862	4407	452557	4760
130	RNA binding motif protein 23	100461	555676	4408	451364	4761
131	RNA binding motif protein 24	112183	379052	4409	368341	4762
132	RNA binding motif protein 24	112183	318204	4410	319551	4763
133	RNA binding motif protein 24	112183	425446	441 1	396898	4764
134	RNA binding motif protein 25	119707	525161	4412	434004	4765
135	RNA binding motif protein 25	119707	525321	4413	436868	4766
136	RNA binding motif protein 25	119707	531500	4414	434333	4767
137	RNA binding motif protein 25	119707	261973	4415	261973	4768
138	RNA binding motif protein 25	119707	527432	4416	431 150	4769
139	RNA binding motif protein 25	119707	526754	4417	436225	4770
140	RNA binding motif protein 25	119707	540173	4418	437934	4771
141	RNA binding motif protein 26	139746	267229	4419	267229	4772
142	RNA binding motif protein 26	139746	327303	4420	327080	4773
143	RNA binding motif protein 26	139746	438724	4421	390222	4774
144	RNA binding motif protein 27	91009	265271	4422	265271	4775
145	RNA binding motif protein 28	106344	223073	4423	223073	4776
146	RNA binding motif protein 33	184863	438356	4424	405793	4777

147	RNA binding motif protein 33	184863	287912	4425	287912	4778
148	RNA binding motif protein 33	184863	401878	4426	384160	4779
149	RNA binding motif protein 33	184863	341 148	4427	341583	4780
150	RNA binding motif protein 34	188739	408888	4428	386226	4781
151	RNA binding motif protein 34	188739	400947	4429	383731	4782
152	RNA binding motif protein 34	188739	429912	4430	413409	4783
153	RNA binding motif protein 34	188739	366606	4431	355565	4784
154	RNA binding motif protein 38	132819	356208	4432	348538	4785
155	RNA binding motif protein 38	132819	440234	4433	407848	4786
156	RNA binding motif protein 38	132819	371219	4434	360263	4787
157	RNA binding motif protein 39	131051	253363	4435	253363	4788
158	RNA binding motif protein 39	131051	407261	4436	384541	4789
159	RNA binding motif protein 39	131051	361 162	4437	354437	4790
160	RNA binding motif protein 39	131051	448303	4438	394824	4791
161	RNA binding motif protein 39	131051	374038	4439	363150	4792
162	RNA binding motif protein 39	131051	528062	4440	436747	4793
163	RNA binding motif protein 39	131051	338163	4441	344581	4794
164	RNA binding motif protein 39	131051	434927	4442	393493	4795
165	RNA binding motif protein 4	173933	532968	4443	432020	4796
166	RNA binding motif protein 4	173933	408993	4444	386561	4797

167	RNA binding motif protein 4	173933	409406	4445	386894	4798
168	RNA binding motif protein 4	173933	483858	4446	435821	4799
169	RNA binding motif protein 4	173933	310092	4447	309166	4800
170	RNA binding motif protein 41	89682	434854	4448	405522	4801
171	RNA binding motif protein 41	89682	372479	4449	361557	4802
172	RNA binding motif protein 41	89682	372487	4450	361565	4803
173	RNA binding motif protein 41	89682	372482	4451	361560	4804
174	RNA binding motif protein 41	89682	203616	4452	203616	4805
175	RNA binding motif protein 42	126254	262633	4453	262633	4806
176	RNA binding motif protein 42	126254	360475	4454	353663	4807
177	RNA binding motif protein 43	184898	331426	4455	33121 1	4808
178	RNA binding motif protein 44	177483	316997	4456	321 179	4809
179	RNA binding motif protein 44	177483	409864	4457	386727	4810
180	RNA binding motif protein 45	155636	286070	4458	286070	481 1
181	RNA binding motif protein 45	155636	455903	4459	415940	4812
182	RNA binding motif protein 46	151962	281722	4460	281722	4813
183	RNA binding motif protein 47	163694	295971	4461	295971	4814
184	RNA binding motif protein 47	163694	381793	4462	371212	4815
185	RNA binding motif protein 47	163694	5 11902	4463	42511 1	4816
186	RNA binding motif protein 47	163694	515053	4464	422564	4817

187	RNA binding motif protein 47	163694	511598	4465	424019	4818
188	RNA binding motif protein 47	163694	513473	4466	421589	4819
189	RNA binding motif protein 47	163694	505414	4467	423527	4820
190	RNA binding motif protein 47	163694	514782	4468	426542	4821
191	RNA binding motif protein 47	163694	319592	4469	320108	4822
192	RNA binding motif protein 47	163694	507180	4470	423398	4823
193	RNA binding motif protein 47	163694	381795	4471	371214	4824
194	RNA binding motif protein 47	163694	505220	4472	425507	4825
195	RNA binding motif protein 48	127993	509224	4473	442073	4826
196	RNA binding motif protein 48	127993	450580	4474	401920	4827
197	RNA binding motif protein 48	127993	265732	4475	265732	4828
198	RNA binding motif protein 4B	173914	525754	4476	433071	4829
199	RNA binding motif protein 4B	173914	310046	4477	310471	4830
200	RNA binding motif protein 5	3756	469838	4478	419534	4831
201	RNA binding motif protein 5	3756	347869	4479	343054	4832
202	RNA binding motif protein 5	3756	441305	4480	390711	4833
203	RNA binding motif protein 5	3756	543047	4481	442591	4834
204	RNA binding motif protein 5	3756	536082	4482	445347	4835
205	RNA binding motif protein 5	3756	417905	4483	406119	4836
206	RNA binding motif protein 5	3756	437500	4484	394622	4837

207	RNA binding motif protein 5	3756	544851	4485	439808	4838
208	RNA binding motif protein 5	3756	539538	4486	440744	4839
209	RNA binding motif protein 6	4534	422955	4487	392939	4840
210	RNA binding motif protein 6	4534	442092	4488	393530	4841
211	RNA binding motif protein 6	4534	425608	4489	408665	4842
212	RNA binding motif protein 6	4534	443081	4490	396466	4843
213	RNA binding motif protein 6	4534	416583	4491	390202	4844
214	RNA binding motif protein 6	4534	43381 1	4492	389763	4845
215	RNA binding motif protein 6	4534	539992	4493	443165	4846
216	RNA binding motif protein 6	4534	266022	4494	266022	4847
217	RNA binding motif protein 7	76053	540163	4495	439918	4848
218	RNA binding motif protein 8A	131795	369307	4496	358313	4849
219	RNA binding motif protein 8A	131795	330165	4497	333001	4850
220	RNA binding motif protein, X-linked	147274	449161	4498	415250	4851
221	RNA binding motif protein, X-linked	147274	320676	4499	359645	4852
222	RNA binding motif protein, X-linked	147274	419968	4500	4051 17	4853
223	RNA binding motif protein, X-linked	147274	431446	4501	4 11989	4854
224	RNA binding motif protein, X-linked 2	134597	305536	4502	339090	4855
225	RNA binding motif protein, X-linked 2	134597	370947	4503	359985	4856
226	RNA binding motif protein, X-linked 2	134597	538614	4504	437425	4857

227	RNA binding motif protein, X-linked-like 1	213516	399794	4505	446099	4858
228	RNA binding motif protein, X-linked-like 1	213516	321792	4506	318415	4859
229	RNA binding motif protein, X-linked-like 2	170748	306904	4507	304139	4860
230	RNA binding motif protein, X-linked-like 3	175718	424776	4508	417451	4861
231	RNA binding motif protein, Y-linked, family 1, member A 1	234414	382707	4509	372154	4862
232	RNA binding motif protein, Y-linked, family 1, member A 1	234414	439108	4510	388006	4863
233	RNA binding motif protein, Y-linked, family 1, member A 1	234414	303902	451 1	303712	4864
234	RNA binding motif protein, Y-linked, family 1, member B	242875	383020	4512	372484	4865
235	RNA binding motif protein, Y-linked, family 1, member D	244395	418956	4513	399181	4866
236	RNA binding motif protein, Y-linked, family 1, member D	244395	382680	4514	372127	4867
237	RNA binding motif protein, Y-linked, family 1, member D	244395	382677	4515	372124	4868
238	RNA binding motif protein, Y-linked, family 1, member E	242389	382658	4516	372104	4869
239	RNA binding motif protein, Y-linked, family 1, member E	242389	382659	4517	372105	4870
240	RNA binding motif protein, Y-linked, family 1, member E	242389	382673	4518	3721 19	4871
241	RNA binding motif protein, Y-linked, family 1, member F	169800	303766	4519	307155	4872
242	RNA binding motif protein, Y-linked, family 1, member F	169800	454978	4520	406005	4873
243	RNA binding motif protein, Y-linked, family 1, member J	226941	414629	4521	405745	4874
244	RNA binding motif protein, Y-linked, family 1, member J	226941	250831	4522	250831	4875
245	RNA binding motif protein, Y-linked, family 1, member J	226941	445779	4523	389621	4876
246	RNA binding motif, single stranded interacting protein 1	153250	348849	4524	294904	4877

247	RNA binding motif, single stranded interacting protein 1	153250	428519	4525	389016	4878
248	RNA binding motif, single stranded interacting protein 1	153250	409075	4526	386347	4879
249	RNA binding motif, single stranded interacting protein 1	153250	409972	4527	387280	4880
250	RNA binding motif, single stranded interacting protein 1	153250	392753	4528	376508	4881
251	RNA binding motif, single stranded interacting protein 1	153250	409289	4529	386571	4882
252	RNA binding motif, single stranded interacting protein 2	76067	262031	4530	262031	4883
253	RNA binding motif, single stranded interacting protein 3	144642	434693	4531	395592	4884
254	RNA binding motif, single stranded interacting protein 3	144642	383767	4532	373277	4885
255	RNA binding motif, single stranded interacting protein 3	144642	383766	4533	373276	4886
256	RNA binding motif, single stranded interacting protein 3	144642	456853	4534	400519	4887
257	RNA binding motif, single stranded interacting protein 3	144642	396583	4535	379828	4888
258	RNA binding motif, single stranded interacting protein 3	144642	273139	4536	273139	4889
259	RNA binding protein S1, serine-rich domain	205937	320225	4537	315859	4890
260	RNA binding protein S1, serine-rich domain	205937	301730	4538	301730	4891
261	RNA binding protein S1, serine-rich domain	205937	397086	4539	380275	4892
262	RNA binding protein with multiple splicing	1571 10	320203	4540	318102	4893
263	RNA binding protein with multiple splicing	1571 10	287771	4541	287771	4894
264	RNA binding protein with multiple splicing	1571 10	339877	4542	340176	4895
265	RNA binding protein with multiple splicing	1571 10	538486	4543	445406	4896
266	RNA binding protein with multiple splicing	1571 10	397323	4544	380486	4897

267	RNA binding protein with multiple splicing 2	166831	300069	4545	300069	4898
268	RNA binding protein, autoantigenic (hnRNP-associated with lethal yellow homolog (mouse))	125970	246194	4546	246194	4899
269	RNA binding protein, autoantigenic (hnRNP-associated with lethal yellow homolog (mouse))	125970	442805	4547	415973	4900
270	RNA binding protein, autoantigenic (hnRNP-associated with lethal yellow homolog (mouse))	125970	448364	4548	413638	4901
271	RNA binding protein, autoantigenic (hnRNP-associated with lethal yellow homolog (mouse))	125970	413297	4549	403744	4902
272	RNA binding protein, autoantigenic (hnRNP-associated with lethal yellow homolog (mouse))	125970	375114	4550	364255	4903
273	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	311745	4551	309117	4904
274	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	550418	4552	450031	4905
275	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	355637	4553	347855	4906
276	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	553186	4554	447753	4907
277	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	436368	4555	402745	4908
278	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	352951	4556	322925	4909
279	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	340209	4557	344196	4910
280	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	547338	4558	447717	4911
281	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	547372	4559	446842	4912
282	RNA binding protein, fox-1 homolog (C. elegans) 1	78328	551752	4560	447281	4913
283	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	397303	4561	380470	4914

284	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	397305	4562	380472	4915
285	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	338644	4563	342831	4916
286	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	408983	4564	386177	4917
287	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	438146	4565	413035	4918
288	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	359369	4566	352328	4919
289	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	262829	4567	262829	4920
290	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	405409	4568	384944	4921
291	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	449924	4569	391670	4922
292	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	414461	4570	407855	4923
293	RNA binding protein, fox-1 homolog (C. elegans) 2	100320	416721	4571	405651	4924
294	RNA binding protein, fox-1 homolog (C. elegans) 3	167281	415831	4572	408395	4925
295	RNA binding protein, fox-1 homolog (C. elegans) 3	167281	453134	4573	393262	4926
296	S1 RNA binding domain 1	68784	535761	4574	263736	4927
297	S1 RNA binding domain 1	68784	263736	4575	441272	4928
298	SERPINE1 mRNA binding protein 1	142864	370995	4576	360034	4929
299	SERPINE1 mRNA binding protein 1	142864	361219	4577	354591	4930
300	SERPINE1 mRNA binding protein 1	142864	370994	4578	360033	4931
301	SERPINE1 mRNA binding protein 1	142864	370990	4579	360029	4932
302	signal recognition particle 14kDa (homologous Alu RNA binding protein)	140319	267884	4580	267884	4933
303	spermatid perinuclear RNA binding protein	165209	407982	4581	384292	4934

304	spermatid perinuclear RNA binding protein	165209	348403	4582	321347	4935
305	spermatid perinuclear RNA binding protein	165209	4791 14	4583	431531	4936
306	spermatid perinuclear RNA binding protein	165209	447404	4584	415968	4937
307	spermatid perinuclear RNA binding protein	165209	360998	4585	354271	4938
308	SRA stem-loop interacting RNA binding protein	119705	557342	4586	450909	4939
309	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	371856	4587	360922	4940
310	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	360426	4588	353604	4941
311	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	371828	4589	360893	4942
312	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	371805	4590	360870	4943
313	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	371802	4591	360867	4944
314	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	456866	4592	398785	4945
315	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	347458	4593	323443	4946
316	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	340954	4594	345425	4947
317	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	371792	4595	360857	4948
318	staufer, RNA binding protein, homolog 1 (Drosophila)	124214	437404	4596	416779	4949
319	staufer, RNA binding protein, homolog 2 (Drosophila)	40341	355780	4597	348026	4950
320	staufer, RNA binding protein, homolog 2 (Drosophila)	40341	524300	4598	428756	4951
321	staufer, RNA binding protein, homolog 2 (Drosophila)	40341	524104	4599	43061 1	4952
322	staufer, RNA binding protein, homolog 2 (Drosophila)	40341	522695	4600	428456	4953
323	staufer, RNA binding protein, homolog 2 (Drosophila)	40341	522509	4601	427977	4954

324	staufen, RNA binding protein, homolog 2 (Drosophila)	40341	521736	4602	428737	4955
325	staufen, RNA binding protein, homolog 2 (Drosophila)	40341	521447	4603	428829	4956
326	TAR (HIV-1) RNA binding protein 1	59588	40877	4604	40877	4957
327	TAR (HIV-1) RNA binding protein 2	139546	456234	4605	416077	4958
328	TAR (HIV-1) RNA binding protein 2	139546	266987	4606	266987	4959
329	TIA1 cytotoxic granule-associated RNA binding protein	116001	433529	4607	401371	4960
330	TIA1 cytotoxic granule-associated RNA binding protein	116001	477807	4608	445092	4961
331	TIA1 cytotoxic granule-associated RNA binding protein	116001	415783	4609	404023	4962
332	TIA1 cytotoxic granule-associated RNA binding protein-like 1	151923	412524	4610	403573	4963
333	TIA1 cytotoxic granule-associated RNA binding protein-like 1	151923	436547	461 1	394902	4964
334	TIA1 cytotoxic granule-associated RNA binding protein-like 1	151923	369086	4612	358082	4965
335	TIA1 cytotoxic granule-associated RNA binding protein-like 1	151923	369093	4613	358089	4966
336	TIA1 cytotoxic granule-associated RNA binding protein-like 1	151923	369092	4614	358088	4967
337	zinc finger CCHC-type and RNA binding motif 1	139168	266529	4615	266529	4968
338	zinc finger RNA binding protein	56097	265069	4616	265069	4969
339	zinc finger RNA binding protein	56097	416900	4617	393243	4970
340	zinc finger RNA binding protein	56097	382126	4618	371560	4971
341	zinc finger RNA binding protein 2	105278	439086	4619	388567	4972
342	zinc finger RNA binding protein 2	105278	262961	4620	262961	4973

343	zinc finger RNA binding protein 2	105278	438164	4621	388974	4974
344	cold shock domain containing El, RNA-binding	9307	261443	4622	261443	4975
345	cold shock domain containing El, RNA-binding	9307	339438	4623	342408	4976
346	cold shock domain containing El, RNA-binding	9307	358528	4624	351329	4977
347	cold shock domain containing El, RNA-binding	9307	369530	4625	358543	4978
348	cold shock domain containing El, RNA-binding	9307	438362	4626	407724	4979
349	cold shock domain containing El, RNA-binding	9307	525878	4627	431562	4980
350	cold shock domain containing El, RNA-binding	9307	525970	4628	432805	4981
351	cold shock domain containing El, RNA-binding	9307	530886	4629	431297	4982
352	cold shock domain containing El, RNA-binding	9307	534389	4630	435185	4983
353	cold shock domain containing El, RNA-binding	9307	534699	4631	432958	4984

[00986] HuR is a stabilizing AREBP. To increase the stability of the mRNA of interest, an mRNA encoding HuR can be co-transfected or co-injected along with the mRNA of interest into the cells or into the tissue. These proteins can also be tethered to the mRNA of interest in vitro and then administered to the cells together. Poly A tail binding protein, PABP interacts with eukaryotic translation initiation factor eIF4G to stimulate translational initiation. Co-administration of mRNAs encoding these RBPs along with the mRNA drug and/or tethering these proteins to the mRNA drug in vitro and administering the protein-bound mRNA into the cells can increase the translational efficiency of the mRNA. The same concept can be extended to co-administration of mRNA along with mRNAs encoding various translation factors and facilitators as well as with the proteins themselves to influence RNA stability and/or translational efficiency.

Example 24. Expression of modified nucleic acid with microRNA binding site

[00987] Human embryonic kidney epithelial cells (HEK293A), antigen presenting cells or cell lines with highly expressed mir-142/146, such as monocyte-derived dendritic

cells (MDDC) or PBMC, are seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). G-CSF mRNA (mRNA sequence is shown in SEQ ID NO: 4258; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-142-5p binding site (G-CSF miR-142-5p) (cDNA sequence is shown in SEQ ID NO: 4985; mRNA sequence is shown in SEQ ID NO: 4986, polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl), G-CSF mRNA having a seed sequence from miR-142-5p binding site (G-CSF miR-142-5p-seed) (cDNA sequence is shown in SEQ ID NO: 4987; mRNA sequence is shown in SEQ ID NO: 4988; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-142-5p binding site without the seed sequence (G-CSF miR-142-5p-seedless) (cDNA sequence is shown in SEQ ID NO: 4989, mRNA sequence is shown in SEQ ID NO: 4990; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-142-3p binding site (G-CSF miR-142-3p) (cDNA sequence is shown in SEQ ID NO: 4991; mRNA sequence is shown in SEQ ID NO: 4992; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a seed sequence from miR-142-3p binding site (G-CSF miR-142-3p-seed) (cDNA sequence is shown in SEQ ID NO: 4993; mRNA sequence is shown in SEQ ID NO: 4994; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-142-3p binding site without the seed sequence (G-CSF miR-142-3p-seedless) (DNA sequence is shown in SEQ ID NO: 4995; mRNA sequence is shown in SEQ ID NO: 4996; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-146a binding site (G-CSF miR-146a) (cDNA sequence is shown in SEQ ID NO: 4997; mRNA sequence is shown in SEQ ID NO: 4998; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a seed sequence from miR-146a binding site (G-CSF miR-146a-seed) (cDNA sequence is shown in SEQ ID NO: 4999; mRNA sequence is shown in SEQ ID NO: 5000; polyA tail at least 140 nucleotides not shown in sequence; 5'Cap, Capl) or G-CSF mRNA having a miR-146a binding site without the seed sequence (G-CSF miR-146a-seedless) (cDNA sequence is shown in SEQ ID NO: 5001; mRNA sequence is shown in SEQ ID NO: 5002; polyA tail at least nucleotides not shown in sequence; 5'Cap, Capl) are tested at a concentration of

250 ng per well in 24 well plates. The mRNA sequences are evaluated with various chemical modifications described herein and/or known in the art including, fully modified with 5-methylcytidine and pseudouridine, fully modified with 5-methylcytidine and 1-methylpseudouridine, fully modified with pseudouridine, fully modified with 1-methylpseudouridine and where 25% of the uridine residues are modified with 2-thiouridine and 25% of the cytosine residues are modified with 5-methylcytidine. The expression of G-CSF in each sample is measured by ELISA.

[00988] Shown in Table 27 are the DNA and mRNA G-CSF sequences with the miR binding sites described above. In the table, the start codon of each sequence is underlined.

Table 27. G-CSF Constructs with miR binding sites

SEQ ID NO.	Description	SEQ
4985	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR-142-5p	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACCAT <u>ATG</u> GCCGGTCCCGCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGAGTCA TTCCTTTTGAAGTGCTCGGAGCAGGTGCGAAAGATTCAAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCAGAGCTTGGGG ATTCCCTGGGCTCCTCTCTCGTCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCCTTTCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTTT GCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCAC CCGCGCTGCAGCCACGCAGGGGGCAATGCCGGCCTTTGCGTC CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTTTGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCCTTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTAGTAGT GCTTTCTACTTTATGTGGTCTTTGAATAAAGCCTGAGTAGGAAG GCGGCCGCTCGAGCATGCATCTAGA
4986	mRNA sequence: G-CSF miR-142-5p	GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCA <u>AUG</u> GCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU AUGGCCUUGCAGUUGCUGCUUUGGCACUCGGCCUCUGGACA GUCCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUU CAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACA UACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCUCGGGCAC AGCUUGGGGAUUCCUGGGCUCUCUCUGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCAGCUCCACUCC GGUUUGUUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGA AUCUCGCCAGAAUUGGGCCCGACGUGGACACGUUGCAGCUC GACGUGGCGGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAG

		GAACUGGGGAUGGCACCCGCGCUGCAGCCCACGCAGGGGGCA AUGCCGGCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUUCAUUAUUUUUGGAAGUCUCG UACCGGGUGCUGAGACAUCUUGCGCAGCCGUGAUAAUAGGCU GCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCU CCCUUGCACCUGUACCUCUAGUAGUGCUUUCUACUUUAUGUG GUCUUUGAAUAAAGCCUGAGUAGGAAG
4987	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR- 142-5p-seed	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCA TTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGG ATTCCCTGGGCTCCTCTCTCGTCTGTCCGTGCGCAGGCTTTGCA GTTGGCAGGGTGCCCTTTCCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTTT GCAACAACCATCTGGCAGCAGATGGAGGAAGTGGGGATGGCAC CCGCGCTGCAGCCCACGCAGGGGGCAATGCCGGCCTTTGCGTC CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTTTGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCCCTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTACTTTA TTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTCGA GCATGCATCTAGA
4988	mRNA sequence: G-CSF miR- 142-5p-seed	GGGAAAUAAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCC CUGCAGUUGCUGCUUUGGCACUCGGCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCA UUCUUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGC GAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAA CUUUGCCAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUG GGGAUUCCCUUGGGCUCCUCUCUGUCCUGUCCGUCGCAGGCU UUGCAGUUGGCAGGGUGCCUUUCCAGCUCCACUCCGUUUUG UUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGAAUCUCG CCAGAAUUGGGCCCGACGCUGGACACGUUGCAGCUCGACGUG GCGGAUUUCGCAACAACCAUCUGGCAGCAGAUUGGAGGAACUG GGGAUGGCACCCGCGCUGCAGCCCACGCAGGGGGGCAAUGCCG GCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGAGUCCUC GUAGCGAGCCACCUUCAAUCAUUUUUGGAAGUCUCGUACCGG GUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUG CCCUUCUUCUCUCCCUUGCACCUGUACCUCUACUUUAUUGGU CUUUGAAUAAAGCCUGAGUAGGAAG
4989	DNA sequence having the T7 polymerase site and restriction	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCA TTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGG

	sites: G-CSF miR- 142-5p- seedless	ATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCTTTCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTTT GCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCAC CCGCGCTGCAGCCACGCAGGGGGCAATGCCGGCCTTTGCGTC CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTTGGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCTTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTAGTAGT GCTTTCTGTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCC GCTCGAGCATGCATCTAGA
4990	mRNA sequence: G-CSF miR- 142-5p- seedless	GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUAUAAGA GCCACC <u>AUG</u> GCCGGUCCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCC CUGCAGUUGCUGCUUUGGCACUCGGCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCA UCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGC GAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUAACAAA CUUUGCCAUCCCCGAGGAGCUCGUACUCUCGGGCACAGCUUG GGGAUUCCCUGGGCUCCUCUCUGUCCUGUCCGUCGCAGGCU UUGCAGUUGGCAGGGUGCCUUUCCAGCUCCACUCCGGUUUG UUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGAUUCUG CCAGAAUUGGGCCCGACGCGUGGACACGUUGCAGCUCAGCUG GCGGAUUUCGCAACAACCAUCUGGCAGCAGAUUGGAGGAACUG GGGAUGGCACCCGCGCUGCAGCCACGCAGGGGGCAUUGCCG GCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGAGUCCUC GUAGCGAGCCACCUCAAUCAUUUUUGGAAGUCUCGUACCGG GUGCUGAGACAUCUUGCGCAGCCG UGAUAUAAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUG CCCUUCUUCUCUCCCUUGCACCUGUACCUCUAGUAGUGCUUU CUGUGGUCUUUGAAUAAAGCCUGAGUAGGAAG
4991	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR- 142-3p	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGAGTCA TTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTGAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATAAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGG ATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCTTTCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTTT GCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCAC CCGCGCTGCAGCCACGCAGGGGGCAATGCCGGCCTTTGCGTC CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTTGGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCTTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTCCATA AAGTAGGAAACACTACATGGTCTTTGAATAAAGCCTGAGTAGG AAGGCGGCCGCTCGAGCATGCATCTAGA
4992	mRNA sequence: G-CSF miR-	GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUAUAAGA GCCACC <u>AUG</u> GCCGGUCCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCC

	142-3p	CUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCA UUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGC GAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUAACAAA CUUUGCCAUCCCCGAGGAGCUCGUACUCUCGGGCACAGCUUG GGGAUUCCCUGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCU UUGCAGUUGGCAGGGUGCCUUUCCCAGCUCCACUCCGGUUUG UUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGAAUCUCG CCAGAAUUGGGCCCCGACGCUGGACACGUUGCAGCUCGACGUG GCGGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAGGAACUG GGGAUGGCACCCGCGCUGCAGCCCACGCAGGGGGCAAUGCCG GCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGAGUCCUC GUAGCGAGCCACCUUCAUAUUUUUGGAAGUCUCGUACCGG GUGCUGAGACAUCUUGCGCAGCCG UGAUAUAAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUG CCCUUCUUCUCUCCCUUGCACCUGUACCUCUCCAUAAGUA GGAAACACUACAUGGUCUUUGAAUAAGCCUGAGUAGGAAG
4993	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR- 142-3p-seed	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCA TTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTCAAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACAAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGG ATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTGCGAGCTTTGCA GTTGGCAGGGTGCCTTTCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTTT GCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCAC CCGCGCTGCAGCCCACGCAGGGGGCAATGCCGGCCTTTGCGTC CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCTTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTACACTA CTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTCGA GCATGCATCTAGA
4994	mRNA sequence: G-CSF miR- 142-3p-seed	GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACC <u>AUG</u>GCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCC CUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCA UUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGC GAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUAACAAA CUUUGCCAUCCCCGAGGAGCUCGUACUCUCGGGCACAGCUUG GGGAUUCCCUGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCU UUGCAGUUGGCAGGGUGCCUUUCCCAGCUCCACUCCGGUUUG UUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGAAUCUCG CCAGAAUUGGGCCCCGACGCUGGACACGUUGCAGCUCGACGUG GCGGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAGGAACUG GGGAUGGCACCCGCGCUGCAGCCCACGCAGGGGGCAAUGCCG GCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGAGUCCUC GUAGCGAGCCACCUUCAUAUUUUUGGAAGUCUCGUACCGG GUGCUGAGACAUCUUGCGCAGCCG UGAUAUAAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUG

		CCCUUCUUCUCUCCCUUGCACCUGUACCUCUACACUACUGGU CUUUGAAUAAAGCCUGAGUAGGAAG
4995	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR- 142-3p- seedless	TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGAGTCA TTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGG ATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCTTTCCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCAGCGCTGGACACGTTGCAGCTCGACGTGGCGGATTTTC GCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCAC CCGCGCTGCAGCCCACGCAGGGGGCAATGCCGGCCTTTGCGTC CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTTGGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCTTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTCCATA AAGTAGGAAATGGTCTTTGAATAAAGCCTGAGTAGGAAGGCG GCCGCTCGAGCATGCATCTAGA
4996	mRNA sequence: G-CSF miR- 142-3p- seedless	GGGAAAUAAAGAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU AUGGCCUUGCAGUUGCUGCUUUGGCACUCGGCCUCUGGACA GUCCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUU CAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACA UACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCUCGGGCAC AGCUUGGGGAUUCUCCUGGGCUCCUCUCUGCCUGCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCAGCUCCACUCC GGUUUGUUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGA AUCUCGCCAGAAUUGGGCCCACGCUGGACACGUUGCAGCUC GACGUGGCGGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAG GAACUGGGGAUGGCACCCGCGCUGCAGCCACGCAGGGGGCA AUGCCGGCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUCAAUCAUUUUUGGAAGUCUCG UACCGGGUGCUGAGACAUCUUGCGCAGCCGUGAUAAUAGGCU GCCUUCUGCGGGGCUUGCCUUCUGGCAUGCCCUUCUUCUCU CCCUUGCACCUGUACCUCUCCAUAAGUAGGAAAUGGUCUU UGAAUAAAGCCUGAGUAGGAAG
4997	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR- 146a	TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGAGTCA TTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGG ATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCTTTCCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCAGCGCTGGACACGTTGCAGCTCGACGTGGCGGATTTTC GCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCAC CCGCGCTGCAGCCCACGCAGGGGGCAATGCCGGCCTTTGCGTC

		CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTTTGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCCTTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTAACCCA TGGAATTCAGTTCTCATGGTCTTTGAATAAAGCCTGAGTAGGAA GGCGGCCGCTCGAGCATGCATCTAGA
4998	mRNA sequence: G-CSF miR- 146a	GGGAAAUAAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU AUGGCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACA GUCCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUU CAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACA UACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCUCGGGCAC AGCUUGGGGAUUCCUGGGCUCUCUCUGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCAGCUCCACUCC GGUUUGUUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGA AUCUCGCCAGAAUUGGGCCCGACGCUGGACACGUUGCAGCUC GACGUGGCGGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAG GAACUGGGGAUGGCACCCGCGCUGCAGCCACGCAGGGGGCA AUGCCGGCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUUCAUAUUUUGGAAGUCUCG UACCGGGUGCUGAGACAUCUUGCGCAGCCGUGAUAAUAGGCU GCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCU CCCUUGCACCUGUACCUCUAACCCAUGGAUUUCAGUUCUCAU GGUCUUUGAAUAAAGCCUGAGUAGGAAG
4999	DNA sequence having the T7 polymerase site and restriction sites: G-CSF- 146a-seed	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCA TTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTGAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACTAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGG ATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCTTTCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTTT GCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCAC CCGCGCTGCAGCCACGCAGGGGGCAATGCCGGCCTTTGCGTC CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTTTGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCCTTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTAGTTCT CTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTCGA GCATGCATCTAGA
5000	mRNA sequence: G-CSF- 146a-seed	GGGAAAUAAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU AUGGCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACA GUCCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUU CAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACA UACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCUCGGGCAC AGCUUGGGGAUUCCUGGGCUCUCUCUGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCAGCUCCACUCC GGUUUGUUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGA

		AUCUCGCCAGAAUUGGGCCCCGACGCUGGACACGUUGCAGCUC GACGUGGCGGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAG GAACUGGGGAUGGCACCCGCGCUGCAGCCCACGCAGGGGGCA AUGCCGGCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUUCAAUCAUUUUUGGAAGUCUCG UACCGGGUGCUGAGACAUCUUGCGCAGCCGUGAUAAUAGGCU GCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCU CCCUUGCACCUGUACCUCUAGUUCUCUGGUCUUUGAAUAAAG CCUGAGUAGGAAG
5001	DNA sequence having the T7 polymerase site and restriction sites: G-CSF- 146a- seedless	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAG CCACC <u>ATG</u> GCCGGTCCC GCGACCCAAAGCCCCATGAACTTAT GGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCC AAGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGAGTCA TTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTGAGGGCG ATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACAAACT TTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGG ATTCCCTGGGCTCCTCTCTCGTCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCCTTTCCAGCTCCACTCCGGTTTGTCTTGT ATCAGGGACTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATT GGGCCCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTTT GCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCAC CCGCGCTGCAGCCCACGCAGGGGGCAATGCCGGCCTTTGCGTC CGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCAC CTTCAATCATTTTTTGAAGTCTCGTACCGGGTGCTGAGACATCT TGCGCAGCCGTGATAATAGGCTGCCTTCTGCGGGGCTTGCTTC TGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTAACCCA TGGAATTCATGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGC CGCTCGAGCATGCATCTAGA
5002	mRNA sequence: G-CSF- 146a- seedless	GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCA <u>U</u> GGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU AUGGCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACA GUCCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUU CAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCGGACA UACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCUCGGGCAC AGCUUGGGGAUUCUCCUGGGCUCCUCUCUGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCAGCUCCACUCC GGUUUGUUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGA AUCUCGCCAGAAUUGGGCCCCGACGCUGGACACGUUGCAGCUC GACGUGGCGGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAG GAACUGGGGAUGGCACCCGCGCUGCAGCCCACGCAGGGGGCA AUGCCGGCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUUCAAUCAUUUUUGGAAGUCUCG UACCGGGUGCUGAGACAUCUUGCGCAGCCGUGAUAAUAGGCU GCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCU CCCUUGCACCUGUACCUCUAACCCAUGGAAUUCAUGGUCUUU GAAUAAAGCCUGAGUAGGAAG

[00989] It is like that the binding site "seed" sequence is sufficient to induce mircoRNA binding, the expression of G-CSF should be down-regulated in cells transfected with miR-142-3p, miR-142-3p-seed, miR-142-5p, miR-142-5p-seed, miR-

146a or miR-146a-seed. Whereas, the miR-142-3p-seedless, miR-142-5p-seedless, miR-146a-seedless should not change the expression of G-CSF, as compared with cells transfected with G-CSF mRNA without microRNA binding sites.

Example 25. APCs specific microRNA binding sites to suppress modified nucleic acid mediated immune stimulation

[00990] The binding sites for microRNAs are used in the 3'UTR of mRNA therapeutics to selectively degrade mRNA therapeutics in the immune cells to subdue unwanted immunogenic reactions caused by mRNA therapeutics delivery.

[00991] A polynucleotide comprising a series of 3'UTR miR binding sites which make the the nucleic acids or mRNA of the present invention more unstable in antigen presenting cells (APCs), such as, but not limited to mir-142-5p, mir-142-3p, mir-146a-5p and mir-146a-3p, encodes an oncology-related polypeptide of the present invention. The addition of miR binding sites in the 3'UTR making a signal sensor polynucleotide unstable would subdue modified mRNA mediated immune stimulation.

[00992] Experiments comparing the cytokine expression (e.g. TNF-alpha) induced by the polypeptide with APCs specific microRNA signature vs. without such signature is performed in vitro by methods described herein and/or known in the art.

Example 26. In Vitro Expression of mRNAs with miR binding sites

[00993] Human embryonic kidney epithelial cells (HEK293A), antigen-presenting cells or cell lines with highly expressed mir-142/146, such as monocyte-derived dendritic cells (MDDC) or PBMC, are seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). Cultured cells are transfected with G-CSF mRNAs with or without microRNA signature, as described in Example 24. The cells are transfected for five consecutive days. The transfection complexes are removed four hours after each round of transfection.

[00994] The culture supernatant is assayed for secreted G-CSF (R&D Systems, catalog #DCS50), tumor necrosis factor-alpha (TNF-alpha) and interferon alpha (IFN-alpha by ELISA every day after transfection following manufacturer's protocols. The cells are analyzed for viability using CELL TITER GLO® (Promega, catalog #G7570) 6 hrs and 18 hrs after the first round of transfection and every alternate day following that. At the same time from the harvested cells, total RNA is isolated and treated with DNASE®

using the RNeasy micro kit (catalog #74004) following the manufacturer's protocol. 100 ng of total RNA is used for cDNA synthesis using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, cat #4368814) following the manufacturer's protocol. The cDNA is then analyzed for the expression of innate immune response genes by quantitative real time PCR using SybrGreen in a Biorad CFX 384 instrument following the manufacturer's protocol.

Example 27. In vivo detection of innate immune response study

[00995] To test the the nucleic acids or mRNA protein expression and in vivo immune response, female BALB/C mice (n=5) are injected intramuscularly with G-CSF mRNA with or without microRNA signatures as described in Example 24. Blood is collected at 8 hours after dosing. The protein levels of G-CSF, TNF-alpha and IFN-alpha is determined by ELISA.

[00996] The difference of cytokine production is seen as measured by mouse TNF-alpha and IFN-alpha level in serum. Injection with G-CSF modified mRNA having miR-142 and miR-146a binding site or binding site seed shows a lower level of cytokine response in vivo.

Example 28. Expression of miR-122 in primary hepatocytes

[00997] Hepatocyte specific miR-122 level in rat and human primary hepatocytes was measured. Hela Cells and primary rat and human hepatocytes were cultured and RNAs were extracted from cell lysates. The miR-122 level in rat and human primary hepatocytes was compared with that in Hela cells. The miR-122 level is about 6 fold increased in primary human hepatocytes and about 12 fold increased in primary rat hepatocytes, respectively, as compared with that in Hela cells.

Example 29. Expression of modified nucleic acid with mir-122 binding site in hepatocytes

[00998] Primary rat and human hepatocytes and Hela cells were seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). G-CSF mRNA having a miR-122 binding site in the 3'UTR (G-CSF miR-122-1X) (mRNA sequence is shown in SEQ ID NO: 4268; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Cap1) fully modified with 5-methylcytidine and pseudouridine (5mC/pU), or fully modified with pseudouridine(pU)

or G-CSF mRNA with four miR-122 binding sites with the seed deleted (G-CSF no seed) (mRNA sequence is shown in SEQ ID NO: 4270; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Cap1) fully modified with 5-methylcytidine and pseudouridine (5mC/pU) or fully modified with pseudouridine (pU) was tested at a concentration of 250 ng per well in 24 well plates. The 24 hours after transfection, the expression of G-CSF was measured by ELISA, and the results are shown in Table 28.

Table 28. G-CSF mir122 expression

	Hela cells	Primary human Hepatocytes	Primary rat Hepatocytes
	Protein Expression (ng/mL)	Protein Expression (ng/mL)	Protein Expression (ng/mL)
G-CSF miR-122 1X (5mC/pU)	167.34	67.60	3.40
G-CSF miR-122 1X (pU)	292.18	116.18	25.63
G-CSF no seed (5mC/pU)	194.78	129.77	8.39
G-CSF no seed (pU)	335.78	462.88	84.93

Example 30. Expression of modified nucleic acids with mir-122 binding sites in hepatocytes

[00999] MicroRNA control gene expression through the translational suppression and/or degradation of target messenger RNA. Mir-122 binding site containing G-CSF mRNA was translationally regulated in hepatocytes.

[001000] Primary rat and human hepatocytes and Hela cells were seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). G-CSF mRNA (G-CSF alpha) (mRNA sequence is shown in SEQ ID NO: 4266; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Cap1) fully modified with 5-methylcytidine and pseudouridine (5mC/pU), G-CSF mRNA having a miR-122 binding site in the 3'UTR (G-CSF miR-122-IX) (mRNA sequence is shown in SEQ ID NO: 4268; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Cap 1) fully modified with 5-methylcytidine and pseudouridine (5mc/pU) or G-CSF mRNA with four miR-122 binding sites with the seed deleted (G-

CSF no seed) (mRNA sequence is shown in SEQ ID NO: 4270; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Cap1) fully modified with 5-methylcytidine and pseudouridine (5mC/pU) was tested at a concentration of 250 ng per well in 24 well plates. 24 hours after transfection, the expression of G-CSF was measured by ELISA. The G-CSF drug (mRNA) levels and protein levels are shown in Table 29.

Table 29. G-CSF drug and protein levels

	Human Hepatocytes		Rat Hepatocytes	
	Drug (mRNA) level (unit normalized to HPRT)	Protein expression (ng/ml)	Drug (mRNA) level (unit normalized to HPRT)	Protein expression (ng/ml)
G-CSF alpha (5mC/pU)	43237.6	247.26	26615.88	784.6
G-CSF miR-122-1X (5mC/pU)	46340.9	74.07	20171.07	40.628
G-CSF no seed (5mC/pU)	70239.7	298.28	23170.47	894.06

Example 31. Modified mRNA sequences with or without Kozak and/or IRES sequences

[001001] Modified mRNA encoding G-CSF with or without a Kozak and/or IRES sequence, and their corresponding cDNA sequences, are shown below in Table 30. In Table 30, the start codon of each sequence is underlined.

Table 30. G-CSF Sequences

	Sequence	SEQ ID NO:
G-CSF with Kozak sequence and IRES and human alpha-globin	Optimized G-CSF cDNA sequence containing a T7 polymerase site, kozak sequence, IRES and Xba1 restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAG AGCCACC TCGTGAGGATCTATTTCCGGTGAATTCCTCGAGACTAGTTCT AGAGCGGCCGCGGATCCCGCCCCTCTCCCTCCCCCCCCCCTA ACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCG TTTGTCTATATGTTATTTCCACCATATTGCCGTCTTTTGCA	5003

3'UTR	ATGTGAGGGGCCGAAACCTGGCCCTGTCTTCTTGACGAGC ATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGT CTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCT TGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCG GAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGC CACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAG TGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATG GCTCACCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCC AGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGG TGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAACGTC TAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAA ACACGATGATAAT <u>ATG</u>GCCGGTCCCGCGACCCAAAGCCCCATGAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTC ATTCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAGG GCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATAC AACTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAG CTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCA GGCTTTGCAGTTGGCAGGGTGCCTTTCCCAGCTCCACTCCGG TTTGTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCGACGCTGGACACGTTGCAGCTCG ACGTGGCGGATTTTCGCAACAACCATCTGGCAGCAGATGGAG GAACTGGGGATGGCACCCGCGCTGCAGCCACGCAGGGGG CAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGT GGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTC TCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCTGACCCCGTACCCCCGTGGT CTTTGAATAAAGTCTGAGTGGGCGGCTCTAGA	
	mRNA sequence (transcribed): GGGAAAUAAAGAGAGAAAAGAAGAGUAAGAAGAAAUAAUAA GAGCCACC UCGUGAGGAUCUAUUUCCGGUGAAUUCCUCGAGACUAGUU CUAGAGCGGCCGCGGAUCCCGCCCCUCUCCUCCCCCCCCC CUAACGUUACUGGCCGAAGCCGCUUGGAAUAAGGCCGGUG UGCGUUUGUCUAUAUGUUAUUUCCACCAUAUUGCCGUCU UUUGGCAAUGUGAGGGCCCGGAAACCUGGCCCUUCUUCU UGACGAGCAUCCUAGGGGUCUUUCCCCUCUCGCCAAAGG AAUGCAAGGUCUGUUGAAUGUCGUGAAGGAAGCAGUUCC UCUGGAAGCUUCUUGAAGACAAACAACGUCUGUAGCGACC CUUUGCAGGCAGCGGAACCCCCACCUGGCGACAGGUGCC UCUGCGGCCAAAAGCCACGUGUAUAAGAUACACCUGCAAA GGCGGCACAACCCCAGUGCCACGUUGUGAGUUGGAUAGUU GUGGAAAGAGUCAAAUGGCUCACCUCAAGCGUAUUAACA AGGGGCUGAAGGAUGCCCAGAAGGUACCCCAUUGUAUGGG AUCUGAUCUGGGGCCUCGGUGCACAUGCUUUACAUGUGUU UAGUCGAGGUUAAAAAACGUCUAGGCCCCCGAACCACGG GGACGUGGUUUUCCUUUGAAAAACACGAUGAUAAU <u>AUG</u>GCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG	5004

	CCCUGCAGUUGCUGCUUUGGCACUCGGCCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCCUUGGGCUCCUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCCGACGUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGACA UCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGC	
G-CSF without a Kozak sequence and with an IRES and human alpha- globin 3'UTR	Optimized G-CSF cDNA sequence containing a T7 polymerase site, IRES and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAAGAAGAGTAAGAAGAAATATAAG A TCGTGAGGATCTATTTCCGGTGAATTCCTCGAGACTAGTTCT AGAGCGGCCGCGGATCCCGCCCTCTCCCTCCCCCCCCCTA ACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCG TTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGA ATGTGAGGGCCCCGAAACCTGGCCCTGTCTTCTTGACGAGC ATTCCTAGGGGTCTTTCCCTCTCGCCAAAGGAATGCAAGGT CTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCT TGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCG GAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGC CACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCAG TGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATG GCTCACCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCC AGAAGGTACCCATTGTATGGGATCTGATCTGGGGCCTCGG TGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTC TAGGCCCCCGAACCACGGGGACGTGGTTTTTCCTTTGAAAA ACACGATGATAAT ATGGCCCGTCCCGCGACCCAAAGCCCCATGAAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTC ATTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTCAAG GCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATAC AAACCTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAG CTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCA GGCTTTGCAGTTGGCAGGGTGCCTTTCCAGCTCCACTCCGG TTTGTTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCTCG ACGTGGCGGATTTGCAACAACCATCTGGCAGCAGATGGAG	5005

	GAAGTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGG CAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGT GGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTTGAAGTC TCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCGTGGT CTTTGAATAAAGTCTGAGTGGGCGGCTCTAGA	
	mRNA sequence (transcribed): GGGAAAUAAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAUA GA UCGUGAGGAUCUAUUUCCGGUGAAUUCUUGAGACUAGUU CUAGAGCGGCCGCGGAUCCCCGCCCCUCUCCUCCCCCCCCC CUAACGUUACUGGCCGAAGCCGCUUGGAAUAAGGCCGGUG UGCGUUUGUCUAUAUGUUAUUUCCACCAUAUUGCCGUCU UUUGGCAAUGUGAGGGCCCCGAAACCUGGCCUGUCUUCU UGACGAGCAUCCUAGGGGUCUUUCCCCUCUCGCCAAAGG AAUGCAAGGUCUGUUGAAUGUCGUGAAGGAAGCAGUCC UCUGGAAGCUUCUUGAAGACAAACAACGUCUGUAGCGACC CUUUGCAGGCAGCGGAACCCCCACCUGGCGACAGGUGCC UCUGCGGCCAAAAGCCACGUGUAUAAGAUACACCUGCAAA GGCGGCACAACCCCAGUGCCACGUUGUGAGUUGGAUAGUU GUGGAAAGAGUCAAAUGGCUCACCUCAGCGUAUUAACA AGGGGCUGAAGGAUGCCCAGAAGGUACCCCAUUGUAUGGG AUCUGAUCUGGGGCCUCGGUGCACAUGCUUUAUGUGUU UAGUCGAGGUUAAAAACGUCUAGGCCCCCGAACCACGG GGACGUGGUUUUCCUUUGAAAAACACGAUGAUAAU <u>AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG</u> CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCUGGGCUCUUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGACA UCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGC	5006
G-CSF without a Kozak sequence	Optimized G-CSF cDNA sequence containing a T7 polymerase site, a Kozak sequence and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAG	5007

and with a human alpha- globin 3'UTR	A ATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTC ATTCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTCAGG GCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATAC AAACTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAG CTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTGCGA GGCTTTGCAGTTGGCAGGGTGCCCTTCCCAGCTCCACTCCGG TTTGTTCCTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCTCG ACGTGGCGGATTTGCGAACAACCATCTGGCAGCAGATGGAG GAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGG CAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGT GGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTTGAAGTC TCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCTTCTGACCCGTACCCCCGTGGT CTTTGAATAAAGTCTGAGTGGGCGGCTCTAGA	
	mRNA sequence (transcribed): GGGAAUAAGAGAGAGAAAAGAAGAGUAAGAAGAAUAUAA GA AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCUGGGCUCCUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCCACGCAGGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGACA UCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGC	5008
G-CSF with an IRES, a human alpha- globin 3'UTR and a	Optimized G-CSF cDNA sequence containing a T7 polymerase site, IRES, a polyA tail of 80 nucleotides and Ascl restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAAG AGCCACC TCGTGAGGATCTATTTCCGGTGAATTCCTCGAGACTAGTTCT AGAGCGGCCGCGGATCCCGCCCCTCTCCCTCCCCCCCCCTA ACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCG	5009

polyA tail of 80 nucleotid es	TTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCA ATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGC ATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGT CTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCT TGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCG GAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGC CACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAG TGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATG GCTCACCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCC AGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGG TGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAACGTC TAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAA ACACGATGATAAT <u>ATG</u>GCCCGTCCCGCGACCCAAAGCCCCATGAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTC ATTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTGAGG GCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATAC AACTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAG CTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCA GGCTTTGCAGTTGGCAGGGTGCCTTTCCCAGCTCCACTCCGG TTTGTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCTCG ACGTGGCGGATTTGCAACAACCATCTGGCAGCAGATGGAG GAACTGGGGATGGCACCCGCGCTGCAGCCACGCAGGGGG CAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGT GGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTC TCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCCCTGCACCCGTACCCCCGTGGT CTTTGAATAAAGTCTGAGTGGGCGGCCAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCGCGCC	
	mRNA sequence (transcribed): GGGAAAUAGAGAGAAAAGAAGAGUAAGAAGAAUUAUAA GAGCCACC UCGUGAGGAUCUAUUUCCGGUGAAUUCCUCGAGACUAGUU CUAGAGCGGCCGCGGAUCCCGCCCCUCUCCCUCCCCCCCC CUAACGUUACUGGCCGAAGCCGCUUGGAAUAAGGCCGGUG UGCGUUUGUCUAUAUGUUAUUUCCACCAUAUUGCCGUCU UUUGGCAAUGUGAGGGCCCGGAAACCUGGCCUGUCUUUCU UGACGAGCAUCCUAGGGGUCUUUCCCCUCUCGCCAAAGG AAUGCAAGGUCUGUUGAAUGUCGUGAAGGAAGCAGUCC UCUGGAAGCUUCUUGAAGACAAACAACGUCUGUAGCGACC CUUUGCAGGCAGCGGAACCCCCACCUGGCGACAGGUGCC UCUGCGGCCAAAAGCCACGUGUAUAAGAUACACCUGCAAA GGCGGCACAACCCAGUGCCACGUUGUGAGUUGGAUAGUU GUGGAAAGAGUCAAAUGGCUCACCUCAAGCGUAUUAACA AGGGGCUGAAGGAUGCCCAGAAGGUACCCCAUUGUAUGGG AUCUGAUCUGGGGCCUCGGUGCACAUGCUUUAUUGUGUU	5010

	UAGUCGAGGUUAAAAAACGUCUAGGCCCCCCGAACCACGG GGACGUGGUUUUCCUUUGAAAAACACGAUGAUAAU <u>AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU</u>AUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCUGGGCUCCUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGACA UCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGC AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AA	
G-CSF without a Kozak sequence and with an IRES, a human alpha- globin 3'UTR and a polyA tail of 80 nucleotid es	Optimized G-CSF cDNA sequence containing a T7 polymerase site, an IRES sequence, a polyA tail of 80 nucleotides and Asc 1 restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAG A TCGTGAGGATCTATTTCCGGTGAATTCCTCGAGACTAGTTCT AGAGCGGCCGCGGATCCCGCCCCTCTCCCTCCCCCCCCCTA ACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCG TTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCA ATGTGAGGGCCCGAAACCTGGCCCTGTCTTCTTGACGAGC ATTCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGT CTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCT TGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCG GAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGC CACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAG TGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATG GCTCACCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCC AGAAGGTACCCATTGTATGGGATCTGATCTGGGGCCTCGG TGCACATGCTTTACATGTGTTTAGTCGAGGTAAAAAACGTC TAGGCCCCCGAACCACGGGACGTGGTTTTCTTTGAAAA ACACGATGATAAT <u>ATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTATGGC</u> <u>CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA</u> AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTC ATTCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTCAGG	501 1

	GCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATAC AAAC TTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAG CTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCA GGCTTTGCAGTTGGCAGGGTGCCTTTCCCAGCTCCACTCCGG TTTGTTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCTCG ACGTGGCGGATTTTCGAACAACCATCTGGCAGCAGATGGAG GAACTGGGGATGGCACCCGCGCTGCAGCCACGCAGGGGG CAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGT GGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTC TCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCCCTGCACCCGTACCCCCGTGGT CTTTGAATAAAGTCTGAGTGGGCGGCCAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCGCGCC	
	mRNA sequence (transcribed): GGGAAAUAGAGAGAAAAGAAGAGUAAGAAGAAAUUAUAA GA UCGUGAGGAUCUAUUUCCGGUGAAUUCUCGAGACUAGUU CUAGAGCGGCCGCGGAUCCCGCCCCUCUCCUCCCCCCCCC CUAACGUUACUGGCCGAAGCCGCUUGGAAUAAGGCCGGUG UGCGUUUGUCUAUAUGUUAUUUCCACCAUAUUGCCGUCU UUUGGCAAUGUGAGGGCCCGGAAACCUGGCCUUCUUCU UGACGAGCAUCCUAGGGGUCUUUCCCCUCUCGCCAAAGG AAUGCAAGGUCUGUUGAAUGUCGUGAAGGAAGCAGUUC UCUGGAAGCUUCUUGAAGACAAACAACGUCUGUAGCGACC CUUUGCAGGCAGCGGAACCCCCACCUGGCGACAGGUGCC UCUGCGGCCAAAAGCCACGUGUAUAAGAUACACCUGCAA GGCGGCACAACCCAGUGCCACGUUGUGAGUUGGAUAGUU GUGGAAAGAGUCAAAUGGCUCACCUCAAGCGUAUUAACA AGGGGCUGAAGGAUGCCCAGAAGGUACCCCAUUGUAUGGG AUCUGAUCUGGGGCCUCGGUGCACAUUCUUACAUGUGUU UAGUCGAGGUAAAAAACGUCUAGGCCCCCCGAACCACGG GGACGUGGUUUUCCUUUGAAAAACACGAUGAUAAU AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCUGGGCUCCUCUCUGUCC UGUCCGUCGAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGUUUGUUCUUGUAUCAGGGACUCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCCGACGUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGACA	5012

	UCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
G-CSF with a human alpha- globin 3'UTR and a polyA tail of 80 nucleotid es	Optimized G-CSF cDNA sequence containing a T7 polymerase site, a polyA tail of 80 nucleotides and Ascl restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAG AGCCACC <u>ATG</u> GCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTC ATTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAGG GCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATAC AAACCTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAG CTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTGCGA GGCTTTGCAGTTGGCAGGGTGCTTTTCCCAGCTCCACTCCGG TTTGTTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCTCG ACGTGGCGGATTTGCAACAACCATCTGGCAGCAGATGGAG GAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGG CAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGT GGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTTGAAGTC TCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCTTTCCTGCACCCGTACCCCCGTGGT CTTTGAATAAAGTCTGAGTGGGCGGCAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCGCGCC	5013
	mRNA sequence (transcribed): GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAUA GAGCCACC <u>AUG</u> GCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCUGGGCUCUUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGACA	5014

	UCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
G-CSF without a kozak sequence and with a human alpha-globin 3'UTR and a polyA tail of 80 nucleotides	Optimized G-CSF cDNA sequence containing a T7 polymerase site, a polyA tail of 80 nucleotides and Ascl restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAG A <u>ATG</u> GCCGGTCCCGCGACCCAAAGCCCCATGAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTC ATTCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTCAGG GCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATAC AAACTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAG CTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTGCGA GGCTTTGCAGTTGGCAGGGTGCTTTTCCAGCTCCACTCCGG TTTGTTCTTGATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCTCG ACGTGGCGGATTTGCAACAACCATCTGGCAGCAGATGGAG GAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGG CAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGT GGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTTGAAGTC TCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCTTCCTGCACCCGTACCCCCGTGGT CTTTGAATAAAGTCTGAGTGGGCGGCAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAGGCGCGCC	5015
	mRNA sequence (transcribed): GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAUA GA <u>AUG</u> GCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCUGGGCUCUUCUCGUCC UGUCCGUCGAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGACA	5016

	UCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	

[001002] These modified mRNA sequences can include at least one chemical modification described herein. The G-CSF modified mRNA sequence can be formulated, using methods described herein and/or known in the art, prior to transfection and/or administration.

[001003] The modified mRNA sequence encoding G-CSF can be transfected *in vitro* to various cell types such as HEK293, HeLa, PBMC and BJ fibroblast and those described in Table 25 using methods disclosed herein and/or are known in the art. The cells are then analyzed using methods disclosed herein and/or are known in the art to determine the concentration of G-CSF and/or cell viability.

[001004] The modified mRNA sequence encoding G-CSF can also be administered to mammals including mat, rats, non-human primates and humans. The serum and surrounding tissue can be collected at pre-determined intervals and analyzed using methods disclosed herein and/or are known in the art to determine the concentration of G-CSF and other pharmacokinetic properties mentioned herein.

Example 32. Modified mRNA sequences miR-122 sequences in an alpha-globin

UTR

[001005] Modified mRNA encoding G-CSF or Factor IX with a mir-122 sequence in a human or mouse alpha-globin 3'UTR, and their corresponding cDNA sequences, are shown below in Table 31. In Table 31, the start codon of each sequence is underlined.

Table 31. G-CSF and FIX Sequences

Description	Sequence	SEQ ID NO:
G-CSF with 1 miR-122 sequence in human alpha-globin 3'UTR	Optimized G-CSF cDNA sequence containing a T7 polymerase site and Xba1 restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATG</u> GCCGGTCCCGCGACCCAAAGCCCCATGAACTTATGGC	5017

	CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCACT CATTCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAG GGCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATA CAAACCTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACA GCTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCCG AGGCTTTGCAGTTGGCAGGGTGCCTTTCCCAGCTCCACTCCG GTTTGTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGA ATCTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCT CGACGTGGCGGATTTTCGCAACAACCATCTGGCAGCAGATGG AGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGG GGCAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGG GTGGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTTGAA GTCTCGTACCGGGTGTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAAC ACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTCTGAGTG GGCGGCTCTAGA	
	mRNA sequence (transcribed): GGGAAAUAGAGAGAAAAGAAGAGUAAGAAGAAAUUAA GAGCCACC <u>AUG</u>CGCGUCCCGCGACCCAAAGCCCCAUGAAACUUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUAUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCCUUGGGCUCCUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGAC AUCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCCCA AACACCAUUGUCACACUCCAGUGGUCUUUGAAUAAAGUCU GAGUGGGCGGC	5018
G-CSF with 1 miR-122 seed sequence in human alpha-globin 3'UTR	Optimized G-CSF cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATG</u>CGCGTCCCGCGACCCAAAGCCCCATGAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCACT	5019

	CATTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAG GGCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATA CAAACCTTTGCCATCCCCGAGGAGCTCGTACTGCTCGGGCACA GCTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGC AGGCTTTGCAGTTGGCAGGGTGCCTTTCCCAGCTCCACTCCG GTTTGTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGA ATCTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCT CGACGTGGCGGATTTGCAACAACCATCTGGCAGCAGATGG AGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGG GGCAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGG GTGGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAA GTCTCGTACCGGGTGTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCACACT CCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTCTAGA	
	mRNA sequence (transcribed): GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAA GAGCCACC <u>AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG</u> CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCUCCUGGGCUCCUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCCACGCAGGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGAC AUCUUGCGCAGCCG UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCCAC ACUCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	5020
G-CSF with 1 miR-122 sequence without the seed in human alpha-globin 3'UTR	Optimized G-CSF cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTATGGC</u> CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCACT CATTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAG GGCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATA CAAACCTTTGCCATCCCCGAGGAGCTCGTACTGCTCGGGCACA GCTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGC	5021

	AGGCTTTGCAGTTGGCAGGGTGCCTTTCCCAGCTCCACTCCG GTTTGTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGA ATCTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCT CGACGTGGCGGATTTTCGCAACAACCATCTGGCAGCAGATGG AGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGG GGCAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGG GTGGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGA GTCTCGTACCGGGTGTGAGACATCTTGCGCAGCCG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAAC ACCATTGTCAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC TCTAGA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAA GAGCCACC <u>AUG</u> GCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUAUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCCUUGGCUCCUCUCUGUCC UGUCCGUCGAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGAC AUCUUGCGCAGCCG UGAUAUAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCCCA AACACCAUUGUCAGUGGUCUUUGAAUAAAGUCUGAGUGG GCGGC	5022
G-CSF with 1 miR-122 sequence in mouse alpha-globin 3'UTR	Optimized G-CSF cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATG</u> GCCGGTCCCGCGACCCAAAGCCCCATGAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCACT CATTCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAG GGCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATA CAAACCTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACA GCTTGGGGATTCCCTGGGCTCCTCTCTCGTCTGTCCGTCCG AGGCTTTGCAGTTGGCAGGGTGCCTTTCCCAGCTCCACTCCG GTTTGTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGA	5023

	ATCTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCT CGACGTGGCGGATTTTCGCAACAACCATCTGGCAGCAGATGG AGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGG GGCAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGG GTGGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAA GTCTCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGCCTTCTGCGGGGCTTGCTTCTGGCCATGCCCTTCTTC TCTCCCTTGACCTGTACCTCTCAAACACCATTGTCACACTC CATGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTC GAGCATGCATCTAGA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAA GAGCCACC <u>AUG</u>GCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUAUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCCGUGGGCUCCUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGAC AUCUUGCGCAGCCG UGAUAAUAG GCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU UCUCUCCCUUGCACCUGUACCUCUCAAACACCAUUGUCAC ACUCCAUGGUCUUUGAAUAAAGCCUGAGUAGGAAG	5024
G-CSF with 1 miR-122 seed sequence in mouse alpha-globin 3'UTR	Optimized G-CSF cDNA sequence containing a T7 polymerase site andXbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATG</u>GCCGGTCCCGCGACCCAAAGCCCCATGAACTTATGGC CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGT CATTCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTGAG GGCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATA CAAACCTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACA GCTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTGCG AGGCTTTGCAGTTGGCAGGGTGCTTTCCAGCTCCACTCCG GTTTGTCTTGATATCAGGGACTGCTGCAAGCCCTTGAGGGA ATCTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCT CGACGTGGCGGATTTTCGCAACAACCATCTGGCAGCAGATGG AGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGG	5025

	GGCAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGG GTGGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAA GTCTCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG GCTGCCTTCTGCGGGGCTTGCTTCTGGCCATGCCCTTCTTC TCTCCCTTGACCTGTACCTCTACACTCCTGGTCTTTGAATA AAGCCTGAGTAGGAAGGCGGCCGCTCGAGCATGCATCTAGA	
	mRNA sequence (transcribed): GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUAUAA GAGCCACC <u>AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG</u> CCCUGCAGUUGCUGCUUUGGCACUCGGCCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCCUGGGCUCCUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGAC AUCUUGCGCAGCCG UGAUAAUAG GCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU UCUCUCCCUUGCACCUGUACCUCUACACUCCUGGUCUUUG AAUAAAGCCUGAGUAGGAAG	5026
G-CSF with 1 miR-122 sequence without the seed in mouse alpha-globin 3'UTR	Optimized G-CSF cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGC</u> CCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCA AGAAGCGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGT CATTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGATTTCAG GGCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATA CAAACCTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACA GCTTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCTGC AGGCTTTGCAGTTGGCAGGGTGCCTTTCCAGCTCCACTCCG GTTTGTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGA ATCTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAGCT CGACGTGGCGGATTTGCAACAACCATCTGGCAGCAGATGG AGGAACTGGGGATGGCACCCGCGCTGCAGCCACGCAGGG GGCAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGG GTGGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAA GTCTCGTACCGGGTGCTGAGACATCTTGCGCAGCCG TGATAATAG	5027

	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTC TCTCCCTTGCACCTGTACCTCTCAAACACCATTGTCATGGTC TTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTCGAGCATG CATCTAGA mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAA GAGCCACC <u>AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG</u> CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGU CCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCG CAGUCAUUCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCG CGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCU CGGGCACAGCUUGGGGAUUCCCUUGGGCUCCUCUCUGUCC UGUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCUUUCCC AGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCA AGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCACGCAGGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACC UUCAAUCAUUUUUGGAAGUCUCGUACCGGGUGCUGAGAC AUCUUGCGCAGCCG UGAUAAUAG GCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU UCUCUCCCUUGCACCUGUACCUCUCAAAACACCAUUGUCAU GGUCUUUGAAUAAAGCCUGAGUAGGAAG	5028
Factor IX with 1 miR- 122 sequence in human alpha-globin 3'UTR	Optimized Factor IX cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATGCAGCGCGTCAACATGATTATGGCCGAATCGCCGGGACT</u> CATCACAATCTGCCTCTTGGGTTATCTCTTGTGCGCAGAATG TACCGTGTTCTTGGATCACGAAAACGCGAACAATAATTCTTA ATCGCCCGAAGCGGTATAACTCCGGGAACTTGAGGAGTTT GTGCAGGGCAATCTTGAACGAGAGTGCATGGAGGAGAAAT GCTCCTTTGAGGAGGCGAGGGAAGTGTTTGAAAACACAGAG CGAACAACGGAGTTTTGGAAGCAATACGTAGATGGGGACC AGTGTGAGTCGAATCCGTGCCTCAATGGGGGATCATGTAAA GATGACATCAATAGCTATGAATGCTGGTGCCCGTTTGGGTTT GAAGGGAAGAACTGTGAGCTGGATGTGACGTGCAACATCA AAAACGGACGCTGTGAGCAGTTTTGTAAGAACTCGGCTGAC AATAAGGTAGTATGCTCGTGACAGAGGGATACCGGCTGGC GGAGAACCAAAAATCGTGCGAGCCCGCAGTCCCGTTCCCTT GTGGGAGGGTGAGCGTGTACAGACTAGCAAGTTGACGAG AGCGGAGACTGTATTCCCCGACGTGGACTACGTCAACAGCA CCGAAGCCGAAACAATCCTCGATAACATCACGCAGAGCACT CAGTCCTTCAATGACTTTACGAGGGTCGTAGGTGGTGAGGA CGCGAAACCCGGTCAGTTCCCCTGGCAGGTGGTATTGAACG GAAAAGTCGATGCCTTTTGTGGAGGTTCCATTGTCAACGAG	5029

	AAGTGGATTGTCACAGCGGCACACTGCGTAGAAACAGGAGT GAAAATCACGGTAGTGGCGGGAGAGCATAACATTGAAGAG ACAGAGCACACGGAACAAAAGCGAAATGTCATCAGAATCA TTCCACACCATAACTATAACGCGGCAATCAATAAGTACAAT CACGACATCGCACTTTTGGAGCTTGACGAACCTTTGGTGCTT AATTCGTACGTACCCCTATTTGTATTGCCGACAAAGAGTAT ACAAACATCTTCTTGAAATTCGGCTCCGGGTACGTATCGGG CTGGGGCAGAGTGTTCATAAAGGGTAGATCCGCACTGGTGT TGCAATACCTCAGGGTGCCCCTCGTGGATCGAGCCACTTGT CTGCGGTCCACCAAATTCACAATCTACAACAATATGTTCTGT GCGGGATTCCATGAAGGTGGGAGAGATAGCTGCCAGGGAG ACTCAGGGGGTCCCCACGTGACGGAAGTCGAGGGGACGTC ATTTCTGACGGGAATTATCTCATGGGGAGAGGAATGTGCGA TGAAGGGGAAATATGGCATCTACACTAAAGTGTACGGTAT GTCAATTGGATCAAGGAAAAGACGAAACTCACG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCTGACCCGTACCCCCCAAAC ACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTCTGAGTG GGCGGCTCTAGA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAGAAAAGAAGAGUAAGAAGAAUUAUA GAGCCACC <u>AUG</u>CAGCGCGUCAACAUGAUUAUGGCCGAAUCGCCGGGAC UCAUCACAAUCUGCCUCUUGGGUUAUCUCUUGUCGGCAGA AUGUACCGUGUUCUUGGAUCACGAAAACGCGAACAAAAU UCUUAUUCGCCCGAAGCGGUUAACUCCGGGAAACUUGAG GAGUUUGUGCAGGGCAAUCUUGAACGAGAGUGCAUGGAG GAGAAAUGCUCUUGAGGAGGCGAGGGAAGUGUUUGAA AACACAGAGCGAACACCGGAGUUUUGGAAGCAAUACGUA GAUGGGGACCAGUGUGAGUCGAAUCCGUGCCUCAAUGGG GGAUCAUGUAAAGAUGACAUCAAUAGCUAUGAAUGCUGG UGCCCGUUUGGGUUUGAAGGGAAGAACUGUGAGCUGGAU GUGACGUGCAACAUCAAAAACGGACGCUGUGAGCAGUUU UGUAAGAACUCGGCUGACAAUAAGGUAGUAUGCUCGUGC ACAGAGGGAUACCGGCUGGCGGAGAACCACAAAAUCGUGCG AGCCCGCAGUCCCGUCCCUUGUGGGAGGGUGAGCGUGUC ACAGACUAGCAAGUUGACGAGAGCGGAGACUGUAUUCGCC GACGUGGACUACGUCAACAGCACCGAAGCCGAAACAAUCC UCGAUAACAUCACGCAGAGCACUCAGUCCUCAAUGACUU UACGAGGGUCGUAGGUGGUGAGGACGCGAAACCCGGUCA GUUCCCCUGGCAGGUGGUUAUUGAACGGAAAAGUCGAUGCC UUUUGUGGAGGUUCCAUUGUCAACGAGAAGUGGAUUGUC ACAGCGGCACACUGCGUAGAAACAGGAGUGAAAAUCACGG UAGUGGCGGGAGAGCAUAACAUAUGAAGAGACAGAGCACA CGGAACAAAAGCGAAAUGUCAUCAGAAUCAUUCACACCA UAACUAUAACGCGGCAAUCAUAAGUACAAUCACGACAUC GCACUUUUGGAGCUUGACGAACCUUUGGUGCUUAAUUCG UACGUCACCCCUAUUUGUAUUGCCGACAAAGAGUAUACAA ACAUCUUCUUGAAAUUCGGCUCGCGGUACGUAUCGGGCUG GGGCAGAGUGUCCAUAAGGGUAGAUCGCGACUGGUGUU	5030

	GCAAUACCUCAGGGUGCCCCUCGUGGAUCGAGCCACUUGU CUGCGGUCCACCAAAUUCACAAUCUACAACAAUAUGUUCU GUGCGGGAUUCCAUGAAGGUGGGAGAGAUAGCUGCCAGG GAGACUCAGGGGGUCCCCACGUGACGGAAGUCGAGGGGAC GUCAUUUCUGACGGGAUUUAUCUCAUGGGGAGAGGAAUG UGCGAUGAAGGGGAAAUAUGGCAUCUACACUAAAGUGUC ACGGUAUGUCAAUUGGAUCAAGGAAAAGACGAAACUCAC G UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCCCA AACACCAUUGUCACACUCCAGUGGUCUUUGAAUAAAGUCU GAGUGGGCGGC	
Factor IX with 1 miR- 122 seed sequence in human alpha-globin 3'UTR	Optimized Factor IX cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC ATGCAGCGCGTCAACATGATTATGGCCGAATCGCCGGGACT CATCACAATCTGCCTCTTGGGTTATCTCTTGTCGGCAGAATG TACCGTGTTCTTGGATCACGAAAACGCGAACAAAATTCTTA ATCGCCCGAAGCGGTATAACTCCGGGAACTTGAGGAGTTT GTGCAGGGCAATCTTGAACGAGAGTGCATGGAGGAGAAAT GCTCCTTTGAGGAGGCGAGGGAAGTGTTTGAAAACACAGAG CGAACAAACGGAGTTTTTGAAGCAATACGTAGATGGGGACC AGTGTGAGTCGAATCCGTGCCTCAATGGGGGATCATGTAAA GATGACATCAATAGCTATGAATGCTGGTGCCCGTTTGGGTTT GAAGGGAAGAACTGTGAGCTGGATGTGACGTGCAACATCA AAAACGGACGCTGTGAGCAGTTTTTGTAAAGAACTCGGCTGAC AATAAGGTAGTATGCTCGTGCACAGAGGGATACCGGCTGGC GGAGAACCAAAAATCGTGCGAGCCCGCAGTCCCGTTCCCTT GTGGGAGGGTGAGCGTGTACAGACTAGCAAGTTGACGAG AGCGGAGACTGTATTCCCCGACGTGGACTACGTCAACAGCA CCGAAGCCGAAACAATCCTCGATAACATCACGCAGAGCACT CAGTCCTTCAATGACTTTACGAGGGTCGTAGGTGGTGAGGA CGCGAAACCCGGTCAGTTCCCCTGGCAGGTGGTATTGAACG GAAAAGTCGATGCCTTTTGTGGAGGTTCCATTGTCAACGAG AAGTGGATTGTACAGCGGCACACTGCGTAGAAACAGGAGT GAAAATCACGGTAGTGGCGGGAGAGCATAACATTGAAGAG ACAGAGCACACGGAACAAAAGCGAAATGTCATCAGAATCA TTCCACACCATAACTATAACGCGGCAATCAATAAGTACAAT CACGACATCGCACTTTTGGAGCTTGACGAACCTTTGGTGCTT AATTCGTACGTCACCCCTATTTGTATTGCCGACAAAGAGTAT ACAAACATCTTCTTGAAATTCGGCTCCGGGTACGTATCGGG CTGGGGCAGAGTGTTCCATAAGGGTAGATCCGCACTGGTGT TGCAATACCTCAGGGTGCCCTCGTGGATCGAGCCACTTGT CTGCGGTCCACCAAATTCACAATCTACAACAATATGTTCTGT GCGGGATTCCATGAAGGTGGGAGAGATAGCTGCCAGGGAG ACTCAGGGGGTCCCCACGTGACGGAAGTCGAGGGGACGTC ATTTCTGACGGGAATTATCTCATGGGGAGAGGAATGTGCGA TGAAGGGGAAATATGGCATCTACACTAAAGTGTCACGGTAT	5031

	GTCAATTGGATCAAGGAAAAGACGAAACTCACG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCTTGACCCGTACCCCCACACT CCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTCTAGA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAA GAGCCACC AUGCAGCGCGUCAACAUGAUUAUGGCCGAAUCGCCGGGAC UCAUCACAAUCUGCCUCUUGGGUUAUCUCUUGUCGGCAGA AUGUACCGUGUUCUUGGAUCACGAAAACGCGAACAAAAU UCUUAUUCGCCCCGAAGCGGUUAUACUCCGGGAAACUUGAG GAGUUUGUGCAGGGCAAUCUUGAACGAGAGUGCAUGGAG GAGAAAUGCUCUUGAGGAGGCGAGGGAAGUGUUUGAA AACACAGAGCGAACACGAGUUUUGGAAGCAAUACGUA GAUGGGGACCAGUGUGAGUCGAAUCCGUGCCUCAAUGGG GGAUCAUGUAAAGAUGACAUCAAUAGCUAUGAAUGCUGG UGCCCGUUUGGGUUUGAAGGGAAGAACUGUGAGCUGGAU GUGACGUGCAACAUCAAAAACGGACGCUGUGAGCAGUUU UGUAAGAACUCGGCUGACAAUAAGGUAGUAUGCUCGUGC ACAGAGGGAUACCGGCUGGCGGAGAACCAAAAAUCGUGCG AGCCCGCAGUCCCGUCCCCUUGUGGGAGGGUGAGCGUGUC ACAGACUAGCAAGUUGACGAGAGCGGAGACUGUAUUCCCC GACGUGGACUACGUCAACAGCACCGAAGCCGAAACAAUCC UCGAUAAACAUACGCAGAGCACUCAGUCCUCAAUGACUU UACGAGGGUCGUAGGUGGUGAGGACGCGAAACCCGGUCA GUUCCCCUGGCAGGUGGUUAUUGAACGAAAAGUCGAUGCC UUUUGUGGAGGUUCCAUGUCAACGAGAAGUGGAUUGUC ACAGCGGCACACUGCGUAGAAACAGGAGUGAAAAUCACGG UAGUGGCGGGAGAGCAUAACAUUGAAGAGACAGAGCACA CGGAACAAAAGCGAAAUGUCAUCAGAAUCAUUCCACACCA UAACUAUAACGCGGCAAUCAUAAGUACAAUCACGACAUC GCACUUUUGGAGCUUGACGAACCUUUGGUGCUUAAUUCG UACGUCACCCCUAUUUGUAUUGCCGACAAAGAGUAUACAA ACAUCUUCUUGAAAUUCGGCUCGCGGUACGUAUCGGGCUG GGGCAGAGUGUCCAUAAGGGUAGAUCGCGACUGGUGUU GCAAUACCUCAGGGUGCCCCUCGUGGAUCGAGCCACUUGU CUGCGGUCCACCAAAUUCACAAUCUACAACAAUAUGUUCU GUGCGGGAUCCAUGAAGGUGGGAGAGAUAGCUGCCAGG GAGACUCAGGGGGUCCCCACGUGACGGAAGUCGAGGGGAC GUCAUUUCUGACGGGAAUUAUCUCAUGGGGAGAGGAAUG UGCGAUGAAGGGGAAUUAUGGCAUCUACACUAAAGUGUC ACGGUAUGUCAAUUGGAUCAAGGAAAAGACGAAACUCAC G UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCCAC ACUCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	5032
Factor IX with 1 miR- 122	Optimized Factor IX cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA	5033

sequence without the seed in human alpha-globin 3'UTR	GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC ATGCAGCGCGTCAACATGATTATGGCCGAATCGCCGGGACT CATCACAATCTGCCTCTTGGGTTATCTCTTGTCTGGCAGAATG TACCGTGTTCTTGGATCACGAAAACGCGAACAAAATTCTTA ATCGCCCGAAGCGGTATAACTCCGGGAACTTGAGGAGTTT GTGCAGGGCAATCTTGAACGAGAGTGCATGGAGGAGAAAT GCTCCTTTGAGGAGGCGAGGGAAGTGTTTGAAAACACAGAG CGAACAAACGGAGTTTTGGAAGCAATACGTAGATGGGGACC AGTGTGAGTCGAATCCGTGCCTCAATGGGGGATCATGTAAA GATGACATCAATAGCTATGAATGCTGGTGCCCGTTTGGGTTT GAAGGGAAGAACTGTGAGCTGGATGTGACGTGCAACATCA AAAACGGACGCTGTGAGCAGTTTTGTAAGAACTCGGCTGAC AATAAGGTAGTATGCTCGTGACAGAGGGATACCGGCTGGC GGAGAACC AAAAATCGTGCGAGCCCGCAGTCCCGTTCCCTT GTGGGAGGGTGAGCGTGTACAGACTAGCAAGTTGACGAG AGCGGAGACTGTATTCCCCGACGTGGACTACGTCAACAGCA CCGAAGCCGAAACAATCCTCGATAACATCACGCAGAGCACT CAGTCCTTCAATGACTTTACGAGGGTTCGTAGGTGGTGAGGA CGCGAAACCCGGTCAGTTCCCTGGCAGGTGGTATTGAACG GAAAAGTCGATGCCTTTTGTGGAGGTTCCATTGTCAACGAG AAGTGGATTGTACAGCGGCACACTGCGTAGAAACAGGAGT GAAAATCACGGTAGTGCGGGAGAGCATAACATTGAAGAG ACAGAGCACACGGAACAAAAGCGAAATGTCATCAGAATCA TTCCACACCATAACTATAACGCGGCAATCAATAAGTACAAT CACGACATCGCACTTTTGGAGCTTGACGAACCTTTGGTGCTT AATTCGTACGTCACCCCTATTTGTATTGCCGACAAAGAGTAT ACAAACATCTTCTTGAAATTTCGGCTCCGGGTACGTATCGGG CTGGGGCAGAGTGTTCCATAAGGGTAGATCCGCACTGGTGT TGCAATACCTCAGGGTGCCCTCGTGGATCGAGCCACTTGT CTGCGGTCCACCAAATTCACAATCTACAACAATATGTTCTGT GCGGGATTCCATGAAGGTGGGAGAGATAGCTGCCAGGGAG ACTCAGGGGGTCCCCACGTGACGGAAGTCGAGGGGACGTC ATTTCTGACGGGAATTATCTCATGGGGAGAGGAATGTGCGA TGAAGGGGAAATATGGCATCTACACTAAAGTGTACGGTAT GTCAATTGGATCAAGGAAAAGACGAAACTCACG TGATAATAG GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCTTGGGCCTCC CCCCAGCCCCTCCTCCCCTTCTGACCCCGTACCCCCCAAAC ACCATTGTCAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC TCTAGA	
	mRNA sequence (transcribed): GGGAAUAAGAGAGAGAAAAGAAGAGUAAGAAGAAUAUAA GAGCCACC AUGCAGCGGUAACAUGAUUAUGGCCGAAUCGCCGGGAC UCAUCACAAUCUGCCUCUUGGGUUAUCUCUUGUCGGCAGA AUGUACCGUGUUCUUGGAUCACGAAAACGCGAACAAAUA UCUUAUUCGCCGAAGCGGUUAACUCCGGGAAACUUGAG GAGUUUGUGCAGGGCAAUCUUGAACGAGAGUGCAUGGAG GAGAAAUGCUCCUUGAGGAGGCGAGGGAAGUGUUUGAA AACACAGAGCGAACACGGAGUUUUGGAAGCAAUACGUA	5034

	GAUGGGGACCAGUGUGAGUCGAAUCCGUGCCUCAAUUGGG GGAUCAUGUAAAGAUGACAUCAAUAGCUAUGAAUGCUGG UGCCCGUUUGGGUUUGAAGGGAAGAACUGUGAGCUGGAU GUGACGUGCAACAUCAAAAACGGACGCUGUGAGCAGUUU UGUAAGAACUCGGCUGACAAUAAGGUAGUAUGCUCGUGC ACAGAGGGAUACCGGCUGGCGGAGAACCAAAAAUCGUGCG AGCCCGCAGUCCCGUCCCUUGUGGGAGGGUGAGCGUGUC ACAGACUAGCAAGUUGACGAGAGCGGAGACUGUAUUCCCC GACGUGGACUACGUCAACAGCACC GAAGCCGAAACAAUCC UCGAUAACAUCACGCAGAGCACUCAGUCCUUC AAUGACUU UACGAGGGUCGUAGGUGGUGAGGACGCGAAACCCGGUCA GUUCCCCUGGCAGGUGGUUAUUGAACGGAAAAGUCGAUGCC UUUUGUGGAGGUUCCA UUGUCAACGAGAAGUGGAUUGUC ACAGCGGCACACUGCGUAGAAACAGGAGUGAAAAUCACGG UAGUGGCGGGAGAGCAUAACA UUGAAGAGACAGAGCACA CGGAACAAAAGCGAAAUGUCAUCAGAAUCAU UCCACACCA UAACUAUAACGCGGCAAUCAUAAGUACAAUCACGACAUC GCACUUUUGGAGCUUGACGAACCUUUGGUGCUUAAUUCG UACGUCACCCCUAUUUGUAUUGCCGACAAAGAGUAUACAA ACAUCUUCUUGAAA UUCGGCUCCGGGUACGUUUCGGGCG GGGCAGAGUGUCCAUAAGGGUAGA UCCGCACUGGUGUU GCAAUACCUCAGGGUGCCCCUCGUGGAUCGAGCCACUUGU CUGCGGUCCACCAAUUCACAAUCUACAACAAUAUGUUCU GUGCGGAU UCCAUGAAGGUGGGAGAGAUAGCUGCCAGG GAGACUCAGGGGGUCCCCACGUGACGGAAGUCGAGGGGAC GUCAUUUCUGACGGGAAUUAUCUCAUGGGGAGAGGAAUG UGCGAUGAAGGGGAAUAUGGCAUCUACACUAAAGUGUC ACGGUAUGUCAAUUGGAUCAAGGAAAAGACGAAACUCAC G UGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCU CCCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCCCA AACACCAUUGUCAGUGGUCUUUGAAUAAAGUCUGAGUGG GCGGC	
Factor IX with 1 miR- 122 sequence in mouse alpha-globin 3'UTR	Optimized Factor IX cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATGCAGCGCGTCAACATGATTATGGCCGAATCGCCGGGACT</u> CATCACAATCTGCCTCTTGGGTTATCTCTTGTCGGCAGAATG TACCGTGTTCTTGGATCACGAAAACGCGAACAAAATTCTTA ATCGCCCGAAGCGGTATAACTCCGGGAACTTGAGGAGTTT GTGCAGGGCAATCTTGAACGAGAGTGCATGGAGGAGAAAT GCTCCTTTGAGGAGGCGAGGGAAGTGTTTGAACACAGAG CGAACACGGAGTTTTGGAAGCAATACGTAGATGGGGACC AGTGTGAGTCGAATCCGTGCCTCAATGGGGGATCATGTAAA GATGACATCAATAGCTATGAATGCTGGTGCCCGTTTGGGTTT GAAGGGAAGAACTGTGAGCTGGATGTGACGTGCAACATCA AAAACGGACGCTGTGAGCAGTTTTGTAAAGAACTCGGCTGAC AATAAGGTAGTATGCTCGTGACAGAGGGATACCGGCTGGC	5035

	GGAGAACCAAAAATCGTGCGAGCCCGCAGTCCCGTTCCCTT GTGGGAGGGTGAGCGTGTCACAGACTAGCAAGTTGACGAG AGCGGAGACTGTATTCCCCGACGTGGACTACGTCAACAGCA CCGAAGCCGAAACAATCCTCGATAACATCACGCAGAGCACT CAGTCCTTCAATGACTTTACGAGGGTTCGTAGGTGGTGAGGA CGCGAAACCCGGTCAGTTCCCCTGGCAGGTGGTATTGAACG GAAAAGTCGATGCCTTTTGTGGAGGTTCCATTGTCAACGAG AAGTGGATTGTACAGCGGCACACTGCGTAGAAACAGGAGT GAAAATCACGGTAGTGGCGGGAGAGCATAACATTGAAGAG ACAGAGCACACGGAACAAAAGCGAAATGTCATCAGAATCA TTCCACACCATAACTATAACGCGGCAATCAATAAGTACAAT CACGACATCGCACTTTTGGAGCTTGACGAACCTTTGGTGCTT AATTCGTACGTCACCCCTATTTGTATTGCCGACAAAGAGTAT ACAAACATCTTCTTGAAATTCGGCTCCGGGTACGTATCGGG CTGGGGCAGAGTGTTCATAAGGGTAGATCCGCACTGGTGT TGCAATACCTCAGGGTGCCCCTCGTGGATCGAGCCACTTGT CTGCGGTCCACCAATTACAAATCTACAACAATATGTTCTGT GCGGGATTCCATGAAGGTGGGAGAGATAGCTGCCAGGGAG ACTCAGGGGGTCCCCACGTGACGGAAGTCGAGGGGACGTC ATTTCTGACGGGAATTATCTCATGGGGAGAGGAATGTGCGA TGAAGGGGAAATATGGCATCTACACTAAAGTGTACGGTAT GTCAATTGGATCAAGGAAAAGACGAAACTCACG TGATAATAG GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTC TCTCCCTTGACCTGTACCTCTCAAACACCATTGTCACACTC CATGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTC GAGCATGCATCTAGA	
	mRNA sequence (transcribed): GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAUA GAGCCACC <u>AUG</u>CAGCGCGUCAACAUGAUUAUGGCCGAAUCGCCGGGAC UCAUCACAAUCUGCCUCUUGGGUUAUCUCUUGUCGGCAGA AUGUACCGUGUUCUUGGAUCACGAAAACGCGAACAAAAU UCUUAUUCGCCCCGAAGCGGUUAUACUCCGGGAAACUUGAG GAGUUUGUGCAGGGCAAUCUUGAACGAGAGUGCAUGGAG GAGAAUUGCUCUUCUUGAGGAGGCGAGGGAAGUGUUUGAA AACACAGAGCGAACACGAGUUUUUGGAAGCAAUACGUA GAUGGGGACCAGUGUGAGUCGAAUCCGUGCCUCAUUGGG GGAUCAUGUAAAGAUGACAUCAAUAGCUAUGAAUGCUGG UGCCCGUUUGGGUUUGAAGGGAAGAACUGUGAGCUGGAU GUGACGUGCAACAUCAAAAACGGACGCGUGUGAGCAGUUU UGUAAGAACUCGGCUGACAUAAGGUAGUAUGCUCGUGC ACAGAGGGAUACCGGCUGGCGGAGAACCAAAAAUCGUGCG AGCCCGCAGUCCCGUCCCUUGUGGGAGGGUGAGCGUGUC ACAGACUAGCAAGUUGACGAGAGCGGAGACUGUAUUCGCC GACGUGGACUACGUCAACAGCACCGAAGCCGAAACAAUCC UCGAUAACAUCACGCAGAGCACUCAGUCCUCAAUGACUU UACGAGGGUCGUAGGUGGUGAGGACGCGAAACCCGGUCA GUUCCCGUGGCAGGUGGUUAUUGAACGGAAAAGUCGAUGCC UUUUGUGGAGGUUCCAUUGUCAACGAGAAGUGGAUUGUC ACAGCGGCACACUGCGUAGAAACAGGAGUGAAAAUCACGG	5036

	UAGUGGCGGGAGAGCAUAACAUAUGAAGAGACAGAGCACA CGGAACAAAAGCGAAAUGUCAUCAGAAUCAUUCCACACCA UAACUAUAACGCGGCAAUAUAAGUACAAUCACGACAUC GCACUUUUGGAGCUUGACGAACCUUUGGUGCUUAAUUCG UACGUCACCCCUAUUUGUAUUGCCGACAAAGAGUAUACAA ACAUCUUCUUGAAAUUCGGCUCGCGGUACGUUUCGGGCG GGGCAGAGUGUCCAUAAGGGUAGAUCGCGCACUGGUGUU GCAAUACCUCAGGGUGCCCCUCGUGGAUCGAGCCACUUGU CUGCGGUCCACCAAUUCACAAUCUACAACAAUAUGUUCU GUGCGGGAUCCAUGAAGGUGGGAGAGAUAGCUGCCAGG GAGACUCAGGGGGUCCCCACGUGACGGAAGUCGAGGGGAC GUCAUUUCUGACGGGAUAUAUCUCAUGGGGAGAGGAUUG UGCGAUGAAGGGGAAAUAUGGCAUCUACACUAAAGUGUC ACGGUAUGUCAAUUGGAUCAAGGAAAAGACGAAACUCAC G UGAUAAUAG GCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU UCUCUCCCUUGCACCUGUACCUCUCAAACACCAUUGUCAC ACUCCAUGGUCUUUGAAUAAAGCCUGAGUAGGAAG	
Factor IX with 1 miR- 122 seed sequence in mouse alpha-globin 3'UTR	Optimized Factor IX cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATGCAGCGCGTCAACATGATTATGGCCGAATCGCCGGGACT</u> CATCACAATCTGCCTCTTGGGTATCTCTTGTGCGCAGAATG TACCGTGTTCTTGGATCACGAAAACGCGAACAAAATTCTTA ATCGCCCGAAGCGGTATAACTCCGGGAACTTGAGGAGTTT GTGCAGGGCAATCTTGAACGAGAGTGCATGGAGGAGAAAT GCTCCTTTGAGGAGGCGAGGGAAAGTGTGTTGAAAACACAGAG CGAACAAACGGAGTTTTTGAAGCAATACGTAGATGGGGACC AGTGTGAGTCGAATCCGTGCCTCAATGGGGGATCATGTAAA GATGACATCAATAGCTATGAATGCTGGTGCCCGTTTGGGTTT GAAGGGAAGAACTGTGAGCTGGATGTGACGTGCAACATCA AAAACGGACGCTGTGAGCAGTTTTGTAAGAACTCGGCTGAC AATAAGGTAGTATGCTCGTGACAGAGGGATACCGGCTGGC GGAGAACC AAAAATCGTGCGAGCCCGCAGTCCCGTTCCCTT GTGGGAGGGTGAGCGTGTACAGACTAGCAAGTTGACGAG AGCGGAGACTGTATTCCCCGACGTGGACTACGTCAACAGCA CCGAAGCCGAAACAATCCTCGATAACATCACGCAGAGCACT CAGTCCTTCAATGACTTTTACGAGGGTCGTAGGTGGTGAGGA CGCGAAACCCGGTCAGTTCCCCTGGCAGGTGGTATTGAACG GAAAAGTCGATGCCTTTTGTGGAGGTTCCATTGTCAACGAG AAGTGGATTGTCACAGCGGCACACTGCGTAGAAACAGGAGT GAAAATCACGGTAGTGGCGGGAGAGCATAACATTGAAGAG ACAGAGCACACGGAACAAAAGCGAAATGTCATCAGAATCA TTCCACACCATAACTATAACGCGGCAATCAATAAGTACAAT CACGACATCGCACTTTTGGAGCTTGACGAACCTTTGGTGCTT AATTCGTACGTACCCCTATTTGTATTGCCGACAAAGAGTAT ACAAACATCTTCTTGAAATTCCGGCTCCGGGTACGTATCGGG CTGGGGCAGAGTGTTCCATAAGGGTAGATCCGCACTGGTGT	5037

	TGCAATACCTCAGGGTGCCCTCGTGGATCGAGCCACTTGT CTGCGGTCCACCAAATTCACAATCTACAACAATATGTTCTGT GCGGGATTCCATGAAGGTGGGAGAGATAGCTGCCAGGGAG ACTCAGGGGGTCCCCACGTGACGGAAGTCGAGGGGACGTC ATTTCTGACGGGAATTATCTCATGGGGAGAGGAATGTGCGA TGAAGGGGAAATATGGCATCTACACTAAAGTGTACGGTAT GTCAATTGGATCAAGGAAAAGACGAAACTCACG TGATAATAG GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTC TCTCCCTTGACCTGTACCTCTACACTCCTGGTCTTTGAATA AAGCCTGAGTAGGAAGGCGGCCGCTCGAGCATGCATCTAGA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUAUAA GAGCCACC <u>AUG</u>CAGCGCGUCAACAUGAUUAUGGCCGAAUCGCCGGGAC UCAUCACAAUCUGCCUCUUGGGUUAUCUCUUGUCGGCAGA AUGUACCGUGUUCUUGGAUCACGAAAACGCGAACAAAAU UCUUAUUCGCCCGAAGCGGUUAUACUCCGGGAAACUUGAG GAGUUUGUGCAGGGCAAUCUUGAACGAGAGUGCAUGGAG GAGAAAUGCUCCUUUGAGGAGGCGAGGGAAGUGUUUGAA AACACAGAGCGAACACCGGAGUUUUGGAAGCAAUACGUA GAUGGGGACCAGUGUGAGUCGAAUCCGUGCCUCAUUGGG GGAUCAUGUAAAGAUGACAUCAAUAGCUAUGAAUGCUGG UGCCCGUUUGGGUUUGAAGGGAAGAACUGUGAGCUGGAU GUGACGUGCAACAUCAAAAACGGACGCUGUGAGCAGUUU UGUAAGAACUCGGCUGACAAUAAGGUAGUAUGCUCGUGC ACAGAGGGAUACCGGCUGGCGGAGAACCAAAAAUCGUGCG AGCCCGCAGUCCCGUCCCCUUGUGGGAGGGUGAGCGUGUC ACAGACUAGCAAGUUGACGAGAGCGGAGACUGUAUUCCCC GACGUGGACUACGUCAACAGCACCGAAGCCGAAACAAUCC UCGAUAACAUCACGCAGAGCACUCAGUCCUCAAUGACUU UACGAGGGUCGUAGGUGGUGAGGACGCGAAACCCGGUCA GUUCCCCUGGCAGGUGGUUAUUGAACGGAAAAGUCGAUGCC UUUUGUGGAGGUUCCAUUGUCAACGAGAAGUGGAUUGUC ACAGCGGCACACUGCGUAGAAACAGGAGUGAAAAUCACGG UAGUGGCGGGAGAGCAUAACAUAUGAAGAGACAGAGCACA CGGAACAAAAGCGAAAUGUCAUCAGAAUCAUUCCACACCA UAACUAUAACGCGGCAAUCAUAAGUACAAUCACGACAUC GCACUUUUGGAGCUUGACGAACCUUUGGUGCUUAAUUCG UACGUCACCCCUAUUUGUAUUGCCGACAAAGAGUAUACAA ACAUCUUCUUGAAAUUCGGCUCCGGGUACGUAUCGGGCUG GGGCAGAGUGUCCAUAAGGGUAGAUCCGCACUGGUGUU GCAAUACCUCAGGGUGCCCCUCGUGGAUCGAGCCACUUGU CUGCGGUCCACCAAAUUCACAAUCUACAACAAUAUGUUCU GUGCGGGAUUCCAUGAAGGUGGGAGAGAUAGCUGCCAGG GAGACUCAGGGGGUCCCCACGUGACGGAAGUCGAGGGGAC GUCAUUUCUGACGGGAAUUAUCUCAUGGGGAGAGGAAUG UGCGAUGAAGGGGAAUUAUGGCAUCUACACUAAAGUGUC ACGGUAUGUCAAUUGGAUCAAGGAAAAGACGAAACUCAC G UGAUAAUAG	5038

	GCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU UCUCUCCCUUGCACCGUACCUCUACACUCCUGGUCUUUG AAUAAAGCCUGAGUAGGAAG	
Factor IX with 1 miR- 122 sequence without the seed in mouse alpha-globin 3'UTR	Optimized Factor IX cDNA sequence containing a T7 polymerase site and XbaI restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAA GAGCCACC <u>ATGCAGCGCGTCAACATGATTATGGCCGAATCGCCGGGACT</u> CATCACAATCTGCCTCTTGGGTTATCTCTTGTCGGCAGAATG TACCGTGTTCTTGATCACGAAAACGCGAACAAAATTCTTA ATCGCCCGAAGCGGTATAACTCCGGGAACTTGAGGAGTTT GTGCAGGGCAATCTTGAACGAGAGTGCATGGAGGAGAAAT GCTCCTTTGAGGAGGCGAGGGAAGTGTTTGAAAACACAGAG CGAACAACGGAGTTTTGGAAGCAATACGTAGATGGGGACC AGTGTGAGTCGAATCCGTGCCTCAATGGGGGATCATGTAAA GATGACATCAATAGCTATGAATGCTGGTGCCCGTTTGGGTTT GAAGGGAAGAACTGTGAGCTGGATGTGACGTGCAACATCA AAAACGGACGCTGTGAGCAGTTTTGTAAGAACTCGGCTGAC AATAAGGTAGTATGCTCGTGACAGAGGGATACCGGCTGGC GGAGAACC AAAAATCGTGCGAGCCCGCAGTCCCGTTCCCTT GTGGGAGGGTGAGCGTGTACAGACTAGCAAGTTGACGAG AGCGGAGACTGTATTCCCGACGTGGACTACGTCAACAGCA CCGAAGCCGAAACAATCCTCGATAACATCACGCAGAGCACT CAGTCCTTCAATGACTTTACGAGGGTCGTAGGTGGTGAGGA CGCGAAACCCGGTCAGTTCCCCTGGCAGGTGGTATTGAACG GAAAAGTCGATGCCTTTTGTGGAGGTTCCATTGTCAACGAG AAGTGGATTGTACAGCGGCACACTGCGTAGAAACAGGAGT GAAAATCACGGTAGTGGCGGGAGAGCATAACATTGAAGAG ACAGAGCACACGGAACAAAAGCGAAATGTCATCAGAATCA TTCCACACCATAACTATAACGCGGCAATCAATAAGTACAAT CACGACATCGCACTTTTGGAGCTTGACGAACCTTTGGTGCTT AATTCGTACGTCACCCCTATTTGTATTGCCGACAAAGAGTAT ACAAACATCTTCTTGAAATTCGGCTCCGGGTACGTATCGGG CTGGGGCAGAGTGTTCCATAAGGGTAGATCCGCACTGGTGT TGCAATACCTCAGGGTGCCCTCGTGGATCGAGCCACTTGT CTGCGGTCCACCAAATTCACAATCTACAACAATATGTTCTGT GCGGGATTCCATGAAGGTGGGAGAGATAGCTGCCAGGGAG ACTCAGGGGGTCCCCACGTGACGGAAGTCGAGGGGACGTC ATTTCTGACGGGAATTATCTCATGGGGAGAGGAATGTGCGA TGAAGGGGAAATATGGCATCTACACTAAAGTGTCACGGTAT GTCAATTGGATCAAGGAAAAGACGAAACTCACG TGATAATAG GCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTC TCTCCCTTGACCTGTACCTCT CAAACACCATTGTCATGGTCTTTGAATAAAGCCTGAGTAGG AAGGCGGCCGCTCGAGCATGCATCTAGA	5039
	mRNA sequence (transcribed): GGGAAUAAGAGAGAGAAAAGAAGAGUAAGAAGAAUAUAA GAGCCACC <u>AUGCAGCGCGUCAACAUGAUUAUGGCCGAAUCGCCGGGAC</u>	5040

UCAUCACAAUCUGCCUCUUGGGUUAUCUCUUGUCGGCAGA AUGUACCGUGUUCUUGGAUCACGAAAACGCGAACAAAAU UCUUAAUCGCCCCGAAGCGGUUAUAACUCCGGGAAACUUGAG GAGUUUGUGCAGGGCAAUCUUGAACGAGAGUGCAUGGAG GAGAAAUGCUCUUCUUGAGGAGGCGAGGGAAGUGUUUGAA AACACAGAGCGAACAAACGGAGUUUUGGAAGCAAUACGUA GAUGGGGACCAGUGUGAGUCGAAUCCGUGCCUCAUUGGG GGAUCAUGUAAAGAUGACAUAUAGCUAUGAAUGCUGG UGCCCCGUUGGGUUUGAAGGGAAGAACUGUGAGCUGGAU GUGACGUGCAACAUCAAAAACGGACGCUGUGAGCAGUUU UGUAAAGAACUCGGCUGACAAUAAGGUAGUAUGCUCGUGC ACAGAGGGAUACCGGCUGGCGGAGAACCAAAAAUCGUGCG AGCCCCGAGUCCCGUUCUUUGUGGGAGGGUGAGCGUGUC ACAGACUAGCAAGUUGACGAGAGCGGAGACUGUAUUCGCC GACGUGGACUACGUCAACAGCACCGAAGCCGAAACAAUCC UCGAUAACAUCACGCAGAGCACUCAGUCCUUCAAUGACUU UACGAGGGUCGUAGGUGGUGAGGACGCGAAACCCGGUCA GUUCCCCUGGCAGGUGGUUAUUGAACGGAAAAGUCGAUGCC UUUUGUGGAGGUUCCAUUGUCAACGAGAAGUGGAUUGUC ACAGCGGCACACUGCGUAGAAACAGGAGUGAAAAUCACGG UAGUGGCGGGAGAGCAUAACAUAUGAAGAGACAGAGCACA CGGAACAAAAGCGAAAUGUCAUCAGAAUCAUUCCACACCA UAACUAUAACGCGGCAAUCAUAUAGUACAAUCACGACAUC GCACUUUUGGAGCUUGACGAACCUUUGGUGCUUAAUUCG UACGUCACCCCUAUUUGUAUUGCCGACAAAGAGUAUAACAA ACAUCUUCUUGAAAUUCGGCUCGCGGUACGUAUCGGGCUG GGGCAGAGUGUCCAUAAGGGUAGAUCCGCACUGGUGUU GCAAUACCUCAGGGUGCCCCUCGUGGAUCGAGCCACUUGU CUGCGGUCCACCAAUUCACAAUCUACAACAAUAUGUUCU GUGCGGGAUCCAUGAAGGUGGGAGAGAUAGCUGCCAGG GAGACUCAGGGGGUCCCCACGUGACGGAAGUCGAGGGGAC GUCAUUUCUGACGGGAUUAUCUCAUGGGGAGAGGAAUG UGCGAUGAAGGGGAAUAUGGCAUCUACACUAAAGUGUC ACGGUAUGUCAAUUGGAUCAAGGAAAAGACGAAACUCAC G UGAUAAUAG GCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU UCUCUCCCUUGCACCUGUACCUCU CAAACACCAUUGUCAUGGUCUUUGAAUAAAGCCUGAGUA GGAAG	

[001006] These modified mRNA sequences can include at least one chemical modification described herein. The G-CSF and/or Factor IX modified mRNA sequence can be formulated, using methods described herein and/or known in the art, prior to transfection and/or administration.

[001007] The modified mRNA sequence encoding G-CSF or Factor IX can be transfected *in vitro* to various cell types such as HEK293, HeLa, PBMC and BJ fibroblast

and those described in Table 25 using methods disclosed herein and/or are known in the art. The cells are then analyzed using methods disclosed herein and/or are known in the art to determine the concentration of G-CSF or Factor IX and/or cell viability.

[001008] The modified mRNA sequence encoding G-CSF or Factor IX can also be administered to mammals including mat, rats, non-human primates and humans. The serum, surrounding tissue and organs can be collected at pre-determined intervals and analyzed using methods disclosed herein and/or are known in the art to determine the concentration of G-CSF or Factor IX and other pharmacokinetic properties mentioned herein.

Example 33. Microphysiological Systems

[001009] The polynucleotides, primary constructs and/or mmRNA described herein are formulated using one of the methods described herein such as in buffer, lipid nanoparticles and PLGA. These formulations are then administered to or contacted with microphysiological systems created from organ chips as described in International Publication Nos. WO2013086502, WO2013086486 and WO2013086505, the contents of each of which are herein incorporated by reference in its entirety.

Example 34. Translation Enhancing Elements (TEEs) in Untranslated Regions

[001010] The 5' and/or 3' untranslated regions (UTRs) in the polynucleotides, primary constructs and/or mmRNA described herein may include at least one translation enhancing element (TEE). Such TEE which may be included in the 5'UTR and/or 3'UTR include, but are not limited to, those listed in Table 32, including portion and/or fragments thereof. The TEE sequence may include at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more than 99% of the TEE sequences disclosed in Table 28 and/or the TEE sequence may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in Table 32.

Table 32. TEE Sequences

TEE Identifier	Sequence	SEQ ID NO
TEE-001	MSCSGCNGMWA	5041

TEE-002	RNSGAGMGRMR	5042
TEE-003	RNSGAGMGRMRRR	5043
TEE-004	RMSCSGCNGMWR	5044
TEE-005	GCGAGAGAA	-
TEE-006	GGGAGCGAA	-
TEE-007	GCGAGAGGA	-
TEE-008	GCGAGCGGA	-
TEE-009	CGGAGCGAA	-
TEE-010	CGGAGCGGA	-
TEE-01 1	ACGAGAGGA	-
TEE-012	ACGAGCGGA	-
TEE-013	GACGAGAGGA	5045
TEE-014	GACGAGAGAA	5046
TEE-015	AGCGAGCG	-
TEE-016	AGGAGAGGA	-
TEE-017	GCCGAGAGA	-
TEE-018	CGAGAGGCA	-
TEE-019	GAGAGGAGC	-
TEE-020	CGCGGCGGA	-
TEE-021	CGCCGCCGC	-
TEE-022	GCGGCTGAA	-
TEE-023	CCGGCTGAA	-
TEE-024	CGCCGCTGAA	5047
TEE-025	CGCCGCGGAA	5048
TEE-026	CGCCGCCGAA	5049
TEE-027	CCCGCGGAA	-
TEE-028	CCCGCCGAA	-
TEE-029	CCCGCTGAA	-
TEE-030	CCCGGCGGA	-
TEE-031	CGCGGCTGA	-
TEE-032	CGGCTGCTA	-
TEE-033	CCCGGCGGA	-
TEE-034	AGCCGCCGCA	5050
TEE-035	ACGCCGCCGA	5051
TEE-036	GGCATTATCGT	5052
TEE-037	GCATTAGTATCT	5053
TEE-038	TCGGTTATTGTT	5054
TEE-039	TCCAATTGGGAA	5055
TEE-040	ATCTATTGGCCA	5056
TEE-041	TTACTGGGTGTT	5057
TEE-042	AGGGTGAAGGTC	5058

TEE-043	GGTGGGTGTGTC	5059
TEE-044	CGCTTCAATGCT	5060
TEE-045	TGCTTCAATGCC	5061
TEE-046	TGTGTCTTTGCA	5062
TEE-047	CACGGGGACAGC	5063
TEE-048	AAGCTGTACATG	5064
TEE-049	GATGGGGGCACA	5065
TEE-050	ATATGTGCCCTT	5066
TEE-051	TCCTTCTGGGTC	5067
TEE-052	GGTGGGTGTGTC	5068
TEE-053	GAATGGATGGGG	5069
TEE-054	CAXGTGATATTC	5070
TEE-055	AGGAGGGTTTGT	5071
TEE-056	TGGGCGAGTGGG	5072
TEE-057	CGGCTCACCAGT	5073
TEE-058	GGTTTCXATAAC	5074
TEE-059	GGTGGGTGTGTC	5075
TEE-060	TTACTGGGTGTT	5076
TEE-061	AAGTCTTTGGGT	5077
TEE-062	CCGGCGGGU	-
TEE-063	CCGGCGGG	-
TEE-064	CCGGCGG	-
TEE-065	CCGGCG	-
TEE-066	CCGGC	-
TEE-067	CGGCGGGU	-
TEE-068	GGGAGACGGCGGCGGTGGCGGCGCGGGCAGAGCAA GGACGCGGCGGATCCCACTCGCACAGCAGCGCACTC GGTGCCCCGCGCAGGGTCG	5078
TEE-069	AAAGAAATGGAATCGAAGAGAATGGAAACAAATGG AATGGAATTGAATGGAATGGAATTGAATGGAATGGG AACG	5079
TEE-070	AAAGAAATGGAATCGAAGAGAATGGAAACAAATGG AATGGAATTGAATGGAATGGAATTGAATGGAATGGG AACG	5080
TEE-071	AGACAGTCAGACAATCACAAAGAAACAAGAATGAA AATGAATGAACAAAACCTTCAAGAAATATGGGATTA TGAAGAGGCCAAATGT	5081
TEE-072	AAAAGGAAATACAAGACAACAAACACAGAAACACA ACCATCGGGCATCATGAAACCTCGTGAAGATAATCA TCAGGGT	5082
TEE-073	AGACCCTAATATCACAGTTAAACGAACTAGAGAAGG AAGAGCAAACAAATTCAAAAGCTAGCGGAAAGCAA GAAATAACTAAGACCAG	5083
TEE-074	AAAGACTTAAACATAAGACCTAAAACCATAAAAACC ACAGAAGAAAACATAGGCAATGCCATTCAGGACATA	5084

	GGCATGGGCAAAGACTTC	
TEE-075	AGCAATAACCAAACAACCTCATTAAAAAGTAGGCAA AGGACATAAACAGACACTTTTCAAAAAGAAGACATAC ACGTGGCCAACAACATATG	5085
TEE-076	AGAAAGAATCAAGAGGAAATGCAAGAAATCCAAAA CACTGTAACAGATATGATGAATAATGAGGTATGCAC TCATCAGCAGACTCGACAT	5086
TEE-077	GCACTAGTCAGATCAAGACAGAAAGTCAACGAACAA AGAACAGACTTAAACTACACTCTAGAACAAATGGAC CTA	5087
TEE-078	AGCAGCCAACAAGCATATGAAATAATGCTCCACAAC ACTCATCATCAGAGAAATGCAAATCAAAACCAAAAT	5088
TEE-079	AATATACGCAAATCAATAAATGTAATCCAGCATATA AACAGTACTAAAGACAAAAACCACATGATTATCTCA ATAGATGCAGAAAAGGCC	5089
TEE-080	ATGTACACAAATCAATAAATGCAGTCCAGCATATAA ACAGAACCAAAACACAAAAACCACATGATTATCTCAA TAGATGCAGAAAAGGCCTTT	5090
TEE-081	TATACCACACAAATGCAAAAAGATTATTAGCAACAAT TATCAACAGCAATATGTCAACAAGTTGACAAACCTA GAGGACATGGAT	5091
TEE-082	AAACACACAAAGCAACAAAAGAAGCAAGCAACAAA AGCATAGATTTATTGAAATGAAAGTACATTCTACAG AGTGGGGGCAGGCT	5092
TEE-083	GAAATCATCATCAAACGGAATCGAATGGAATCATTG AATGGAATGGAATGGAATCATCATGGAATGGAAACG	5093
TEE-084	AACAGAATGGAATCAAATCGAATGAAATGGAATGG AATAGAAAGGAATGGAATGAAATGGAATGGAAAGG ATTGGAATGGAATGCAATCG	5094
TEE-085	TACAAAGAACTCAAACAAATCAGCAAGAACAAAAA CAATCCCAACAAAATGTTGGACAAAGACATGAATAG ACAATTCTCGAAAGAAGATGTACAAATGGCT	5095
TEE-086	TGTTGAGAGAAATTAACAAAAGCACAGATAAATGGA AAAACGTGTTTCATAGATTGAAAGACTTCATGTTGTAT GGTGTC	5096
TEE-087	AAACGATTGGACAGGAATGGAATCACCATCGAATGG AAACGAATGGAATCTTCGAATGGAATTGAATGAAAT TATTGAACGGAATCAAATAGAATCATCATTGAACAG AATCAAATTGGATCAT	5097
TEE-088	AACAATAAACAAACTCCAAGTAGACACAATAGTCAA ATTGCTGAAAATGAAATATAAAGGAACAATCTCGAT GGTAGCCCAAGGA	5098
TEE-089	AAATCAATAAATGTAATTCAGCATATAAACAGAACC AAAGACAAAAACCACATGATTATCTCAATAGATGCA GAAAAGGCCTTT	5099
TEE-090	GCTCAAGGAAATAAAATAGGACACAAAGAAATGGA AAAACATTCCATACTCATGGATAGAAAGAATCAATA TCATGAAATGGCC	5100
TEE-091	AACATACGCAAATCAATAAATGTAATCCAGCATATA AACAGAACCAAGACAAAAACCACATGATTATCTCA	5101

	ATAGATGCAGAAAAGGCC	
TEE-092	AACAATCACTAGTCCTTAAGTAAGAGACAACACCTT TTGTCACACACAGTTTGTCTAACTTTATCTTGGTAA TTGGGGAGACC	5102
TEE-093	AGAAAACACACAGACAACAAAAAACACAGAACGAC AATGACAAAATGGCCAAGC	5103
TEE-094	ACACAACAACCAAGAAACAACCCCATTAAGAAGTGG GAAAAATACATGAATAAACACATCTCAAAAGAAGAC AAACAAGTGGCTAAC	5104
TEE-095	ACAGCAGAAAACGAACATCAGAAAATCACTCTACAT GATGCTTAAATACAGAGGGCAAGCAACCCAAGAGA AAACACCACTTCCTAAT	5105
TEE-096	GAATAGAACAGAATGGAATCAAATCGAATGAAATG GAATGGAATAGAAAGGAATGGAATGAAATGGAATG GAAAGGATTGGAATGGAATG	5106
TEE-097	TAAGCAGAGAAAATATCAACACGAAAATAATGCAA GGAGAAAAATACAGAACAATCCAAAATGTGGCC	5107
TEE-098	GAACAATCAATGGAAGCAGAAACAAATAAACCAAG GTGTGCATCAAGGAATACATTACGCATGATGGCTG TATGAGTAAAATG	5108
TEE-099	GATCAATAAATGTAATTCATCATATAAACAGAGAAC TAAAGACAAAAACACATGATTATCGCAATACATGCA GAAAAGGCC	5109
TEE-100	GACAAGAGTTCAGAAAGGAAGACTACACAGAAATA CGCATTTTAAAGTCACTGACATGGAGATGACACTTA AAACCATGAACATGGATGGG	5110
TEE-101	AAGCAAAGAAAGAATGAAGCAGCAAAAGAACGAAA GCAGGAATTTATTGAAAACCAAAGTACACTCCACAG TATGGGAGCGGACCCGAGCA	5111
TEE-102	ACCAACATAAGACAAAGAAACATCCAGCAGCTGCCT ATGGCAAAAGATTACAATGTGTCAAACAAGAGGGCA ATG	5112
TEE-103	GGACAAATTGCTAGAAATAAACAAATTACCAAAAAT GATTCAAGTAGAGACAGAGAATCAAAATAGAACTAC ACATAAGTGGGCCAAG	5113
TEE-104	AACATAATCCATCAAATAAACAGAACCAAAAGACAAA AACCACATGATTATCTCAATAGATGCAGAAAAGGCC TTC	5114
TEE-105	AAAATCAATATGAAAACAAACACAAGCAGACAAAG AAAATTGGGCAAAAGGTTTGAGCAGACACTTCACCA AAGAAGTACAAATGGCAAATCAGCA	5115
TEE-106	AACCAAATTAGACAAATTGGAATCATTACACATAA CAAAAGTAATAAACTGTCAGCCTCAGTAGTATTCATT GTACATAAACTGGCC	5116
TEE-107	AAGGAATTTAAGCAAATCAACAAGCAAAACCAAAT AATCCCATTA AAAAGTGGGTAAAGGACATGAATACA CACTTGTCAATAGAGGACATTCAAGTGGCCAAC	5117
TEE-108	TAACCTGATTTGCCATAATCCACGATACGCTTACAAC AGTGATATACAAGTTACATGAGAAACACAAACATTT TGCAAGGAACTGTGGCCAGATG	5118

TEE- 109	AACTAACACAAGAACAGAAAACCAAACATCACATGT TCTCACTCATAAGCGGGAGCTGAACAATGAGAACAC ACGGACACAGGGAGAGGAACATG	5119
TEE-1 10	TAAACTGACACAAACACAGACACACAGATACACACA TACATACAGAAATACACATTCACACACAGACCTGGT CTTTGGAGCCAGAGATG	5120
TEE-1 11	ATCAACAGACAACAGAAACAAATCCACAAAGCACTT AGTTATTAGAACTGTCATACAGACTGTACAACAACC ACATTTACCAT	5121
TEE- 112	AAATAAGCCAACGGTCATAAATTGCAAAGCCTTTTA CAATCCAAACATGATGGAACGATATGCCATTTTGA AGGTGATTTGAAAAGCACATGGTTT	5122
TEE-1 13	AAACAGTTCAAAAATTATTGCAACAAAATGAGAGAG ATGAGTTTATCTTGCAAACATAATGGATGGTAGCAGT GACAGTGGCAAAACGTGGTTTGATTCT	5123
TEE- 114	TAAGCAACTTCAGCAAAGTCTCAGGATACAAAATCA ATGTACAAAAATCACAAGCATTCTTATACACCAACA ACAGACAAACAAGAGTGCCAAATCATG	5124
TEE-1 15	AGCAAACAACAACAACAACAACAACAATATGACAG GAACAAAACGTCACATATCAACATTAACAAGAATG TAAACAGCCTAAATGCTTCACTTAAAAGTTATAGAC AGGGGCTGGGCATGGTGGCTCACGCC	5125
TEE-1 16	GGAAATAACAGAGAACACAAACAATGGGAAAACA TTCCATGTTTCATGGATAGGAAGAATCAATATTGTGA AAATGGCCATACT	5126
TEE-1 17	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GTACAAAAATCACAAGCATTCTTATACACCAATAAC AGACAAACAGAGAGCC	5127
TEE-1 18	AGATAAGAATAAGGCAAACATAGTAATAGGGAGTTC ATGAATAACACACGGAAAGAGAACTTACAGGGCTGT GATCAGGAAACG	5128
TEE-1 19	AGGAAATAAAAGAAGACACAAACAATGGAAGAAC ATTCCATGCTTATGGATAGGGAGAATCAGTATCGTG AAAATGGCCATACT	5129
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TEE- 121	AATGGACTCGAATGAAATCATCATCAAACGGAATCG AATGGAATCATTGAATGGAATGGAATGGAATCATCA TGGAATGGAAACG	5131
TEE- 122	AAGATTTAAACATAAGACCTAAAACGACAAAAATCC TAGGAGAAAACCTAAGCAATACCATTCAGGACATAG GCATGGGCAAAGACTTCATG	5132
TEE- 123	TAATGAGAAGACACAGACAACACAAAGAATCACAG AAACATGACACAGGTGACAAGAACAGGCAAGGACC TGCAGTGCACAGGAGCC	5133
TEE- 124	TAAACGTTAGACCTAAAACCATAAAAACCCTAGAAG AAAACCTAGGCATTACCATTCAGGACATAGGCATGG GCAAGGAC	5134
TEE- 125	GAATTGAATTGAATGGAATGGAATGCAATGGAATCT	5135

	AATGAAACGGAAAGGAAAGGAATGGAATGGAATGG AATG	
TEE- 126	GTAATGGAATGGAATGGAAGGAATCGAAACGAAA GGAATGGAGACAGATGGAATGGAATGGAACAGAG	5136
TEE- 127	AGAGAAATGCAAATCAAAACCACAATGGAATACCAT CTCACGCCAGTCAGAATGGCAATTATTAaaaaaatca CAACAATTAATGATGGCAAGGCTGTGG	5137
TEE- 128	AACATACACAAATCAATAAACGTAATCCAGCTTATA AACAGAACCAAGACAAAAACCACATGATTATCTCA ATAGATGCGGAAAAGGCC	5138
TEE- 129	TAAACAGAACCAAGACAAAAATCACATGATTATCT CAATAGATGCAGAAAAGGCC	5139
TEE- 130	AATGGAATGCAATCGAATGGAATGGAATCGAACGGA ATGGAATAAAATGGAAGAAAACCTGGCAAGAAATGG AATCG	5140
TEE-131	AGATAAAAAGAACAGCAGCCAAAATGACAAAAGCA AAAAGCAAAATCGTGTTAGAGCCAGGTGTGGTGATG TGTGCT	5141
TEE- 132	AGGAAAGTTTTCAATATGAGAAAGATACAAACCAAC AGAATAAGCAAACCTGGATAAACAGAAAATACAGAG AGAGCCAAGG	5142
TEE- 133	GCAATCTCAGGATACAAAATCAATGTGCAAAAATCA CAAGCATTCTCATACACCAATAACAGACAAACAGAG CCAAATCATG	5143
TEE- 134	AGCATTTCATATCTTGCAGTGTTGGGAAAGAGTGAGA GGTTGTGATGTCAAGAAGGATAGGTCAGAAGTGGAA GGTATGGGGGATTGTGCCTGCTGTCATGGCT	5144
TEE- 135	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GTGCAAAAATCACAAGCATTCTTATACACCAATAAC AGACAAACAGAGAGCC	5145
TEE- 136	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GTGCAAAAATCACAAGCATTCTTATACACCAACAAC AGACAAACAGAGAGCC	5146
TEE- 137	TAAGCCGATAAGCAACTTCAGCAAAGTCTCAGGAGA CAAAATCAATGTGCAAAAATCACAAGCATTCTTAT ACACTAATAACAGACAAACAGAGAGCCAAATCATG	5147
TEE- 138	AACGTGACATACATACAAAAAGTTTTTAGAGCAAGT GAAATTTTAGCTGCTATATGTTAATTGGTGGTAATCC C	5148
TEE- 139	TACGCAAATCGATAAATGTAATCCAGCATATAAACA GAACCAAAGACAAAAACCACATGATTATCTCAATAG ATGCAGAAAAGGCC	5149
TEE- 140	GCAATCGAATGGAATGGAATCGAACGGAATGGAATA AAATGGAAGAAAACCTGGCAAGAAATGGAATCG	5150
TEE- 141	TTGAATCGAATGGAATCGAATGGATTGGAAAGGAAT AGAATGGAATGGAATGGAATTGACTCAAATGGAATG	5151
TEE- 142	TAAAGAAAAACAAACAAACAGAAATCAATGAAAT CCCATTCAAAGGTCAGCAACCTCAAAGACTGAAGGT AGATAAGCCCACAAGGATG	5152
TEE- 143	GTCATATTTGGGATTTATCATCTGTTTCTATTGTTGTT	5153

	GTTTTAGTACACACAAAGCCACAATAAATATTCTAG GCT	
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TEE- 145	AACCCACAAAGACAACAGAAGAAAAGACAACAGTA GACAAGGATGTCAACCACATTTTGAAGAGACAAGT AATCAAACACATGGCA	5155
TEE- 146	AAAGACCGAAACAACAACAGAAACAGAAACAACA ACAATAAGAAAAAATGTAAAGCAAAACAATGATTG CACAACCTTACATGATTACTGAGTGTTCTAATGGT	5156
TEE- 147	AATCAGTAAACGTAATACAGCATATAAACAGAACCA AAGACAAAAACCACATGATTATCTCAATAGATGCAG AAAAGGCC	5157
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TEE- 154	TAAGACACATAGAAAACATAAAGCAAAATGGCAGA TGTAATGCAACCTATCAATCAAAACATTACGAATG GCTT	5164
TEE- 155	TGAAACAAATGATAATGAAAATACAACATACCAAAC ATACGAGATACAGTAAAAGCAGTACTAAGATGCAAG TATATATTGCTACAAGTGCCTAC	5165
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TEE- 158	AAGGATTCTGAATGGAATGCAATCGAATGGAATGGAA TCGAACGGAATGGAATAAAATGGAAGAAAACCTGGC AAGAAATGGAATCG	5168
TEE- 159	GATCATCAGAGAAACAGAGAAATGCAAATTAACC ACAATGAGATACTATCTCCACACAAGTCAGAAATGGC	5169

	TAT	
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TEE- 168	AGCAGTGCAAGAACAACATAACATACAAGTAAACA AACACATGGGGCCAGGTAATAAAAAGTCAGGCTCAA GAGGTCAG	5178
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TEE- 173	GGAATAACAACAACAACAACCAAAAGACATATAGA AAACAAACAGCACGATGGCAGATGTAAAGCCTACC	5183
TEE- 174	AAACGCAGAAACAAATCAACGAAAGAACGAAGCAA TGAAAGACAAAGCAACAAAAGAATGGAGTAAGAAA GCACACTCCACAAAGTGGAAGCAGGCTGGGACA	5184
TEE- 175	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GTGCAAAAATCACAAGCATTCCTATACACCAACAAC AGACAAACAGAGAGCC	5185
TEE- 176	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GGGAAAAAATCACAAGCATTCCTATACATCAATAAC AGACAAACAGAGAGCC	5186

TEE- 177	ACACATTTCAAGGAAGGAAACAAGAACAGACAGAA ACACAACATACTTCATGAAACCACATTTTAGCATCCT GGCCGAGTATTCATCA	5187
TEE- 178	AGCAACTTCAGCAAAGTCTCAGGACACAAAATCAAT GTGCAAAAATCACAAGCATTCTTATACACCAATAAC AGACAAACAGAGAGCC	5188
TEE- 179	TATTTTACCAGATTATTCAAGCAATATATAGACAGCT TAAAGCATACAAGAAGACATGTATAGATTTACATGC AAACACTGCACCACCTTTACATAAGGGACTTGAGCAC	5189
TEE- 180	CCCAACTTCAAATTATACTACAAGGCTACAGTAATC AAAAAAGCATAGTACTATTACAAAAACAGACACACA GGCCAATGGAATACAAT	5190
TEE-181	AGAAAGGATTCTGAATGGAATGAAAAAGAATTGAAT GGAATAGAACAGAATGGAATCAAATCGAATGAAAT GGAATGGAATAGAAAGGAATGGAATG	5191
TEE- 182	GTTTACAGTCAAGTGTACAAACAGAATATAAGCAAA CAAAAGAGAACATATACTTACAAACTATGCTAAGTG CCATGAAGGAAAAG	5192
TEE- 183	AAGAGTATTGAAGTTGACATATCTAGACTGATCAAG AACAAAGACAAAAGGTACAGATTATCAAGAAAATG AGCGGGCAAAGCAAGATGGCC	5193
TEE- 184	AGTAGAATTGCAATTGCAAATTTACACATATACTCA CACACAAGTACACACATCCACTTTTACAACTAAAAA AACTAGCACCCAGGACAGGTGCAGTGGCT	5194
TEE- 185	TGAATGCTATAGAGCAGTAAAAACAAATAAATGAAC TACATTACAGCTACTTACAACCATATGAAAGAATAT AACCATAACAATGATGAGTGGACAAAAGCTAAGTGT GAAAGAATGCATAGTGCTACAGCAGCCAACATTTAC AGC	5195
TEE- 186	GAATGGAATCAAATAGAAATGGAATCGAAACAAATG GAATGGAATGGAATGGGAGCTGAGATTGTGTCACTG CAC	5196
TEE- 187	TAAAAGTGTGCTCAACATCATTGATCATCAGAGAAA TGCAAATCAAACTACAATGAGATATCATCTCATCC CAGTCAAAGTGGCT	5197
TEE- 188	TCAGACCATAGCAGATAACATGCACATTAGCAATAC GATTGCCATGACAGAGTGGTTGGTG	5198
TEE- 189	ACAAACAATCCAATTCGAAAATGGGCAAGATATTTT ACCAAAGACATGAGCTGATATTTTAC	5199
TEE- 190	AGGAAAAACAACAACAACAGGAAAAACAACCTC AGTATGAAGACAAGTACATTGATTTATTCAACATTTA CTGATCACTTTTCAGGTGGTAGGCAG	5200
TEE-191	AACAAAACAAAAACCCAACTCAATAACAAGAAGAC AAACAACCCAATTTAAAATGAGCAAAGAAGCTTGATA AACATGTCTCCAAAGAAGATACGGCCAAAGAGCAC	5201
TEE- 192	ATACAACTAAAGCAAATATAAGCAACTAAAGCAACA GTACAACTAAAGCAAAACAGAACAAGACTGCCAGG GCCTAGAAAAGCCAAGAAC	5202
TEE- 193	AACAACAACAACAACAGGAAAAACAACCTCAGTATG AAGACAAGTACATTGATTTATTCAACATTTACTGATC	5203

	ACTTTTCAGGTGGTAGGCAGACC	
TEE-194	AGAGAGTATTCATCATGAGGAGTATTACTGGACAAA TAATTCACAAACGAACAAACCAAAGCGATCATCTTT GTACTGGCTGGCTA	5204
TEE-195	AGTAAATCACCATAAAGAAGGTAAGAGTTCATTAC AAAAACAACAAACTGAAGAATCAGGCCATAGTA	5205
TEE-196	AAAATAGAATGAAAGAGAATCAAATGGAATTGAATC GAATGGAATCGAATGGATTGGAAAGGAATAGAATG GAATGGAATGGAATG	5206
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TEE-199	CCATAACACAATTA AAAACAACCTAAATGTCTAATA GAAGAACTGTT CAGACCGGCATGGTGGCTTATA CC	5209
TEE-200	TGGATTT CAGATATTTAACACAAAATAGTCAAAGCA GATAAATACTAGCAACTTATTTTTAATGGGTAACATC ATATGTTCTGTCCTT	5210
TEE-201	ATCATTGAATGCAATCACATGGAATCATCACAGAAT GGAATCGTACGGAATCATCATCGAATGGAATTGAAT GGAATCATCAATTGGACTCGAATGGAAACATCAAAT GGAATCGATTGGAAGTGTCGAATGGACTCG	521 1
TEE-202	AGAAACAGCCAGAAAACAATTATTACCTACAGCATT AAAAC TATTCAAATGACAGCATATTTTT CAGCAGAA ATCATGAAGGCCAGAAGGACGTGTCAT	5212
TEE-203	AAAATGATCATGAGAAAATT CAGCAACAAAACCATG AAATTGCAAAGATATTACTTTTGGGATGGAACAGAG CTGGAAGGCAAAGAG	5213
TEE-204	AACCACTGCTCAAGGAAATAAGAGAGAACACAAAC AAATGAAAAAACATTCCATGCTCATGGATAGGAAGA ATCAG	5214
TEE-205	TACTCTCAGAAGGGAAGCAGATATTCAGCATAAATC ATATTGTTTGTACAAAGAGTCTGGGCATGGTGAATG ACACT	5215
TEE-206	TATAGTTGAATGAACACACATACACACACATGCC ACAAAACAAAAACAAAGTTATCCTCACACACAGGAT AGAAACCAAACCAAATCCCAACACATGGCAAGATGA T	5216
TEE-207	GCTCAAAGAAATCAGAAATGACACAAGCAAATGGA AAAACATGCCATGTT CATGAATATGAAGAATCAATA TTGTTAAATGGCCATACTGCTCA	5217
TEE-208	GGATACAAAATCAATGTACAAAATCACAAGCATTC TTATACACCAATAACAGACAAACAGAGAGCC	5218
TEE-209	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GTACAAAATCACAAGCATTCTTATACACCAACAAC AGACAAACAGAGAGCC	5219
TEE-210	AGGAGAATAGCAGTAGAATGACAAAATTAGATTTTC ACATGAAACTTGATGACAGTGTAGGAAATGGACTGA	5220

	AAGGACAAGAC	
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TEE-2 12	AAGTTCAAACATCAGTATTAACCTTGAACATCAATG GCCTACATGCATCACTTAAAACATACAGACAGGCAA ATTGGGTTAAGAAAACAAACAAGCAAACAAAACAT GTTCCAAACATTTGTTGGCTAT	5222
TEE-213	AAGAAACAATCAAAAGGAAGTGCTAGAAATAAAAC ACACTGTAATAGAAAAGAAGAATGCCTTATGGGCTT ATCAATAGACTAGACATGGCCAGG	5223
TEE-214	AAAGAAAGACAGAGAACAACGTAATTCAAGATGA CTGATTACATATCCAAGAACATTAGATGGTCAAAGA CTTTAAGAAGGAATACATTCAAAGGCAAAAAGTCAC TTACTGATTTTGGTGGAGTTTGCCACATGGAC	5224
TEE-215	AGCAACTTCAGCAAAGTTTCAGGATACAAAATCAAT GTGCAAAAATCACAAGCATTCTTATACACCAACAAC AGACAAACAGAGAGCC	5225
TEE-216	AGAATCAAATGGAATTGAATCGAATGGAATCGAATG GATTGGAAAGGAATAGAATGGAATGGAATGGAATG	5226
TEE-217	AAACAGAACCACAGATATCTGTAAAGGATTACACTA TAGTATTCAACAGAGTATGGAACAGAGTATAGTATT CAACAGAGTATGCAAAGAACTAAGGCCAGAAAG	5227
TEE-218	AAAAAATGTTCAACATCACTAGTCAGCAGAGAAATG CAATCAAATCACAATGAGATAACTTCTCACACCA GACAGCATGGC	5228
TEE-219	GAATCCATGTTTCATAGCACAACAACCAAACAGAAGA AATCACTGTGAAATAAGAAACAAAGCAAAACACAG ATGTCGACACATGGCA	5229
TEE-220	AGGATACAAAATCAAAGTGCAAAAATCACAAGCATT CTTATACACCAATAACAGACAAACAGAGAGCC	5230
TEE-221	AACAGATTTAAACAACCAACAAGCAAAAAACGAA CAACTCCATTCAAACATGGACAAAAGACACGAACAG ACACTTTTCAAAGAAGACATACATGTGGCC	5231
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TEE-223	TAAACAACGAGAACACATGAACACAAAGAGGGGAA CAACAGACACCAAGACCTTCTTGAGGGTGGAGGATG GGAGGAGGGAG	5233
TEE-224	GGTTCAACTTACAATATTTTGAAGTTGACAACAGTGCA AAAGCAATACACGATTAGTAGAAACACACTTCCAAT GCCCATAGGACCATTCTGC	5234
TEE-225	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GAGCAAAAATCACAAGCATTCTTACACACCAATAAC AGACAAACAGAGAGCC	5235
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TEE-227	TGAAAATACAAATGACCATGCAAGTAATTCGCAGG GAGAGAGCGGATATGAACAAACAGAAGAAATCAGA	5237

	TGGGATAGTGCTGGCGGGAAGTCA	
TEE-228	GCAAATGATTATAAGTGCTGTTATAGAAACATTCAA AGACCAGAAAAGGACCACAATGGCTGACCAC	5238
TEE-229	AGTCAATAACAAGAAGACAAACAACCCAATTACAAA ATGGGATATGAATTTAATAGATGTTACTCCAAGGAA GATACACAAATGGCCAAC	5239
TEE-230	ATGGTTAAAACCTCAACAATGAAAACACAAACAGCGC AATTTAAAAATGGGCAAAATGACAGGCCAGACCCAG TGGCTCATGCG	5240
TEE-231	TAACACTCACAGAACTCAACAAAACACTATACATG CATTTACCAGTTTATTATAAAGATACAAGTCAGGAA CAGCCAAATGGAAGAAATGTAAATGGCAAG	5241
TEE-232	AACAGACCATAAATAAACACAGAAGACACACGAGT GTAAAGTCAGTGCCCCGCTGCGAATTAATCGGGGT GATGTGATGGCGAGTGAGTGGGTAGTT	5242
TEE-233	GAATAGAATAGAATGGAATCATCGAATGGAATCGAA TGGAATCATCATGATATGGAATTGAGTGGAATC	5243
TEE-234	GGAATCTATAATACAGCTGTTTATAGCCAAGCACTA AATCATATGATACAGAAAACAAATGCAGATGGTTTG AAGGGTGGG	5244
TEE-235	AAGATAGAGTTGAAACAGTGGACAATTAAAGAGTAA TTTGGAAGAATGGTGAAATTACAGCCATGCTTTGAA TCAGGCGGGTTCCTGCGC	5245
TEE-236	TGAAAAGAAGAATGACCATAAGCAAGCAGATGAAA AACAAAACAGAATTTTACAGACGTCTTGGAAGTAT ATCTTGGGC	5246
TEE-237	AGGAATCTATAATACAGCTGTTTATAGCCAAGCACT AAATCATATGATACAGAAAACAAATGCAGATGGTTT GAAGGGTGGG	5247
TEE-238	AGGAAAAGAAAGAAATAGAAAATGCGAAATGGTAA GAAAAAACAGCATAATAAACATTTGTATGGTGTGA TGGACAATGCATT	5248
TEE-239	TAACAGTACCAAAAAACAGTCATAATCTTCAAGAGC TTAAATTTAGCATGAAAGGAAGACATTCATCAAAGA ATCACACAAAGGAATGTAAAATTAAATGGAGATTAG TGCCAGGAAAGAGC	5249
TEE-240	GCAAAACACAAACAACGCCATAAAAAACTGGGCAA AGGATATGAACAGACATTTTTTCAAAACAAAACATAC TTATGGCCAAC	5250
TEE-241	AACAAAATTGAACAACATGCAAAGAAACATAAACG AAGCAATGAAAGTGTGCAGATCCACTGAAATGAAAG TGCTGTCCAGAGTGGGAGCCAGCTCGAGA	5251
TEE-242	GAATGGAATCAACATCAAACGGAATCAAACGGAATT ATCGAATGGAATCGAAGAGAATCATCGAATGGCCAC GAATGGAATCATCTAATGGAATGGAATGGAATAATC CATGG	5252
TEE-243	TACAAGAAAATCACAGTAACATTTATAAAACACAGA AGTGTGAACACACAGCTATTGACCTTGAAAACAGTG AAAGAGGGTCAGCTGTAGAACTAAGACATAAGCAA AGTTTTTCAATCAAGAATACATGGGTGGCC	5253

TEE-244	AAGAATTGGACAAAACACACAAACAAAGCAAGGAA GGAATGAAAGGATTTGTTGAAAATGAAAGTACACTC CACAGTGTGGGAGCAG	5254
TEE-245	ACAGTTAACAAAAACCGAACAAATCTAATTACGAAAT GAACAAAAGATATGAACAGACATTTACCCGAGAGT ATACAGGGGCCAGGCATGGT	5255
TEE-246	AAACGCACAAACAAAGCAAGGAAAGAATGAAGCAA CAAAAGCAGAGATTTATTGAAAATGAAAAATACACT CCACAGGGTGGG	5256
TEE-247	CACCATGAGTCATTAGGTAAATGCAAATCAAAACCA CAATGAAATACTTCACACCCATGAAGATGGCTATAA TAAAAAACAGACA	5257
TEE-248	AGCAACTTCAGCAAAGTCTCAGGAGACAAAATCAAT GTACAAAAATCACAAGCATTCTTATACACCAATAAC AGACAAACAGAGAGCC	5258
TEE-249	TGACATGCAAGAAATAAGGAAGTGCAAAAACAAAC AAACAAACAACAACAACAACAACAACAACAAC AAAAAACAGTCCCAAAAGGATGGGCAG	5259
TEE-250	AGACTTGAAAAGCACAGACAACGAAAGCAAAAATG GACAAATGGAATCACATCAAGCTAAAAGGTTTTCGA TGGCAAAGG	5260
TEE-251	GCAAAAGAAACAATCAGTAGAGTAAACAGACAACCT CATAGAATGCAAGAAAATCATCGCAATCTGTACATC CAACAAAGGGCT	5261
TEE-252	ACAAAATCAAACCTAACCTCGATAAGAATGCAAGTGA ATCAAAATGAGTTTCAAGGGGTTGTGGCTAGTACAC GCTTTCTACAGCTG	5262
TEE-253	ACAAACCACTGCTCAAGGAAATAAGGACACAAACA AATGGAACAACATTCCGTGCTCATGGATAGGAAGAA TCAATATCGTGAAAATGGCCATACT	5263
TEE-254	GAACGATTTATCACTGAAAATTAATACTCATGCAAG TAGTAAACGAATGTAATGACCATGATAAGGAGACGG ACGGTGGTGATAGT	5264
TEE-255	AGCAGAAGAAATAACTGAAATCAGAGTGAAACTGA ATCAAATTGAGATGCAAAAATACATACGAAATGGCC AG	5265
TEE-256	TGAATAGACACACAGACCAATGGAACAGAATAGAG AACACAGAATAAATCTGCACACTTATAGCCAGCTGA TTTTTGACAAATTTGCCAAG	5266
TEE-257	AGCAACTTCAGCAGTCTCAGTATACAAAAACAATGT GCAAAAATCACAAGCATTCTATATGCCAATAACAG ACAAACAGAGAGCC	5267
TEE-258	ACCAATCAAGAAAACAATGCAACCCACAGAGAATG GACAAAAGCAAGGCAGGACAATGGCT	5268
TEE-259	GCCACAATTTTGAAACAACCATAATAATGAGAATAC ACAAGACAACTCCAATAATGTGGGAAGACAACTTT GCAATTCACATCATGGC	5269
TEE-260	GAAAATGAACAATATGAACAAACAAACAAAATTACT ACCCTTACGAAAGTACGTGCATTCTAGTATGGTGAC AAAAAGGAAA	5270

TEE-261	TATGCAAATCAATAAACATAATCCATCACATAAAACA GAAACAAAGACAAAATGACATGATTATCTCAATAGA TGCAGAAAAGGCC	5271
TEE-262	CACCCATCTGTAGGACCAGGAAGCCTGATGTGGGAG AGAACAGCAGGCTAAATCCAGGGTTGGTCTCTACAG CAGAGGGAATCACAAGCCTGTTAGCAAGTGAAGAAC CAACACTGGCAAGAGTGTGAAGGCC	5272
TEE-263	AGGATACAAAATCAATGTACAAAAATCACAAACATT CTTATACACCAACAACAGACAAACAGAGAGCCAAAT CATGGGTG	5273
TEE-264	AGGAAAATGCAAATCAGAACGACTATAACACACCAT CTCAAACCTCGTTAGGATGGCTATTATCAAAAAGTCA AGAGATAACAAATGTGGGCAAGGG	5274
TEE-265	GTAACAAAACAGACTCATAGACCAATAGAACAGAAT AGAGAATTCAGAAATAAGACTGCACTTCTATGACCA TGTGATCTTAGACAAACCT	5275
TEE-266	AAAGGAAAACCTACAAAACACTGCTGAAAGAAATCAT TGACAACACAAACAAATGGAAACACATCCCAAGATC ATGGGTGGGTGGAATCAAT	5276
TEE-267	ACACACATACCAACAGAACATGACAAAAGAACAAA ACCAGCCGCATGCATACTCGATGGAGACAAAGGTAA CACTGCAGAATGGTGAAGGAAGAACAGTCATTTTAA TGACAGTGTGGCT	5277
TEE-268	AACTAAGACAACAGATTGATTTACACTACTATTTTCA CACAGCCAAAAATATCACTATGGCAATCGTCAAAAG GTCAATTCAAAGATGGGACAGT	5278
TEE-269	GATCAGCTTAGAATACAATGGAACAGAACAGATTAG AACAATGTGATTTTATTAGGGGCCACAGCACTGTTG ACTCAAGTACAAGTTCTGACTCATGTAGAACTAACA CTTTT	5279
TEE-270	GAATGGAATCAAATCGAATGAAATGGAATGGAATAG AAAGGAATGGAATGAAATGGAATGGAAAGGATTCTG AAT	5280
TEE-271	AAATGAACAAAACCTAGAGGAATGACATTACCTGACT TCAAATTATACTACAGAGCTATAGTAACCAAAACAG CATGGTACAGGCAT	5281
TEE-272	GGACAACATACACAAATCAGTCAAGATACATCATTT CAACAGAATGAAAGACAAAACCATTTGATCACTTC AATCGATGATGAAAAAGCA	5282
TEE-273	AACTTCAGCAAATTCCTCAGGATACAAAATCAATGTG CAAAAACCACAAGCATTCTTATACACCAATAATAGA CAGTGAGCCAAAT	5283
TEE-274	TATGACTTTTACAAATTACAGAAAAAGACACCCATT TGACAAGGGAACCTGAAGGTGGTGAAGACATACTGGC AGGCTAC	5284
TEE-275	AACAGCAATAGACACAAAGTCAGCACTTACAGTACA AAAATAATGGCAAAAGCACATGAAGTGGGACAT	5285
TEE-276	TGTAACACTGCAAACCATAAAAACCGTAGAAGAAAA CCTAGACAATACTATTTCAGGACATAGGCATGGGCAA AGAC	5286

TEE-277	GAAGAAGAAAAAACATGGATATACAATGTCAACAG AAATCAAGGAGAAACGGAATTTACCAATCAATTTA GTGATCTGGGTT	5287
TEE-278	AAAACACACAAACATACATGTGGATGCACATATAAA CATGCACATACACACACACATAAATGCACAAACACA CTTAACACAAGCACACATGCAAACAAACACATGG	5288
TEE-279	TAGAAGGAATTTGATACATGCTCAGAAATACAGGCA AAGGAAGTAGGTGCCTGCCAGTGAACACAGGGGAA CTATGGCTCCTA	5289
TEE-280	TGACTAAACAGAGTTGAACAAGAACAAAAAGCAAA TTTGCAGAAATGAAATACATACTAATTGAAAGTCCA TGGACAGGCTCAACAGATGATATAGATACAGCTAAA GAGATAATTAGTGAAATGGATCAG	5290
TEE-281	AAGTAATAAGACTGAATTAGTAATACAAAGTGTCTC AACAAAGAAAATTGCGGGACTGTTCATGCTCATGGA CAGGAAGAATCAATATCATGAAAATGGCC	5291
TEE-282	ACAGACAGAGATTTAAAACAATAAACAAGCAGTAA GCAAACACAGATAACAAAATGACATGATCCAACAAA TACTCAGAAGGAGACTTAGAAATGAATTGAGGGTC	5292
TEE-283	AGAAAAAACAAACAGCCCATTTAAAGGTAGACAA AGGACATGAACACTTTTCAAAAGAAGACATACATGT GGCCAAACAGCATG	5293
TEE-284	AAAAATGACCAGAGCAATAGAATGCATTGACCAGAT AAAGACCTTCACGTATGTTGAACTAAAATGTGTGGT GCAGGTG	5294
TEE-285	AATCAGTCTAGATCTTAAAGGAACACCAGAGGGAGT ATTTAAATGTGCCCAATAAGCAAGAATTATGGTGAT GTGGAAGTA	5295
TEE-286	GAATGGAATGGAAAGGAATCGAAACGAAAGGAATG GAGACAGATGGAATGGAATGGAACAGAGAGCAATG G	5296
TEE-287	GGAATGGAATGAACACGAATGTAATGCAACCCAATA GAATGGAATCGAATGGCATGGAATATAAAGAAATGG AATCGAAGAGAATGGAACAAATGGAATGGAATTG	5297
TEE-288	AGGACATGAATAGACAATTCTCAAAAGAAGATACAC AAGTGGCAAACAAACACATGAAAAAAGACTCAACA TTAGTAATGACCATGGAAATGCAAATC	5298
TEE-289	TCCAGTCGATCATCATATAGTCAGCACTTATCATACA CCAAGCCGTGTGCAAGGAAAGGGAATACAACCATGA ACATGATAGATGGATGGTT	5299
TEE-290	TACAGATAAGAAAATTGAGACTCAAGAGTATTACAT AAATTGTTTCAGCTACCACAGCAAAAAATGGTATGG TTGGGAATCAAGCTCAGGG	5300
TEE-291	AGCCTATCAAAAAGTGGGCTAAGAATATGAATACAC AATTCTCAAAAGAAGATATACAAATGGGCAACAAAC ATATGAAAACATACTCAACATCACTAATGATCAGGG AAATG	5301
TEE-292	GAAAATGAACAATATGAACAAACAAACAAAATTACT ACCCTTACGAAAGTACGTGCATTCTAGTATGGTGAC AAAAAGGAAAG	5302

TEE-293	ACATACGCAAATCAATAAACATAATCCATCACATAA ACAGAACCAAAGACAAAAATCACATGATTATCTCAA TAGATGCAGAAAAGGCCTTCGAC	5303
TEE-294	AAGAGTATCAACAGTAAATTACATTAGCAGAAGAAT CAACAAACATGAAAATAGAAATTATGGTAGCCAAAG AACAG	5304
TEE-295	AATCGAATGGAATCAACATCAAACGGAAAAAACG GAATTATCGAATGGAATCGAAGAGAATCATCGAATG GACC	5305
TEE-296	GAAAGGAATAGAATGGAATGGATCGTTATGGAAAG ACATCGAATGGGATGGAATTGACTCGAATGGATTGG ACTGGAATGGAACGGACTCGAATGGAATGGACTGGA ATG	5306
TEE-297	TAAGCAATTTTCAGCAGTCTCAGGATACAAAATCAAT GTGCAAAAATCACAAGCATTCTTATACACCAACAAC AGACAAACAGAGAGCCAAATCG	5307
TEE-298	AACGGAATCAAACGGAATTATCGAATGGAATCGAAG AGAATCATCGAATGGCCACGAATGGAATCATCTAAT GGAATGGAATGGAATAATCCATGGACCCGAATG	5308
TEE-299	ACATCAAACGGAATCAAACGGAATTATCGAATGGAA TCGAAAAGAATCATCGAACGGACTCGAATGGAATCA TCTAATGGAATGGAATGGAAG	5309
TEE-300	ATCGAATGGAATCAACATCAAACGGAAAAAACGG AATTATCAAATGGAATCGAAGAGAATCATCGAATGG ACC	5310
TEE-301	GAATAATCATTGAACGGAATCGAATGGAAACATCAT CGAATGGAAACGAATGGAATCATCATCGAATGGAAA TGAAAGGAGTCATC	531 1
TEE-302	CATCAAACGGAATCAAACGGAATTATCGAATGGAAT CGAAAAGAATCATCGAACGGACTCGAATGGAATCAT CTAATGGAATGGAATGGAAGAATCCATGGACTCGAA TG	5312
TEE-303	AAACGGAATCAAACGGAATTATCGAATGGAATCGAA GAGAATCATCGAATGGACTCGAATGGAATCATCTAA TGGAATGGAATGGAAGAATCCATGG	5313
TEE-304	ATACACAAATCAATAAATGTAATCCAGCATATAAAC AGAACCAAAGACAAAAACCATATGATTATCTCAATG GATGCAGAAAAGGCC	5314
TEE-305	AATCGAATAGAATCATCGAATGGACTCGAATGGAAT CATCGAATGTAATGATGGAACAGTC	5315
TEE-306	TGGAATGGAATCATCGCATAGAATCGAATGGAATTA CCATCGAATGGGATCGAATGGTATCAACATCAAACG CAAAAAACGGAATTATCGAATGGAATCGAAGAGA ATCTTCGAACGGACCCG	5316
TEE-307	ATGGAATGGAATGGAATGGAATTAAATGGAATGGAA AGGAATGGAATCGAATGGAAAGGAATC	5317
TEE-308	GTCGAAATGAATAGAATGCAATCATCATCAAATGGA ATCCAATGGAATCATCATCAAATAGAATCGAATGGA ATCATCAAATGGAATCGAATGGAGTCATTG	5318
TEE-309	TGGAATTATCGAAAGCAAACGAATAGAATCATCGAA	5319

	TGGACTCGAATGGAATCATCGAATGGAATGGAATGGAACAG	
TEE-310	AAAGGAATGGAATGCAATGGAATGCAATGGAATGCACAGGAATGGAATGGAATGGAATGGAAAGGAATG	5320
TEE-311	AATCTAATGGAATCAACATCAAACGGAAAAAACGGAAATTATCGAATGGAATCGAAGAGAATCATCGAATGGACC	5321
TEE-312	TACACAACAAAAGAAATACTCAACACAGTAAACAGACAACCTTCAGAACAGGAGAAAATATTTGCAAATACATCTAACAAAGGGCTAATATCCAGAATCT	5322
TEE-313	TGCAATCCTAGTCTCAGATAAAACAGACATTAAACC AACAAAGATCAAAGAGACAAAGAAGGCCATTAC	5323
TEE-314	GAATCGAATGGAATCAACATCAAACGGAAAAAACGGAATTATCGAATGGAATCGAAAAGAATCATCGAATGGACC	5324
TEE-315	AATGGAATCGAATGGAATGCAATCCAATGGAATGGAATGCAATGGAATGGAATCGAACGGAATGCAGTGGAAGGGAATGG	5325
TEE-316	GAACACAGAAAAATTTCAAAGGAATAATCAACAGG GATTGATAACTAACTGGATTTAGAGAGCCAAGGCAAAGAGAATCAAAGCACAGGGCCTGAGTCGGAG	5326
TEE-317	AGTTGAATAGAACCAATCCGAATGAAATGGAATGGAATGGAACGGAATGGAATTGAATGGAATGGAATGGAATGCAATGGA	5327
TEE-318	AACTCGATTGCAATGGAATGTAATGTAATGGAATGGAATGGAATTAACGCGAATAGAATGGAATGGAATGTAATGGAACGGAATGGAATG	5328
TEE-319	AAGCGGAATAGAATTGAATCATCATTGAATGGAATC GAGTAGAATCATTGAAATCGAATGGAATCATAGAATGGAATCCAAT	5329
TEE-320	AATGGAATCGAAAGGAATAGAATGGAATGGATCGTTATGGAAAGATATCGAATGGAATGGAATTGACTCGAATGGAATGGACTGGAATGGAACG	5330
TEE-321	TAACGGAATAATCATCGAACAGAATCAAATGGAATCATCATTGAATGGAATTGAATGGAATCTTCGAATAGACATGAATGGACCATCATCG	5331
TEE-322	AACGGAATCAAACGGAATTATCGAATGGAATCGAATAGAATCATCGAACGGACTCGAATGGAATCATCTAATGGAATGGAATGGAAG	5332
TEE-323	ATTGGAATGGAACGGAACAGAACGGAATGGAATGGAATAGAATGGAATGGAATGGAATGGAATGGTATGGAATGGAATGGAATGGTACG	5333
TEE-324	AATCCACAAAGACAACAGAAGAAAAGACAACAGTAGACAAGGATGTCAACCACATTTTGAAGAGACAAGTAATCAAACACATGGCA	5334
TEE-325	GAATCGAATGGAATCAACATCAAACGGAAAAAACGGAATTATCGAATGGAATCGAAAAGAATCATCGAACGGACTCGAATGGAATCATCTAATGGAATGGAATGGAAGAATCCATGG	5335
TEE-326	AATGGAATCGAATGGAATCATCATCAAATGGAATCT	5336

	AATGGAATCATTGAACGGAATTGGATGGAATCGTCA T	
TEE-327	CAACATCAAACGGAAAAAACGGAATTATCGAATGG AATCGAAGAGAATCATCGAATGGACC	5337
TEE-328	CACAACCAAAGCAATGAAAGAAAAGCACAGACTTAT TGAAATGAAAGTACACACCACAGAATGGGAGCAGG CTCAAGCAAGC	5338
TEE-329	ATCAAAGGGAATCAAGCGGAATTATCGAATGGAATC GAAGAGAATCATCGAATGGACTCGAATGGAATCATG TGATGGAATGGAATGGAATAATCCACGGACT	5339
TEE-330	GGAATCGAATGGAATCAATATCAAACGGAGAAAAA CGGAATTATCGAATGGAATCGAAGAGAATCATCGAA TGGACC	5340
TEE-331	AGGAATGGACACGAACGGAATGCAATCGAATGGAA TGGAATCTAATAGAAAGGAATTGAATGAAATGGACT GG	5341
TEE-332	GGAAGGGAATCAAATGCAACAGAATGTAATGGAAT GGAATGCAATGGAATGCAATGGAATGGAATGGAATG CAATGGAATGG	5342
TEE-333	AAATTGGATTGAATCGAATCGAATGGAAAAAATGAA ATCAAATGAAATTGAATGGAATCGAAATGAATGTAA ACAATGGAATCCAATGGAATCCAATGGAATCGAATC AAATGGTTTTGAGTGGCGTAAAATG	5343
TEE-334	AATGGAAGGGAATGGAATGGAATCGAATCGAATGG AACAGAATTCAATGGAATGGAATGGAATGGAATGGA ATCGAATGGAATGG	5344
TEE-335	GAAAAATCATTGAACGGAATCGAATGGAATCATCAT CGGATGGAAACGAATGGAATCATCATCGAATGGAAA TGAAAGGAGTCATC	5345
TEE-336	GGAATCGAATGGAATCAACATCAAACGGAGAAAAA CGGAATTATCGAATGGAATCGAAGAGAATCATCGAA TGGACC	5346
TEE-337	AAAGAAATGTCACTGCGTATACACACACACGCACAT ACACACACCATGGAATACTACTCAGCTATACAAAGG AATGAAATAATCCACAGCCAC	5347
TEE-338	GGAATCGAATGGAATCAATATCAAACGGAAAAAAA CGGAATTATCGAATGGAATCGAAGAGAATCATCGAA TGGACC	5348
TEE-339	TGAACGGAATCGAATGGAATCATCATCGGATGGAAA CGAATGGAATCATCATCGAATGGAAATGAAAGGAGT CATC	5349
TEE-340	GAATAGAACGAAATGGAATGGAATGGAATGGAATG GAAAGGAATGGAATGGAATGGAACG	5350
TEE-341	TGGAATTATCGTCGAATAGAATCGAATGGTATCAAC ATCAAACGGAAAAAACGGAATTATCGAATGGAATC GAAGAGAATCATCGAACGGACTCGAATGGAATCATC TAATGGAATGGAATGGAATAATCCATGG	5351
TEE-342	GACAAAAAGAATCATCATCGAATAGAATCAAATGGA ATCTTTGAATGGACTCAAAGGAATATCGTCAAATG GAATCAAAAGCCATCATCGAATGGACTGAAATGGAA	5352

	TTATCAAATGGACTCG	
TEE-343	AACCAAACCAAGCAAACAAACAAACAGTAAAACT CAATAACAACCAACAAACAGGAAATACCAGGTAATT CAGATTATCTAGTTATGTGCCATAGT	5353
TEE-344	GAATGAATTGAATGCAAACATCGAATGGTCTCGAAT GGAATCATCTTCAAATGGAATGGAATGGAATCATCG CATAGAATCGAATGGAATTATCAACGAATGGAATCG AATGGAATCATCATCAGATGGAATGAATGGAATCG TCAT	5354
TEE-345	TGGAATGGAATCAAATCGCATGGAATCGAATGGAAT AGAAAAGAATCAAACAGAGTGGAATGGAATGGAAT GGAATGGAATCATGCCGAATGGAATG	5355
TEE-346	AAATGGAATAATGAAATGGAATCGAACGGAATCATC ATCAAAAGGAACCGAATGAAGTCATTGAATGGAATC AAAGGCAATCATGGTCTGAATGGAATCAAATGGAAC AGCATTGAATAGAATTGAATGGAGTCATCACATGGA ATCG	5356
TEE-347	GAATTAACCCGAATAGAATGGAATGGAATGGAATGG AACAGAACGGAACGGAATGGAATGGAATGGAATGG AATGGAATG	5357
TEE-348	AAGATATACAAGCAGCCAACAAACATACGAAAGAA TGCTCAACATCACTAATCCTCAGAGAAATTTAAATCA AAACCACAATGAGTTACAATCTCATACCAGTCAGAA T	5358
TEE-349	AGATAAGTGGATGAACAGATGGACAGATGGATGGAT GGATGGATGGATGGATGGATGCCTGGAAGAAAGAA GAATGGATAGTAAGCTGGGTATA	5359
TEE-350	AGAATTACAAACCACTGCTCAACAAAATAAAAAGAGT ACACAAACAAATGGAAGAATATTCCATGCTTATGGA TAGGAAGAATCAATATTGTGAAAATGGCCATACT	5360
TEE-351	CATCGAATGGACTCGAATGGAATAATCATTGAACGG AATCGAAGGGAATCATCATCGGATGGAAACGAATGG AATCATCATCGAATGGAAATG	5361
TEE-352	AAAGGAATCAAACGGAATTATCGAATGGAATCGAAA AGAATCATCGAACGGACTCGAATGGAATCATCTAAT GGAATGGAATGGAAGAATCCATGGACTCGAATG	5362
TEE-353	GGATATAAACAAGAAAACAATAATCACAACCTCAAT ATCAAAGTGCAATGATGGTGCAAAATGCAAGTATGG TGGGGACAGAGAAAGGATGC	5363
TEE-354	AACATCAAACGGAACAAAAACGGAATATCGAATGG AATCGAAGAGAATCATCGAATGGACC	5364
TEE-355	TAAAATGGAATCGAATGGAATCAACATCAAATGGAA TCAAATGGAATCATTGAACGGAATTGAATGGAATCG TCAT	5365
TEE-356	AATCATCATCGAATGGAATCGAATGGTATCATTGAA TGGAATCGAATGGAATCATCATCAGATGGAATGAA TGGAATCGTCAT	5366
TEE-357	CAATGCGTCAAGCTCAGACGTGCCTCACTACGGCAA TGCGTCAAGCTCAGGCGTGCCTCACTAT	5367
TEE-358	TAAGCTGATAAGCAACTTTAGCAAAGTCTCAGGATA	5368

	CAAAATCAATGTACAAAAATCACAAGCATTCTTATA CACCAACAACAGACAGACGGAGAGCCAAA	
TEE-359	AATCAAAGAATTGAATCGAATGGAATCATCTAATGT ACTCGAATGGAATCACCAT	5369
TEE-360	ATGAACACGAATGTAATGCAATCCAATAGAATGGAA TCGAATGGCATGGAATATAAAGAAATGGAATCGAAG AGAATGGAAACAAATGGAATGGAATTGAATGGAAT GGAATTG	5370
TEE-361	ATCAAACGGAATCAAACGGAATTATCGAATGGAATC GAAGAGAATCATCGAACGGACTCGAATGGAATCATC TAATGGAATGGGATGG	5371
TEE-362	AATGGAAAGGAATCAAATGGAATATAATGGAATGCA ATGGACTCGAATGGAATGGAATGGAATGGACCCAAA TGGAATGGAATGGAATGGAATG	5372
TEE-363	GGAATACAACGGAATGGAATCGAAAAAAATGGAAA GGAATGAAATGAATGGAATGGAATGGAATGGAATG GATGGGAATGGAATGGAATGG	5373
TEE-364	GAATCAAGCGGAATTATCGAATGGAATCGAAGAGAA TCATCGAAAGGACTCGAATGGAATCATCTAATGGAA TGGAATGGAATAATACACGGACC	5374
TEE-365	AAGATAACCTGTGCCCAGGAGAAAAACAATCAATGG CAACAAAAGCAGAAACAACACAAATGATACAATTA GCAGACAGAAACATTGAGATTGCTATT	5375
TEE-366	AATGGACTCCAATGGAATAATCATTGAACGGAATCT AATGGAATCATCATCGGATGGAAATGAGTGGAAATCA TCATCGAATGGAATCG	5376
TEE-367	AATCTATAAACGTAATCCATCACATAAACAGGACCA AAGAGAAAAACCGCATGATTATCTCAAGAATGCAGA AAAGGCC	5377
TEE-368	TAATTGATTGAAATTAATGGAATTGAATGGAATGC AATCAAATGGAATGGAATGTAATGCAATGGAATGTA ATAGAATGGAAAGCAATGGAATG	5378
TEE-369	AAAGGAATGGACTTGAACAAAATGAAATCGAACGAT AGGAATCGTACAGAACGGAAAGAAATGGAACGGAA TGGAATG	5379
TEE-370	TGAGCAGGGAACAATGCGGATAAATTTACAAATAC AATGTTGAGCAAAAGAAAGACACAAAAGAATACAC ACATACACACCATATGGGCTAGG	5380
TEE-371	AATGGAATCGAACGGAATCATCATCAAACGGAACCG AATGGAATCATTGAATGGAATCAAAGGCAATCATGG TCGAATG	5381
TEE-372	AATGGAATGGAATGTACAAGAAAGGAATGGAATGA AACCGAATGGAATGGAATGGACGCAAAATGAATGG AATGGAAGTCAATGG	5382
TEE-373	AACGGAAAAAACGGAATTATCGAATGGAATCGAA GAGAATCATCGAATGGACC	5383
TEE-374	GGAATAATCATTGAACGGAATCGAATGGAATCATCA TCGGATGGAAACGAATGGAATCATCATCGAATGGAA ATGAAAGGAGTCATC	5384
TEE-375	GGAACGAAATCGAATGGAACGGAATAGAATAGACT	5385

	CGAATGTAATGGATTGCTATGTAATTGATTCTGAATGG AATGGAATCG	
TEE-376	TGAAAGGAATAGACTGGAACAAAATGAAATCGAAT GGTAGGAATCATAACAGAACAGAAAGAAATGGAACG GAATGGAATG	5386
TEE-377	AACCCGAATAGAATGGAATGGAATGGAATGGAACG GAACGGAATGGAATGGAATGGATTGGAATGGAATG GAATG	5387
TEE-378	AAAGAGAATCAAATGGAATTGAATCGAATGGAATCG AATGGATTGGAAGGAATAGAATGGAATGGAATGG AATGGAATGGAATGGAATG	5388
TEE-379	AATGGAATCATCAGTAATGGAATGGAAAGGAATGGA AAGGACTGGAATGGAATGGAATGGAATGGAATGGAATG	5389
TEE-380	GGAACAAAATGAAATCGAACGGTAGGAATCGTACA GAACGGAAGAAATGGAACGGAATGGAATGCACTC AAATGGAAGGAGTCCAATGGAATCGAAAGGAATA GAATGGAATG	5390
TEE-381	AGAATGAGATCAAGCAGTATAATAAAGGAAGAAGT AGCAAAATTACAACAGAGCAGTGAAATGGATATGCT TTCTGGCAATAATTGTGAAAGGTCTGGTAATGAGAA AGTAGCAACAGCTAGTGGCTGCCAC	5391
TEE-382	AACAAATGGAATCAACATCGAATGGAATCGAATGGA AACACCATCGAATTGAAACGAATGGAATTATCATGA AATTGAAATGGATGGACTCATCATCG	5392
TEE-383	TAACATGCAGCATGCACACACGAATACACAACACAC AAACATGTATGCACGCACACGTGAATACACAACACA CACAAACATGCATGCATGCATACATGAATACACAGC ACACAAATATCCAGCAT	5393
TEE-384	GAATGGAATCAACATCAAACGGAAAAAAAACGGAA TTATCGAATGGAATCGAATAGAATCATCGAATGGAC C	5394
TEE-385	AATCGAATGAAATGGAGTCAAAAGGAATGGAATCG AATGGCAAGAAATCGAATGTAATGGAATCGCAAGGA ATTGATGTGAACGGAACGGAATGGAAT	5395
TEE-386	AATGGAATTGAACGGAACATCAGCGAATGGAATCG AAAGGAATCATCATGGAATAGATTCTGAATGGAATGG AAAGGAATGGAATGGAATG	5396
TEE-387	ATGGAATCAACATCAAACAGAATCAAACGGAATTAT CGAATGGAATCGAAGACAATCATCGAATGGACTCGA ATGGAATCATCTAATGGAATGGAATGGAAGAATCCA TGGTCTCGAATGCAATCATCATCG	5397
TEE-388	GAATAATCATTGAACGGAATCGAATGGAATCATCTT CGGATGGAAACGAATGGAATCATCATCGAATGGAAA TGAAAGGAGTCATC	5398
TEE-389	AATGGACTCGAATGGAATAATCATTGAACGGAATCG AATGGAATCATCATCGGATGGAAATGAGTGGAAATCA TCATCGAATGGAATCG	5399
TEE-390	AAATGAAATCGAACGGTAGGAATCGTACAGAACGG AAAGAAATGGAACGGAATGGAATGCAATCGAATGG AAAGGAGTCCAATGGAAGGGAATCGAAT	5400

TEE-391	TACCAAACATTTAAAGAACAAATATCAATCCTACGC AAACCATTCTGAAACACAGAGATGGAGGATATACAG CGAAACTCATTCTACATGGCC	5401
TEE-392	TATTGGAATGGAATGGAATGGAGTCGAATGGAACGG AATGCACTCGAATGGAAGGCAATGCAATGGAATGCA CTCAACAGGAATAGAATGGAATGGAATGGAATGG	5402
TEE-393	GGAATTTAATAGAATGTACCCGAATGGAACGGAATG GAATGGAATTGTATGGCATGGAATGGAA	5403
TEE-394	GCAATCCAATAGAATGGAATCGAATGGCATGGAATA TAAAGAAATGGAATCGAAGAGAATGGAGACAAATG GAATGGAATTGAATGGAATGGAATTG	5404
TEE-395	AATGGAATCGAATGGAATCATCATCAAATGGAATCT AATGGAATCATTGAACGGAATTAAATGGAATCGTCA TCGAATGAATTCAATGCAATCAACGAATGGTCTCGA ATGGAACCAC	5405
TEE-396	AATTGCAAAAAGAAACACACATATACACATATAAAAC TCAAGAAAGACAAAACCTAATGATGATAGAAAT CAGAAAAGTACAGTACATTGGTTGTCTTGGTGGG	5406
TEE-397	TGACATCATTATTATCAAGAAACATTCTTACCACTGT TACCAACTTCCCAACACAGACTATGGAGAGAGAGAT AAGACAGAATAGCATT	5407
TEE-398	AAAGAATTGAATTGAATAGAATCACCAATGAATTGA ATCGAATGGAATCGTCATCGAATGGAATCGAAGGGA ATCATTGGATGGGCTCA	5408
TEE-399	ATCATCGAATGGAATCGAATGGAATCAATATCAAAC GGAAAAAACGGAATTATCGAATGGAATCGAATAG AATCATCGAATGGACC	5409
TEE-400	GAATGAAATCGTATAGAATCATCGAATGCAACTGAA TGGAATCATTAAATGGACTTGAAAGGAATTATTATG GAATGGAATTG	5410
TEE-401	TAAGCAACTTCAGCAAAGTCTCAGGATACAAAATCA ATGTGCAAAAATCTCAAGCATTCTTATACACGAACA ACAGACAAACAGAGAGCT	541 1
TEE-402	ACTCAAAGGAATTGATTGGAATGGAATAGAATGGC AAGGAATAGTATTGAATTGAATGGAATGGAATGGAC CCAAATG	5412
TEE-403	GAATGGAATTTAAAGGAATAGAATGGAAGGAATCG GATGGAATGGAATGGAATAGAATGGAGTCGAATGG AATAGAATCGAATGGAATGGCATTG	5413
TEE-404	TGAGAAAATGATGGAAGAGGAATAAAACGAAAC AAAACCACAGGAACACAGGTGCATGTGAATGTGCAC AGACAAAGATACAGGGCGGACTGGGAAGGAAGTTT CTGCACCAGAATTTGGGG	5414
TEE-405	AACAAAAAATGAGTCAAGCCTTAAATAAAATCAGAG CCAAAAAGAAGACATTACATCTGATAAGACAAAAA TTCAAAGGACCATC	5415
TEE-406	AACCCAGTGGAATTGAATTGAATGGAATTGAATGGA ATGGAAAGAATCAATCCGAGTCGAATGGAATGGTAT GGAATGGAATGGCATGGAATCAAC	5416
TEE-407	ATCAACATCAAACGGAAAAAACGGAATTATCGAA	5417

	TGGAATCGAAGAGAATCATCGAATGGACC	
TEE-408	AAGGAATGGAATGGTACGGAATAGAATGGAATGGA ACGAATTGTAATGGAATGGAATTTAATGGAACGGAA TGGAATGGAATGGAATCAACG	5418
TEE-409	AACGGAATGGAAAGCAATTTAATCAAATGCAATACA GTGGAATTGAAGGGAATGGAATGGAATGGC	5419
TEE-410	AATCGAATGGAACGGAATAGAATAGACTCGAATGTA ATGGATTGCTATGTAATTGATTGGAATGGAATGGAA TCGAATGGAATGCAATCCAATGGAATGGAATGCAAT GCAATGGAATGGAATCGAACGGAATGCAGTGGAAG GGAATGG	5420
TEE-41 1	TAGCAACATTTTAGTAACATGATAGAAACAAAACAG CAACATAGCAATGCAATAGTAACACAACAGCAACAT CATAACATGGCAGCA	5421
TEE-412	AATGGAATCGAAGAGAATGGAACAAATGGAATGG AATTGAATGGAATGGAATTGAATGGAATGGGAAGGA ATGGAGTG	5422
TEE-413	AGCAAACAAGTGAATAAACAAGCAAACAAGTGAAC AAGCAAACAAGTGAATAAACAAGCAAACAAGTGAA CAAGCAAACAAGTGAATAAACAAGCAAACAAGTGA ACAAGGAAACAAGTGAATAAACAAGGCTCT	5423
TEE-414	AATGGAATCAACACGAGTGCAATTGAATGGAATCGA ATGGAATGGAATGGAATGGAATGAATTCAACCCGAA TGGAATGGAAGGAATGGAATC	5424
TEE-415	GAATCGAATGGAATCAACATCAAACGGAAAAAAAC GGAATTATCGAATGGAATCGAAGAGAATCATCGAAT GGACC	5425
TEE-416	AACACGAATGTAATGCAATCCAATAGAATGGAATCG AATGGCATGGAATATAAAGAAATGGAATCGAAGAG AATGGAACAAACGGAATGGAATTGAATGGAATGG AATTGAATGGAATGGGAACGAATGGAGTGAAATTG	5426
TEE-417	GAATGGAACGGAATAGAACAGACTCGAATGTAATGG ATTGCTATGTAATTGATTGGAATGGAATCGA ATGGAATGCAATCCAATGGAATGGAATGCAATGCAA TGGAATGGAATCGAATGGAATGCAGTGGAAGGGAAT GG	5427
TEE-418	GAATCGAATGGAATCAATATCAAACGGAAAAAAAC GGAATTATCGAATGGAATCGAAGAGAATCATCGAAT GGACC	5428
TEE-419	ATAAACATCAAACGGAATCAAACGGAATTATCGAAT GGAATCGAAGAGAATAATCGAATGGACTCAAATGGA GTCATCTAATGGAATGGTATGGAAGAATCCATGGAC TCCAACGCAATCATCAGCGAATGGAATC	5429
TEE-420	AAAAGAAAAGACAAAAGACACCAATTGCCAATACT GAAATGAAAAACAGGTAATACTATTGATCCCATG GACATTAAAATGATGTTGAAGGAACACCAC	5430
TEE-421	AATGTCAAGTGAATCGAGTGGAATCATCGAAAGAA ATCGAATGGAATCGAAGGGAATCATTGGATGGGCTC AAAT	5431
TEE-422	ATCATCGAATGGAATAGAATGGTATCAACATCAAAC	5432

	GGAGAAAAACGGAATTATCGAATGGAATCGAAGAG AATCTTCGAACGGACC	
TEE-423	GAATGGAATCATCGCATAGAATCGGATGGAATTATC ATCGAATGGAATCGAATGGTATCAACATCAAACGGA AAAAAACGGAATTATCGAATGGAATCGAATTGAATC ATCGAACGGACCCG	5433
TEE-424	AATGGACTCGAATGGAATAATCATTGAACGGAATCG AATGGAATCATCATCGGATGGAAATGAATGGAATAA TCCATGGACTCGAATGCAATCATCATCGAATGGAAT CGAATGGAATCATCGAATGGACTCG	5434
TEE-425	AATGCAATCATCAACTGGCTTCGAATGGAATCATCA AGAATGGAATCGAATGGAATCATCGAATGGACTC	5435
TEE-426	AAGAGACCAATAAGGAATAAGTAAGCAACAAGAGG AAGGAGAAAAGGGCAAGAGAGATGACCAGAGTT	5436
TEE-427	TGGAATCATCATAAAATGGAATCGAATGGAATCAAC ATCAAATGGAATCAAATGGAATCATTGAACGGAATT GAATGGAATCGTCAT	5437
TEE-428	GGAATCATCGCATAGAATCGAATGGAATTATCATCG AATGGAATCGAATGGAATCAACATCAAACGAAAAA AAACCGGAATTATCGAATGGAATCGAAGAGAATCAT CGAACGGACC	5438
TEE-429	AAATCATCATCGAATGGGATCGAATGGTATCCTTGA ATGGAATCGAATGGAATCATCATCAGATGGAAATGA ATGGAATCGTCAT	5439
TEE-430	GGAATGTAATAGAACGGAAAGCAATGGAATGGAAC GCACTGGATTGAGTGAATGGAATCTATTGGAATG GAATCGAATGGAATGGTTTGGCATGGAATGGAC	5440
TEE-431	AAACAATGGAAGATAATGGAAGATATCGAATGGA ATAGAATGGAATGGAATGGACTCAAATGGAATGGAC TTTAATGGAATGG	5441
TEE-432	GGAACGAAATCGAATGGAACGGAATAGAATAGACT CGAATGTAATGGATTGCTATGTAATTGATTGGAATG AATGGAATCGAATGGAATGCAATCCAATGGAATGGA ATGCAATGCAATGAATGGAATGGAATGGAATGGAAT GGAA	5442
TEE-433	AAACCGAATGGAATGGAATGGACGCAAAATGAATG GAATGGAAGTCAATGGACTCGAAATGAATGGAATGG AATGGAATGGAATG	5443
TEE-434	GGAATCGAATGGAATCAACATCAAACGGAAAAAAA CAGAATTATCGTATGGAATCGAATAGAATCATCGAA TGGACC	5444
TEE-435	CAACCCGAGTGAATAAAATGGAATGGAATGGAATG AAATGGAATGGATCGGAATGGAATCCAATGGAATCA ACTGGAATGGAATGGAATGGAATG	5445
TEE-436	TATCATCGAATGGAATCGAATGGAATCAACATCAA CGGAAAAAACGGAATTATCGAATGGAATCGAAGA GAATCATCGAATGGACC	5446
TEE-437	CGGAATAATCATTGAACGGAATCGAATGGAATCATC ATCGGATGGAAACGAATGGAATCATCATCGAATGGA AATGAAAGGAGTCATC	5447

TEE-438	CAACACACAGAGATTAAAAACAAACAAACAAT CCAGCCCTGACATTTATGAGTTTACAGACTGGTGG GAGGCAGAGAAG	5448
TEE-439	CACTACAAACCACGCTCAAGGCAATAAAAGAACACA AACAAATGGAAAAACATTCCATGCTCATGGATGGG	5449
TEE-440	AATCGAATGGAATTAACATCAAACGGAAAAAACG GAATTATCGAATGGAATCGAAGAGAATCATCGAATG GACC	5450
TEE-441	TGGAAAAAGAAATCAAATTGAATGGCATCGAACGGAAT GGGATGGAATGGAATAGACCCAGATGTAATGGACTC GAATGGAATG	5451
TEE-442	GACTAATATTCAGAATATACAAGGAACTCAAACAAC TCAACAGTAGAAAAAAAACCTGAATAGACATTTCT CAAAAGAAGACATACAAATGGCC	5452
TEE-443	GGTCCATTTCGATGATTCTCTTCGATTCCATTTCGATAA TTCCGTTTTTCCCGTTTGATGTTGATTCC	5453
TEE-444	GGAACGAAATCGAATGGAACGGAATAGAATAGACT CGAATGTAATGGATTGCTATGTAATTGATTGGAATGG AATGGAATCGAATGGAATGCAATCCAATGGAATGGA ATGCAATGCAATGAATGGAATGGAATGGAATGGAAT GGA	5454
TEE-445	AGCAACTTCAGTAAAGTGTGAGGATACAAAATCAAT GTGCAAAAATCACAAGCATTCTTATACATCAATAAC AGACAAACAGAGAGCCAAA	5455
TEE-446	GAATAATCATTGAACGGAATCGAATGGAATCATCAT CGGATGGAAACGAATGGAATCATCATCGAATGGAAA TGAAAGGAGTCATC	5456
TEE-447	TAATCATCTTCGAATTGAAAACAAAGCAATCATTA ATGTACTCTAACGGAATCATCGAATGGACC	5457
TEE-448	GGAATCGAATGGAATCAACATCAAACGGAAAAAAA CGGAATTATCGAATGGAATCGAAGAGAATCATCGAA TGGACC	5458
TEE-449	AGAGAAAAGATGATCATGTAACCATTGAAAAGACAA TGTACAAAATAATACTAATCACACAGGACCAGAAA GCAATTTAGACCAT	5459
TEE-450	AATGGAATCGAATGGAATCAACATCAAACGGAAAA AACGGAATTATCGAATGGAATCAAAGAGAATCATCG AATGGACC	5460
TEE-451	AATGGAATTATCATCGAATGGAATCGAATGGAATCA ACATCAAACGGAAAAAACGGAATTATCGAATGGA ATCGAAGAGAATCATCGAATGGACC	5461
TEE-452	GTCAACACAGGACCAACATAGGACCAACACAGGGTC AACACAGGACCAACATAGGACCAACACAGGGTCAA CACAAGACCAACATGGGACCAACACAGGGTCAACAT AGGACCAACATGGGACCAACACAGGGTCAACACAG GACCAAC	5462
TEE-453	GAATCAACTCGATTGCAATCGAATGGAATGGAATGG TATTAACAGAATAGAATGGAATGGAATGGAATGGAA CGGAACG	5463
TEE-454	ACTCGAATGCAATCAACATCAAACGGAATCAAACGG	5464

	AATTATCGAATGGAATCGAAGAGAATCATCGAACGG ACTCGAATGGAATCATCTAATGGAATGGAATGG	
TEE-455	AATGGAATGGAATAATCGACGGACCCGAATGCAATC ATCATCGTACAGAATCGAATGGAATCATCGAATGGA CTGGAATGGAATGG	5465
TEE-456	AATACAAACCACTGCTCAACGAAATAAAAAGAGGATA CAAACAAATGGAAGAACATTCTATGCTCATGGGTAG GATGAATTCATATCGTGAAAATGGCCATACTGCC	5466
TEE-457	AAACACGCAAACACACACACAAGCACACTACCACAC AAGCGGACACACATGCAAACACGCGAACACACACA CATATACACACAAGCACATTACAAAACACAAGCAAA CACCAGCAGACACACAAACACACAAACATACATGG	5467
TEE-458	AATCGAACGGAATCAACATCAAACGGAAAAAAAC GGAATTATCGAATGGAATCGAAGAGAATCATCGAAT GGACC	5468
TEE-459	TAATTGATTCTGAATGGAATGGAATAGAATGGAATTG AATGGAATGGACCATAATGGATTGGACTTTAATAGA AAGGGCATG	5469
TEE-460	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GTACAAAAGTCACAAGCATTCTTATACACCAACAAA AGACAAACAGAGAGCC	5470
TEE-461	ACATCAAACGGAAAAAAACAAAACGGAATTAT CGAATGGAATCGAAGAGAATCATCGAATGGACC	5471
TEE-462	GAAATTCCAATTAATAATGAAATCGACTTATCTTAAC AAATATAGCAATGCTGACAACACTTCTCCGGATATG GGTACTGCT	5472
TEE-463	ACATCTCACTTTTAGTAATGAACAGATCATTGAGACA GAAAATTAGCAAAGAACATCAGAGTTAAACTACAC TCTAAACCAAATGGACCTA	5473
TEE-464	GAAGAAAGCATTTCATTCAAGACATCTAACTCGTTGA TATAATGCATACAGTTCAAAATGATTACACTATCATT ACATCTAGGGCTTTC	5474
TEE-465	ACACACACATTCAAAGCAGCAATATTTACAACAGCC AAAAGGTGGAAACAATTGAGCAATTG	5475
TEE-466	ATCATCGAATAGAATCGAATGGTATCAACACCAAAC GGAAAAAACGGAATTATCGAATGGAATCGAAGAG AATCTTCGAACGGACC	5476
TEE-467	ATCAACATCAAACGGAAAAAACGGAATTATCGAATG GAATCGAAGAGAATCATCGAACGGACC	5477
TEE-468	AATCGAAAGGAATGTCATCGAATGGAATGGACTCAA ATGGAATAGAATCGGATGGAATGGCATCGAATGGAA TGGAATGGAATTGGATGGAC	5478
TEE-469	AACATGAACAGTGGAAACAATCAGTGAACCAATACAA GGGTAAATAAGCTAGCAATTAAGCTGTATCACT GGTCTAAAGATAGAAGATCAAGTAGAAAATCAGCGC AAGAGGAAAGATATACGAAAATAATGGCC	5479
TEE-470	CGAATGGAATCATTATGGAATGGAATGAAATGGAAT AATCAAATGGAATTGAATGGAATCATCGAATGGAAT CGAACAAAATCCTCTTTGAATGGAATAAGATGGAAT CACCAAATGGAATTG	5480

TEE-471	AAGGGAATTGAATAGAATGAATCCGAATGGAATGGA ATGGAATGGAATGGAATGGAATGGAATGGAATGGA ATGGAATG	5481
TEE-472	GAATGGAATCGAATCAAATTAAATCAAATGGAATGC AATAGAAGGGAATACAATGGAATAGAATGGAATGG AATGGAATGGACT	5482
TEE-473	AAACGGAATCAAACGGAATTATCGAATGGAATCGAA GAGAATCATCGAACGGACTCGAATGGAATCATCTAA TGGAATGGAATGGAAGAATCCATGGACT	5483
TEE-474	ATGGAATCAACATCAAACGGAAAAAAAAACGGAAT TATCGAATGGAATCGAAGAGAATCATCGAATGGACC AGAATGGAATCATCTAATGGAATGGAATGG	5484
TEE-475	AATGGAATCATCATCGAATGGAATCGAATGGAATCA TGGAATGGAATCAAATGGAATCAAATGGAATCGAAT GGAATGGAATGGAATG	5485
TEE-476	AACGGAATCAAACGGAATTACCGAATGGAATCGAAT AGAATCATCGAACGGACTCGAATGGAATCATCTAAT GGAATGGAATGGAAG	5486
TEE-477	AAACGGAATCAAACGGAATTATCGAATGGAATCGAA AAGAATCATCGAACGGACTCGAATGGAATCATCTAA TGGAATGGAATGGAAGAATCCATGG	5487
TEE-478	GAATGATACGGAATACAATGGAATGGAACGAAATG AAATGGAATGGAATGGAATGGAATGGAATGGAATG G	5488
TEE-479	ACAGCAAGAGAGAAATAAAACGACAAGAAACTAC AAAATGCCTATCAATAGTTACTTTAAATATCAGTGGA CCAAATCAGTGAAACAAAAGACACAGAGTGGC	5489
TEE-480	AATGGACTCGAATGGATTAATCATTGAACGGAATCG AATGGAATCATCATCGGATGGTAATGAATGGAATCA TCATCGAATGGAATCGG	5490
TEE-481	GAATGGAATCGAAAGGAATGTCATCGAATGGAATGG AATGGAACGGAATGGAATCGAATGGAATGGACTCGA ATGGAATAGAATCGAATGCAATGGCATCG	5491
TEE-482	ATCGAATGGAATCAACATCAGACGGAAAAAAAAACGG AATTATCAAATGGAATCGAAGAGAATCATCGAATGG ACC	5492
TEE-483	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAAT GTGCAAAAATCAAAGCATTCCTTATGCACCAATAAC AGACACAGAGCCAAAT	5493
TEE-484	AATGGAATGGAACGCAATTGAATGGAATGGAATGGA ACGGAATCAACCTGAGTCAAATGGAATGGAATGGAA TGGAATG	5494
TEE-485	GGAACGAAATCGAATGGAACGGAATAGAATAGACT CGAATGTCATGGATTGCTATGTAATTGATTGGAATGG AATGGAATCG	5495
TEE-486	TAGCAGGAAACAGCAAACCTCAAATTAAGTAATTTCA AGAGCGTATCATCAATGAACTATTTCAAAGATGTG GGCAAGAT	5496
TEE-487	GAATTGAAAGGAATGTATTGGAATAAAATGGAATCG AATAGGTTGAAATACCATAGGTTGGAATTGAATGGA	5497

	ATGGGAGGGACACCAATGGAATTG	
TEE-488	AAGCAACTTCAGCAAAGTCTCGGGATACAAAATCAA TGTGCAAAAATCACAAGCATTCTTATACACCACTAA CAGACAAATGGAGAGTC	5498
TEE-489	GAATGGAATCAACATCAAACGGAAAAAACGGAAT TATCGAATGGAATCGAAGAGAATCATCGAATGGACC AGAATGGAATCATCTAATGGAATGGAATGGAATAAT CCATGG	5499
TEE-490	AAAAGCAATTGGACTGATTTTAAATATACGTGGCAA CAAGGATAAACTGCTAATGATGGGTTTGCAAATACA GATCG	5500
TEE-491	AATGGAATCAACATCGAACGGAAAAAACGGAATT ATCGAATGGAATCGAAGAGAATCATCGAATGGACC	5501
TEE-492	AAACGGAATTATCAAATGGAATCGAAGAGAATCATC GAACGGACTCGAATGGAATCATCTAATGGAATGGAA TGGAAG	5502
TEE-493	TGCAAGATAACACATTTTAGTTGACACCATTGAAAA CAGTTTTAACCAAGAATATTAGAACCAATGAAGCAG AGAAATCAAAGGGTGGATGGAAGTCCAAAGGAT G	5503
TEE-494	TAGAACAGAATTGAATGGAATGGCATCAAATGGAAT GGAAACGAAAGGAATGGAATTGAATGGACTCAAAT GTTATGGAATCAAAGGGAATGGACTC	5504
TEE-495	AAGAGAATCATCGAATGGAATCGAATGGAATCAACA TCAAACGGAAAAAACGGAATTATCGAATGGAATCG AAGAGAATCATCGAATGGACC	5505
TEE-496	ATCAACATCAAACGGAAAAAACGGAATTATCGAAT GGAATCGAAGAGAATCATCGAATGGACC	5506
TEE-497	GAATCAACATCAAACGGAAAAAACCGAATTATCGA ATGGAATCGAAGAGAATCATCGAATGGACC	5507
TEE-498	ATCAACATCAAACGGAATCAAACGGAATTATCGAAT GGAATCGAAGAGAATCATCAAATGGACTCGAATGGA ATCATCTAATGGAATGGAATGGAAGAATCCATGG	5508
TEE-499	ATCGAATGGAATCATTGAATGGAAGGAATGGAATC ATCATGGAATGGAACGAATGGAATCACTGAATGGA CTCGAATGGGATCATCA	5509
TEE-500	ATTCAGCCTTTAAAAAAGAAGACAGTCCTGTCATTT GTGACAATATGAATGAAACAGACATCACATTAAATG AAATGAGCCAGGCGCAG	5510
TEE-501	GAATGAAATGAAATCAAATGGAATGTACATGAATGG AATAGAAAAGAATGCATCTTTCTCGAACGGAAGTGC ATTGAATGGAAGGAATCTACTGGAATGGATTGGA TGGAATGGAATGGGATGGAATGGTATGG	551 1
TEE-502	AACATCAAACGGAATCAAACGGAATTATCGAATGGA ATCGAAGAGAATCATCGAACGGACTCGAATGGAATC ATCTAATGGAATGGAATGGAAGAATCCATGGACTCG AATGCAATCATCATCGAATGAAATCGAATGGAATCA TCGAATGGACTCG	5512
TEE-503	ATGGAATTCAATGGAATGGACATGAATGGAATGGAC TTCAATGGAATGGTATCAAATGGAATGGAATTCAGT	5513

TEE-504	AATGGAAAGGAATCGAATGGAAGGGAATGAAATTG AATCAACAGGAATGGAAGGGAATAGAATAGACGGC AATGGAATGGACTCG	5514
TEE-505	AGCAACTTCAGCAAAGTATCAGGATACAAAATCAAT GTACAAAAATCCCAAGCATTCTTATACACCAACAAC AGACAAACAGAGAGCC	5515
TEE-506	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCGAT GTGCAAAAATCACAAGCATTCTTATACACCAACAAC AGATAAACAGAGAGCC	5516
TEE-507	AACGGAAAAAAAACGGAATTATCGAATGGAATCGA AGAGAATCATCGAATGGACCAGAATGGAATCATCTA ATGGAATGGAATGGAATAATCCATGGACTCGAATG	5517
TEE-508	GGAATCAAACGGAATTATCGAATGGAATCGAAGAGA ATCATAGAACGGACTCAAATGGAATCATCTAATGGA ATGGAATGGGAGAATCCATGGACTCGAATG	5518
TEE-509	AATGGAATCAATATCAAACGGAAAAAAAACGGAATTA TCGAATGGAATCGAAGAGAATCATCGAATGGACC	5519
TEE-510	AACGGAATCAAACGGAATTATCGAATGGAATCGAAA AGAATCATCGAACGGACTCGAATGGAATCATCTAAT GGAATGGAATGGAAGAATCCATGG	5520
TEE-51 1	AAACGGAATTATCGAATGGAATCAAAGAGAATCATC GAATGGCCACGAATGGAATCATATAATGGAATGGAA TGGAATAATCCATGGACC	5521
TEE-512	AATGGAATCGAATGGATTGATATCAAATGGAATGGA ATGGAAGGGAATGGAATGGAATGGAATTGAACCAA ATGTAATGGATTG	5522
TEE-513	TAAAAGACGGAACAGATAGAAAGCAGAAAGGAAAG GTGAATTGCATTACCACTATTCATACTGCCACACACA TGACATTAGGCCAAGTC	5523
TEE-514	AATGGAATCGAATGGAACAATCAAATGGACTCCAAT GGAGTCATCTAATGGAATCGAGTGAATCATCGAAT GGACTCG	5524
TEE-515	TAACACATAAACAAACACAGAGACAAAATCTCCGAG ATGTTAATCTGCTCCAGCAATACAGAACAATTTCTAT TACCAACAGAATGCTTAATTTTTCTGCCT	5525
TEE-516	GGAATCGAATGGAATCAACATCAAACGGAAAAAAA CGGAATTATCGAATGGAATCAAAGAGAATCATCGAA TGGACC	5526
TEE-517	AGAATGGAAAGGAATCGAAACGAAAGGAATGGAGA CAGATGGAATGGAATG	5527
TEE-518	GAATCATCATAAAATGGAATCGAATGGAATCAACAT CAAATGGAATCAAATGGTCTCGAATGGAATCATCTT CAAATGGAATGGAATGG	5528
TEE-519	AACAACAATGACAAACAAACAACGACAAAGAC ATTTATTTGGTTCACAAATCTCCAGGGTGTACAAGAA GCATGGTGCCAGCATCTGCTCAGCTTCTGATGAGGG CTCTGGGAAGCTTTTACTC	5529
TEE-520	AACGGACTCGAACGGAATATAATGGAATGGAATGGA TTCGAAAGGAATGGAATGGAATGGACAGGAAAAGA ATTGAATGGGATTGGAATGGAATCG	5530

TEE-521	AACATCAAACGAAATCAAACGGAATTATCAAATTGA ATCGAAGAGAATCATCGAATTGCCACGAATGCAATC ATCTAATGGTATGGAATGGAATAATCCATGGACCCA GATG	5531
TEE-522	AGAAATTAACAGCAAAAAGAAGGATGCAGTGCAACTC AGGACAACACATACAATTCAAGCAACAAATGTATAG TGGCTGGGCACCAAGGATACAG	5532
TEE-523	GCAATAAAATCGACTCAGATAGAGAAGAATGCAATG GAATGGAATGGAATGGAATGGAATGGGATGGAATG GTATGGAATGG	5533
TEE-524	AATGGACTCGAATGAAATCATCATCAAACGGAATCG AATGGAATCATTGAATGGAAAGGATGGGATCATCAT GGAATGGAAACGAATGGAATCACTG	5534
TEE-525	CCACATAAAACAAAACACTACAAGACAATGATAAAGTT CACAACATTAACACAATCAGTAATGGAAAAGCCTAG TCAATGGCAG	5535
TEE-526	TGGAATGGAATGGAATGGAATCAAATCGCATGGTAA TGAATCAAATGGAATCAAATCGAATGGAAATAATGG AATCGAAGGGAAACGAATGGAATCGAATTGCACTGA TTCTACTGACTTCGAGGAAAATGAAATGAAATGCGG TGAAGTGAATGG	5536
TEE-527	GAATGTTATGAAATCAACTCGAACGGAATGCAATAG AATGGAATGGAATGGAATGGAATGGAATGGAATGG	5537
TEE-528	AATGGAATCATTGAATGGAATGGAATGGAATCATCA AAGAAAGGAATCGAAGGGAATCATCGAATGGAATC AAACGGAATCATCGAATGGAATGGAATGGAATG	5538
TEE-529	GGAATCAACATCAAACGGAAAAAAACGGAATTATC GAATGGAATCGAAGAGAATCATCGAATGGACC	5539
TEE-530	GGAATAATCATCATCAAACAGAACCAAATGGAATCA TTGAATGGAATCAAAGGCAATCATGGTCAATG	5540
TEE-531	GCATAGAATCGAATGGAATTATCATTGAATGGAATC GAATGGAATCAACATCAAACGGAAAAAAACGGAAT TATCGAATGGAATCGAAGAGAATCATCGAATGGACC C	5541
TEE-532	AATGGAATCGAAGAGAATCATCGAACGGACTCGAAT GGAATCATCTAATGGAATGGAATGGAATAATCCATG GACCCGAATG	5542
TEE-533	AAATGAATCGAATGGAATTGAATGGAATCAAATAGA ACAAATGGAATCGAAATGAATCAAATGGAATCGAAT CGAATGGAATTGAATGGCATGGAATTG	5543
TEE-534	AGTTAATCCGAATAGAATGGAATGGAATGCAATGGA ACGGAATGGAACGGAATGGAATGGAATGGAATGGA ATGGAATG	5544
TEE-535	ATCACAATCACACAACACATTGCACATGCATAACAT GCACTACAATACACACACAACACATACACAACACA CATGCAATACAACACAAAACGCAACACAACATATAC ACAACACACAGCACACACATGCC	5545
TEE-536	AAAGACTTAAACGTTAGACCTAAAACCATAAAAACC CTAGAGGAAAACCTAGGCATTACCATTGAGGACTTA GGCATGGGCAAGGAC	5546

TEE-537	AAAGTCCAAAGATGAACAAAATATCCAGAAGGAAA ACAAATGCACTTGGGGAGTGGGAAAAGAAAACCAAG ACTGAGCAATGCGTCAAGCTCAGACGTGCCTCACTA CG	5547
TEE-538	AAACGGAATCAAACGGAATTATCGAATGGAGTCGAA AAGAATCATCGAACGGACTCGAATGGAATCATCTAA TGGAATGGAATGGAAGAATCCATGG	5548
TEE-539	AATTGATTGCAAATTAATGGAATTGAATGGAATGCA ATCAAATGGAATGGAATGTAATGCAATGGAATGTAA TAGAATGGAAAGCAATGGAATG	5549
TEE-540	TACAGAACACATGACTCAACAACAGCAGAAAGCATA TTCTTTTCAAATGCACATGAAACATTATCATGATGGA CCAAAT	5550
TEE-541	GGAACAAAATGAAATCGAACGGTAGGAATCATACA GAACAGAAAGAAATGGAACGGAATGGAATG	5551
TEE-542	AACGGAAAAAACGGAATTATCGAATGGAATCGAAG AGAATCATCGAATGGAATCGAATGGAGTCATCG	5552
TEE-543	AATCGAACGGAATCAACATCAAACGGAAAAAAACG GAATTATCGAATGGAATCGAAGAGAATCATCGAATG GACC	5553
TEE-544	AGAATGGAATGCAATAGAATGGAATGCAATGGAATG GAGTCATCCGTAATGGAATGGAAAGGAATGCAATGG AATGGAATGGAATGG	5554
TEE-545	ATGGAATCAACATCAAACGGAATCAAACGGAATTAT CGAATGGAATCGAAGAGAATCATCGAACGGATTCTGA ATGGAATCATCTAATGGAATGGAATGGAAGAATCCA TGGACTCGAATGCAATCATCAGCGAATGGAATCGAA TGGAATCATCGAATGGACTCG	5555
TEE-546	GGAATAAAACGGACTCAATAGTAATGGATTGCAATG TAATTGATTGATTTCGAATGGAATCGCATGGAATGT AATGGAATGGAATGGAATGGAAGGC	5556
TEE-547	AATGGAATCAACATCAAACGGAAAAAAACGGAATT ATCGTATGGAATCGAAAAGAATTATCGAATGGACC	5557
TEE-548	TCAAACGGAAAAAAACGGAATTATCGAATGGAATCG AAGAGAATCATCGAATGGACC	5558
TEE-549	ACATCAAACGGAATCAAACGGAATTATCGAATGGAA TCGAAAAGAATCATCGAACGGACTCGAATGGAATCA TCTAATGGAATGGAATGGAAGAATCCATGGACTCGA ATG	5559
TEE-550	TGGAATCGAATGGAATCAACATCAAACGGAAAAAA ACGGAATTATCGAATGGAATCGAAGAGAATCATCGA ATGGACC	5560
TEE-551	AATGGAATCGAATGCAATCATCGAACGGAATCGAAT GGCATCACCGAATGGAATGGAATGGAATGGAATGGA ATGG	5561
TEE-552	AGAATTGATTGAATCCAAGTGAATTGAATGGAATG GAATGGATTAGAAAGGAATGGAATGGATTGGAATGG ATTGGAATGGAAAGG	5562
TEE-553	AACTGCATCAACTAACAGGCAAAATAACCAGCTAAT ATCATAATGACAGGATTAAATTCACAAATGACAATA	5563

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TEE-554	GTAAACAAACAATCAAGCAAGTAAGAACAGAAATA ACAGCATTGGCTTTTGAGTTAATGACAAGAACACTC GGCATGGGAGCCTGGGTGAGCAAATCACAGATCTTC	5564
TEE-555	AAAGGAATGGACTGGAACAAAATGAAATCGAACGG TAGGAATCGTACAGAACGGACAGAAATGGAACGGC ATGGAATGCACTCG	5565
TEE-556	GAATCAACCCGAGCGGAAAGGAATGGAATGGAATG GAATCAACACGAATGGAATGGAACGGAATGGAATG GGATGGGATGAAATGGAATGG	5566
TEE-557	AAGAAATGGAATCGAAGAGAATGGAAACAAACGGA ATGGAATTGAATGGAATGGAATTGAATGGAATGGGA	5567
TEE-558	GACATGCAAACACAACACACAGCACACATGGAACAT GCATCAGACATGCAAACACAACACACATACCACACA TGGCATATGCATCAGACGTGCCTCACTAC	5568
TEE-559	AAAGGAATGCACTCGAATGGAATGGACTTGAATGGA ATGTCTCCGAATGGAACAGACTCGTATGAAATGGAA TCGAATGGAATGGAATCAAATGGAATTGATTTGAGT GAAATGGAATCAAATGGAATGGCAACG	5569
TEE-560	GGAACAAAATGAAATCGAACGGTAGGAATCGTACA GAACGGAAAGAAATGGAACGGAATGGAATGCACTC GAATGGAAAGGAGTCCAAT	5570
TEE-561	AAATTGATTGAAATCATCATAAAATGGAATCGAAGG GAATCAACATCAAATGGAATCAAATGGAATCATTGA ACGGAATTGAATGGAATCGTCAT	5571
TEE-562	AGAATGGAAAGCAATAGAATGGAACGCACTGGATTC GAGTGCAATGGAATCAATTGGAATGGAATCGAATGG AATGGATTGGCA	5572
TEE-563	AACACCAAACGGAAAAAACGGAATTATCGAATGG AATCGAAGAGAATCTTCGAACGGACCCGAATGGGAT CATCTAATGGAATGGAATGGAATAATCCATGG	5573
TEE-564	AATGGAGACTAATGTAATAGAATCAAATGGAATGGC ATCGAATGGAATGGACTGGAATGGAATGTGCATGAA TGGAATGGAATCGAATGGATTG	5574
TEE-565	AAATCGAATGGAACGCAATAGAATAGACTCGAATGT AATGGATTGCTATGTAATTGATTCGAATGGAATGGA ATCGACTGGAATGCAATCCAATGGAATGGAATGCAA TGCAATGGAATGGAATCGAACGGAATGCAGTGGAAAG GGAATGG	5575
TEE-566	AATCAACAAGGAACTGAAACAAGTAAACAAGAAAA CAAATAACACCATAAAACATGGGCAAAGGACATAA ACAGACATTTTTCAAAAAAGACATACAAATGGCCGA G	5576
TEE-567	AATGGAATCAACATCAAACGGAAAAAACGGAATT ATCGAATGGAATCGAAGAGAATCATCGAATGGACCC AGGCTGGTCTTGAAGTCC	5577
TEE-568	ATTGAATGGGCTAGAATGGAATCATCTTTGAACGGA ATCAAAGGGAATCATCATCGAATGGAATCGAATGGA AATGTCAACG	5578
TEE-569	AATGGACTCGAATGGAATCAACATCAAATGGAATCA	5579

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TEE-571	ACTCGAGTGGAAATTGACTGTAACAAAATGGAAAGTA ACGGATTGGAATCGAATGGAACGGAATGGAATGGA ATGGACAT	5581
TEE-572	TACAACTTTAAAAAATGATCAACAGATACACAGTT AGCAAGAAAGAATTGAGGGCAAAGAATATGCCAGA CAAACCTCAAGAGGAAGATGATGGTAGAGATAGGTCA CATTGGAGTGTCA	5582
TEE-573	AAATCAACAACAAACGGAAAAAAAAGGAATTATCG AATGGAATCAAAGAGAATCATCGAATGGACC	5583
TEE-574	AACGGAATCAAACGGAATTATCGAATGGAATCGAAA AGAATCATCGAACGGACTCGAATGGAATCATCTAAT GTAATGGAATGGAAGAATCCATGGACTCGAATG	5584
TEE-575	AACGGAAAAAACGGAATTATCGAATGGAATCGAA GAGAATCATCGAATGGACCAGAATGGAATCATCTAA TGGAATGGAATGGAATAATCCATGGACTCGAATG	5585
TEE-576	CAACATCAAACGGAAAAAACGGAATTATGGAATG GAATCGAAGAGAATCATCGAATGGACCCGAATGGAA TCATCTGAAATATAATAGACTCGAAAGGAATG	5586
TEE-577	ATGGAATCGAATGGAATGGACTGGAATGGAATGGAT TCGAATGGAATCGAATGGAACAATATGGAATGGTAC CAATG	5587
TEE-578	GAATGGAATCAACATCAAACGGAAAAAACGGAAT TATCGAATGGAATCGAAGAGAATCATCGAATGGACC	5588
TEE-579	AAATGGACTCGAATGGAATCATCATAGAATGGAATC GAATGCAATGGAATGGAATCTTCCGGAATGGAATGG AATGGAATGGAATGGAG	5589
TEE-580	GAATCATCATAAAATGGAATCGAATGGAATCAACAT CAAATGGAATCAAATGGAATCATTGAACGGAATTGA ATGGAATCGTCAT	5590
TEE-581	ATCGAATGGAATCAACATCAAACGGAAAAAACGG AATTATCGAATGGAATCGAAGAGAATCATCGAATGG ACC	5591
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TEE-583	AGAAACAGAAAACAGTCAAACCAATGGGCAATCCAT ATCAGATGCAGTATTATGAACAGAAGTGTAAGAAT GCACCAGGCACAATGGC	5593
TEE-584	GATTGGAACGAAATCGAATGGAACGGAATAGAATA GACTCGAATGTAATGGATTGCTATGTAATTGATTGCA ATGGAATGGAATCGAATGGAATGCAATCCAATGGAA TGGAATGCAATGCAATGGAATGG	5594
TEE-585	ATGGAATGGAATAATCAACGTACTCGAATGCAATCA TCATCGTATAGAATCGAATGGAATCATCGAATGGAC	5595

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TEE-587	AGAAAAGATAACTCGATTAAACAAATGAACAAACACC TGAATACACAAGTCTCAAAAGAAGACATAAAAAATGG CCAAC	5597
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TEE-589	AATGGAATCAACATCAAACGGAATCAAGCGAAATTA TCGAATGGAATCGAAGAGAATCATCGAATGGACTCG AATGGAATCATCTAATGGAATGGAATGGGAT	5599
TEE-590	AAACACAGTACAAATACTAATTCAAATCAAACCTTAC TCAAAGTCATAATCAAACATGCCAGACGGGCTGAGG GGCAGCATTA	5600
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TEE-594	CAATCAGAGCGGACACAAACAAATTGCATGGGAAG AATCAATATCGTGAAAATGGCC	5604
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TEE-597	CAATCAAAACAGCAATGAGATACCATTTTACACCAA TCAAAATGGCTACTAAAAAGTCAAAAGCAAATGCC	5607
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TEE-599	AATGGAATGGAATCATCGCATAGAATGGAATGGAAT TATCATCGAATTGAATCGAATGGTATCAACATCAAA CGGAAAAAACGGAAATATCGAATGGAATCGAAGA GAATCATCGAACGGACC	5609
TEE-600	GAAAAACAAAACAAAACAAAACAAAACAATCAA AAAAGTGGTAGCAGAAACCAGAAAGTCCATGTATAT AGCTAATTGGCCTGGTTGT	5610
TEE-601	AGACCTTTCTCAGAAGACACACAAATTGCCAACAGG TATATGAAAAAATGTTCAATATCACTAATCATCAGG	561 1

	GCGATGCC	
TEE-602	CATGGAATCGAATGGAATTATCATCGAATGGAATCG AATGGTACCAACACCAAACGGAAAAAACGGAATT ATCGAATGGAATCGAAGAGAATCTTCGAACGGACC	5612
TEE-603	AGAGCAGAAACAAATGGAATTGAAATGAAGACAAC AATCAAAAGCATCAATGAAATGAAAAGTTGGGTTTT GGAAGAGAGAAACAAT	5613
TEE-604	ACACAAACACACACACACACACACACACACACACAC ACACACACACACACACACACACACACACACACATAC	5614
TEE-605	AACAAACAAATGAGATGATTTTCAGATAGTGATAAAC ACTATAACATAATTAATTCGTGCCAATCAGAGCATA ACAGTGGTGTGGTGGCTGTGGAACAGATAGCAGAC	5615
TEE-606	AATGGAATCGAGTGGAAATGGAAGGCAATGGAATAG AATGGAATGGAATCGAAAGGAACGGAATGGAATGG AATGGAATG	5616
TEE-607	AGAAATGGAATCGGAGAGAATGGAAACAAATGGAA TGGAATTGAATGGAATGGAATTGAATGGAATGGGAA CG	5617
TEE-608	AAGAGAACTGCAAAACACTGCTCAAAGAAATCAGA GATGACAAAAACACATGGAAAAACGTTTCATGCTCA TGGATTGGAAGACTTA	5618
TEE-609	AATCAACACGAATAGAATGGAACGGAATGGAATGG AATGGAATGGAATGGAATGGAGTGGAAATGGAACAG AATGGAGTGGAAAT	5619
TEE-610	AACATCAAACGAAATCAAACGGAATTATCAAATTGA ATCGAAGAGAATCATCGAATTGCCACGAATGCAACC ATCTAATGGTATGGAATGGAATAATCCATGGACCCA GATG	5620
TEE-61 1	CGGAATTATCATCGAATGTAATCGAATGGAATCAAC ATCAAACGGAAAAAAACGGAATTATCGAATGGAATC GAAGAGAATCATCGAATGGACC	5621
TEE-612	TGGACACACACGAACACACACCTACACACACGTGGA CACACACGGACACATGGACACACACGAACACATGGA CACACACACGGGGACACACACAGACACACACAGAG ACACACACGGACACATGG	5622
TEE-613	ATCAAACGGAATCAAACGGAATTATCGAATGGAATC GAAGAGAATCATCGAATGGACTCGAATGGAATCATC TAATGGAATGGAATGGAAGAATCCATGG	5623
TEE-614	AAATGGAATGGAATGCACTTGAAAGGAATAGACTGG AACAAAATGAAATCGAACGGTAGGAATCATAACAGA ACAGAAAGAAATGGAACGGAATGGAATG	5624
TEE-615	ACCACACACAAAATACACCACACACCACACACACAC CACACACTATACACACACCACACACCACACACAC	5625
TEE-616	AAAGAAATAGAAGGGAGTTGAACAGAATCGAATGG AATCGAATCAAATGGAATCGAATGGCATCAAATGGA ATCGAATGGAATGTGGTGAAGTGGATTGG	5626
TEE-617	GGAATCATCATAAAATGGAATCGAATGGAATCATCA TCAAATGGAATCAAATGGAATCATTGAACGGAATTG AATGGAATCGTCAT	5627
TEE-618	AAAGATCAATGTACAAAAATCAGCAGCATTTCTATA	5628

	AACCAACAATGTCCAGGCTGAGAGAGAAATCAAGA AAACAATTC	
TEE-619	TGGAATGGAATGGAATGAAATAAACACGAATAGAAT GGAACGGAATGGAACGGAATGGAATGGAATGGAAT GGAAAG	5629
TEE-620	TAATCAGCACAAATCAACTGTAGTCACAAAACAAATA GTAACGCAATGATAAAGAAACAGAGAACTAGTTCAA ATAAACATGATAAGATGGGG	5630
TEE-621	AAGCGGAATTATCAAATGGAATCGAAGAGAATGGA AACAAATGGAATGGAATTGAATGGAATGGAATTGAA TGGAATG	5631
TEE-622	AATGGAATCAACATCAAACGGAAAAAACGGAATT ATCGAATGGAATCGAAGAGAATCATCGAATGGACC	5632
TEE-623	ACTTGAATCGAATGGAAAGGAATTTAATGAACTTAA ATCGAATGGAATATAATGGTATGGAATGGACTCATG GAATGGAATGGAAAGGAATC	5633
TEE-624	TGGAATCATCATCGAAAGCAAGCGAATGGAATCATC AAATGGAAACGAATGGAATCATCGAATGGACTCGGA TGGAATTGTTGAATGGACT	5634
TEE-625	TGGAATCAACATCAAACGGAAAAAACGGAATTATC GAATGGAATCGAAGAGAATCATCGAATGGACC	5635
TEE-626	TAAGTGAATTGAATAGAATCAATCTGAATGTAATGA AATGGAATGGAACGGAATGGAATGGAATGGAATGG AATGGAATGGAATGG	5636
TEE-627	AGGAAAATTTAATCAGCAGGAATAGAAACACACTTG AGAAATCCATGTGGAATGAAAAGAGAATGGCTGAGC AGCAACAGATTGTCAAAAAGGAAATC	5637
TEE-628	AACATCAAACGGAAAAAACGGAATTATCGAATG GAATCGAAGAGAATCATCGAATGGACC	5638
TEE-629	TAATTGAGAATAAGCATTCCAGTGGAAAAAAACTA AACAATTTGTTGTAAAACATCCTTAAAAGCATCAGA AAGTTAATACAGCAATGAAGAATTACAGGACCAAAT TAAGAATGGTATGGAAGCCTGTTA	5639
TEE-630	TATCATCGAATGGAATCGAATGGAATCAACATCAAA CGGAAAAAACGGAATTATCGAATTGAATCGAAGAG AATCATCGAATGGACC	5640
TEE-631	AGCAAAACAAACACAATCTGTCGTTTCATGGTACTAC GACATACTGGGAGAGATATTCAAATGATCACACAAA ACAACATG	5641
TEE-632	AAGGATTCGAATGGAATGAAAAAGAATTGAATGGA ATAGAACAGAATGGAATCAAATCGAATGAAATGGA GTGGAATAGAAAGGAATGGAATG	5642
TEE-633	AACGGAATCAAACGGAATTATCGAATGGAATCGAAG AGAATCATCGAACGGACTCGAATGGAATCATCTAAT GGAATGGAATGGAAGAATCCATGGACTCGAATGCAA TCATCATCGAATGGAATCGAACGGAATCATCGAATG GCC	5643
TEE-634	AATCAACTAGATGTCAATGGAATGCAATGGAATAGA ATGGAATGGAATTAACACGAATAGAATGGAATGGAA TGGAATGGAATGG	5644

TEE-635	AATGGACTCGAATGGAATAATCATTGAACGGAATCG AATGGAATCATCATCGGATGGAAATGAATGGAATCA TCATCGCATGGAATCG	5645
TEE-636	GAATGGAATGATACGGAATAGAATGGAATGGAACG AAATGGAATTGAAAGGAAAGGAATGGAATGGAATG GAATGG	5646
TEE-637	AATCATCATCGAATGGAATCGAATGGTATCATTGAG TGGAATCGAATGGAATCATCATCAGATGGAAATGAA TGGAATCGTCAT	5647
TEE-638	GAATCAAATCAATGGAATCAAATCAAATGGAATGGA ATGGAATTGTATGGAATGGAATGGCATGG	5648
TEE-639	TAATGCAGTCCAATAGAATGGAATCGAATGGCATGG AATATAAAGAAATGGAATCGAAGAGAATGGGAACA AATGGAATGGAATTGAGTGAATGGAATTGAATGGA ATGGGAACGAATGGAGTG	5649
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TEE-642	GCCAACAATCATATGAGAAAAAGCTCAACATCACTG ATCATTTAGGAATGCAAATCAAACCACAATGAGA TACTATCA	5652
TEE-643	AATCAAATGGAATGAAATCGAATGGAATTGAATCGA ATGGAATGCAATAGAATGTCTTCAAATGGAATCGAA TGGAATTTGGTGAAGTGGACGGGAGTG	5653
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TEE-645	AGCAACTTCAGCAAAGTCTCAGCATACAAAATCAAT GTGCAAAAATCACACGCATTCCTATACACCAATAAC AGACAAACAGAGAGCC	5655
TEE-646	GAATCAAATGGAATGGACTGTAATGGAATGGATTG AATGGAATCGAATGGAGTGGACTCAAATGGAATG	5656
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TEE-650	AACGGAATCACGCGGAATTATCGAATGGAATCGAAG AGAATCATCGAATGGACTCGAATGGAATCATCTAAT GGAATGGAATGG	5660
TEE-651	GGAATCAACTCGATTGCAATGGAATGCAATGGAAAG GAATGGAATGCAATTAAAGCGAATAGAATGGAATGG AATGGAATGGAACGGAATGGAATG	5661
TEE-652	AAAACAAACAACAACGACAAATCATGAGACCAGAG TTAAGAAACAATGAGACCAGGCTGGGTGTGGTG	5662
TEE-653	AATCGAAAGGAATGCAATATTATTGAACAGAATCGA	5663

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TEE-655	AACCCGAGTGCAATAGAATGGAATCGAATGGAATGG AATGGAATGGAATGGAATGGAATGGAGTC	5665
TEE-656	GAATGGAATTGAAAGGAATGGAATGCAATGGAATG GAATGGGATGGAATGGAATGCAATGGAATCAACTCG ATTGCAATG	5666
TEE-657	GAAAAAACGGAATTATCGAATTGAATCAAATAGAA TCATCGAACGGACCAAAATGGAATCATCTAATGGAA TGGAATGGAATAATCCATGGACTCTAATG	5667
TEE-658	TGGAATCATCTAATGGAATGGAATGGAATAATCCAT GGACTCGAATGCAATCATCATAAAATGGAATCGAAT GGAATCAACATCAAATGGAATCAAATGGGATCATTG AACGGAATTGAATGGAATCGTCAT	5668
TEE-659	GAAAAAACGGAATTATCGAATTGAATCGAATAGAA TCATCGAACGGACCAGAATGGAATCATCTAATGGAA TGGAATGGAATAATCCATGGACTCGAATG	5669
TEE-660	AACCACTGCTTAAGGAAATAAGAGAGAACACAAAC AAATGGAAAAACGTTCCATGCTCATGGATAGGAGAA TCAATATCGTGAAAATGGCC	5670
TEE-661	TATCGAATGGAATGGAAAGGAGTGGAGTAGACTCGA ATAGAATGGACTGGAATGAAATAGATTGGAATGGAA TGGAATGGAATGAAGTGGACTCG	5671
TEE-662	GTATCAACATCAAACGGAACGGAATTATCGA ATGGAATCATCTAATGGAATGGAATGGAATAATCCA TGGACTCGAATG	5672
TEE-663	TAAATGGAGACATCATTGAATACAATTGAATGGAAT CATCACATGGAATCGAATGGAATCATCGTAAATGCA ATCAAGTGAATCAT	5673
TEE-664	GAATGGAATTGAAAGGTATCAACACCAAACGGAAA AAAAAACGGAATTATCGAATGGAATCGAAGAGAATC ATCGAACGGACC	5674
TEE-665	AGCAATTTGAGCAAAGTCTCAGGATACAAAATCAAT GTACAAATTCACAAGCATTCTTATGGACCAACAACA G	5675
TEE-666	GGAATCGAATGGCATCAACATCAAACGGAAAAAAA CGGAATTATCGAATGGAATCGAATGGAATCATC	5676
TEE-667	AAACAAAACACAGAAATGCAAAGACAAAACATAAA ACGCAGCCATAAAGGACATATTTTAGATAACTGGGG AAATTTGTATGGGCTGTGT	5677
TEE-668	AATGGAATCAACATCAAACGGAATCAAACGGAATTA TCGAATGGAATCGAAGAGAATCATCGAACGGACTCG AATGGAATCATCTAATGGAATGGAATGGAAG	5678
TEE-669	AATCGAATGGAATCAGCATCAAACGGAAAAAACG GAATTATCGAATGGAATCGAAGAGAATCATCGAATG GACC	5679
TEE-670	AAACGGAATTATAGAATGGACTGGAAGAGAATCATC	5680

	GAACGGACTAGAATGGAATCATCTAATCGAATGGAA TGGAACAATCCATGGTCTAGCA	
TEE-671	TGAACAGAGAATTGGACAAAACGCACAAAGTAAAG AAAAAGAATGAAGCAACAAAAGCAGAGATTTATTG AAAACAAAAGTACACACCACACAGGGTGGGAGTGG	5681
TEE-672	ATCATAACGACAAGAACAAATTCACACACAACAATA TTAACTTCAAATCCAAATGGGTAAATGCTCCAATTA AAGGATGCAGACGGGCAAATTGGATA	5682
TEE-673	ATCATAACGACAAGAACAAATTCACACACAACAATA TTCACCTCAAATCCAAATGGGTAAATGCTCCAATTA AAGGATGCAGACGGGCAAATTGGATA	5683
TEE-674	GAATGGAATCGAATGGATTGATATCAACTGGAATGG AATGGAAGGGAATGGAATGGAATGGAATTGAACCA AATGTAATGACTTGAATGGAATG	5684
TEE-675	GAATCAACATCAAACGGAAAAAACGGAATTATCGA ATGGAATCGAAGAGAATCATCGAATGGACC	5685
TEE-676	GGAATCAACATCAAACGGAAAAAACGGAATTATCG AATGGAATCGAAGAGAATCATCGAATGGACC	5686
TEE-677	ATGGAATCAACATCAAACGGGAATCAAACGGAATTAT CGAATGGAATCAAAGAGAATCATCGAACGGACTCGA ATGGAATCATCTAATGGAATGGAATGGAAGAATCCA TGGACTCGAATGCAATCATCATCGAAT	5687
TEE-678	GGAATGGAATGGAATGGAGCCGAATGGAATGGAAT GTACTCAAATGGAATGC	5688
TEE-679	AAAACACCTAGGAATACAGATAACAAGGGACATTAA CTACCTCTTAAAGAGAACTACAAACCACTGCTCAAG GAAATGAGAGAGGACACAAACACATGGAAAAACAT TCCATCCTCATGGATAGGAAGAATCAATATTGTGAA AATGGCC	5689
TEE-680	AACACGACTTTGAGAAGAGTAAGTGATTGTTAATTA AAGCAAGAGAATTATTGATGTATCACAGTCATGAGA AATATTGGAAGGAATATGGTCCATAC	5690
TEE-681	ACACATATCAAACAAACAAAAGCAATTGACTATCTA GAAATGTCTGGGAAATGGCAAGATATTACA	5691
TEE-682	GGAATCATCATATAATGGAATCGAATGGAATCAACA TCAAATGGAATCAAATGGAATCATTGAACGGAATTG AATGGAATCGTCAT	5692
TEE-683	AATGGAATCAACATCAAACGGAATCAAATGGAATTA TCGAATGGAATCGAAGAGAATCATCGAATTGTCACG AATGGAATCATCTAATGGAATGGAATGGAATAATCC ATGGCCCCTATGCAATGGACTCGAATGAAATCATCA TCAAACAGAATCGAATGGAATCATCTAATGGAATGG AATGGCATAATCCATGGACTCGAATG	5693
TEE-684	TAAAATGAAACAAATATACAACACGAAGGTTATCAC CAGAAATATGCCAAAACCTTAAATATGAGAATAAGAC AGTCTCAGGGGCCACAGAG	5694
TEE-685	AAAATACAGCGTTATGAAAAGAATGAACACACACAC ACACACACACACAGAAAATGT	5695
TEE-686	CAAACAAATAGGTACCAAACAAATAACAACATAAAC CTGACAACACACTTATTTACAAGAGACATCCCTTATA	5696

	TGAAAGGGTACAGAAAAGTCGATGGTAAGATGATGG GGAAAGGTATACCAACCACTAGCAGAAGG	
TEE-687	TGGAATCGAATGGAATCAATATCAAACGGAAAAAAA CGGAATTATCGAATGGAATCGAAAAGAATCATCGAA TGGGCCCGAATGGAATCATCT	5697
TEE-688	ACAAATGGAATCAACAACGAATGGAATCGAATGGA AACGCCATCGAAAGGAAACGAATGGAATTATCATGA AATTGAAATGGATG	5698
TEE-689	AATCAATAAATGTAAACCAGCATATAAACAGAACCA ACGACAAAAACCATGATTATCTCAATAGATGCAG AAAAGGCC	5699
TEE-690	AAAATAAACGCAAATTTAAATCACAAGATACCAACA CATTCCCACGGCTAAGTACGAAGAACAAGGGCGAAT GGTCAGAATTAAGCTCAAACCT	5700
TEE-691	CAACATCAAACGGAATCAAACGGAATTATCGAATGG AATCGAAGAGAATCATCGAATGGACTCGAATGGAAT CATCTAATGGAATGGAATGGAAG	5701
TEE-692	ACATCAAACGGAAAAAAACGGAATTATCGAATGGA ATCGAAGAGAATCATCGAATGGACC	5702
TEE-693	AATGGACTCGAATAGAATTGACTGGAATGGAATGGA CTCGAATGGAATGGAATGGAATGGAAGGGACTCG	5703
TEE-694	AAGAAAGACAGAGAACAAACGTAATTCAAGATGAC TGATTACATATCCAAGAACATTAGATGGTCAAAGAC TTTAAGAAGGAATACATTCAAAGGCAAAACGTCCT TACTGATTTTGGTGGAGTTGCCACATGGAC	5704
TEE-695	GAATGGAATCGAATGGAATGAACATCAAACGGAAA AAAACGGAATTATCGAATGGAATCAAAGAGAATCAT CGAATGGACCCG	5705
TEE-696	ATGGACTCGAATGTAATAATCATTGAACGGAATCGA ATGGAATCATCATCGGATGGAAACGAATGGAATCAT CATCGAATGGAATCGAATGGGATC	5706
TEE-697	GAAATGGAATGGAAAGGAATAAAATCAAGTGAAAT TGGATGGAATGGATTGGAATGGATTGGAATG	5707
TEE-698	AAACGGAAAAAAAACGGAATTATCGAATGGAATCG AAGAGAATCATCGAACGAACCAGAATGGAATCATCT AATGGAATGGAATGGAATAATCCATGG	5708
TEE-699	ATTAACCCGAATAGAATGGAATGGAATGGAATGGAA CGGAACGGAATGGAATGGAATGGAATGGAATGGAA TGGATCG	5709
TEE-700	AACATCAAACGGAAAAAAACGGAATTATCGTATGGA ATCGAAGAGAATCATCGAATGGACC	5710
TEE-701	GAATAGAATTGAATCATCATTGAATGGAATCGAGTA GAATCATTGAAATCGAATGGAATCATCATCGAATGG AATTGGGTGGAATC	571 1
TEE-702	CACCGAATAGAATCGAATGGAACAATCATCGAATGG ACTCAAATGGAATTATCCTCAAATGGAATCGAATGG AATTATCG	5712
TEE-703	AATGCAATCGAATAGAATCATCGAATAGACTCGAAT GGAATCATCGAATGGAATGGAATGGAACAGTC	5713
TEE-704	AAATCATCATCGAATGGAATCGAATGGTATCATTGA	5714

	ATGGAATCGAATGGAATCATCATCAGATGGAAATGA ATGGAATCGTCAT	
TEE-705	GAATGGAATCGAAAGGAATAGAATGGAATGGATCGT TATGGAAAGACATCGAATGGAATGGAATTGACTCGA ATGGAATGGACTGGAATGGAACG	5715

Example 35. In Vitro Expression of modified nucleic acids with miR-122

[001011] MicroRNA controls gene expression through the translational suppression and/or degradation of target messenger RNA. The expression of G-CSF mRNA and Factor IX mRNA with human or mouse alpha-globin 3' untranslated regions (UTRs) were down regulated in human primary hepatocytes using miR-122 sequences in the 3'UTR.

[001012] Primary human hepatocytes were seeded at a density of 350000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL).

[001013] G-CSF mRNA having a human alpha-globin 3'UTR (G-CSF Hs3'UTR; mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a mouse alpha-globin 3'UTR (G-CSF Mm3'UTR; mRNA sequence shown in SEQ ID NO: 5717; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine. G-CSF mRNA containing a human 3'UTR having a miR-122 sequence in the 3'UTR (G-CSF Hs3'UTR miR-122; mRNA sequence shown in SEQ ID NO: 5018; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-122 seed sequence in the 3'UTR (G-CSF Hs3'UTR miR-122 seed; mRNA sequence shown in SEQ ID NO: 5020; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF Hs3'UTR miR-122 seedless; mRNA sequence shown in SEQ ID NO: 5022; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine. G-CSF mRNA containing a mouse 3'UTR having a miR-122 sequence in the 3'UTR (G-CSF Mm3'UTR miR-122; mRNA sequence shown in SEQ ID NO: 5024; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-122 seed sequence in the 3'UTR (G-CSF Mm3'UTR miR-122 seed; mRNA sequence shown in SEQ ID NO: 5026; polyA tail of

approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF Mm3'UTR miR-122 seedless; mRNA sequence shown in SEQ ID NO: 5028; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001014] Factor IX mRNA having a human alpha-globin 3'UTR (Factor IX Hs3'UTR; mRNA sequence shown in SEQ ID NO: 5718; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a mouse alpha-globin 3'UTR (Factor IX Mm3'UTR; mRNA sequence shown in SEQ ID NO: 5719; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine. Factor IX mRNA containing a human 3'UTR having a miR-122 sequence in the 3'UTR (Factor IX Hs3'UTR miR-122; mRNA sequence shown in SEQ ID NO: 5030; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-122 seed sequence in the 3'UTR (Factor IX Hs3'UTR miR-122 seed; mRNA sequence shown in SEQ ID NO: 5032; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-122 sequence without the seed sequence in the 3'UTR (Factor IX Hs3'UTR miR-122 seedless; mRNA sequence shown in SEQ ID NO: 5034; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine. Factor IX mRNA containing a mouse 3'UTR having a miR-122 sequence in the 3'UTR (Factor IX Mm3'UTR miR-122; mRNA sequence shown in SEQ ID NO: 5036; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-122 seed sequence in the 3'UTR (Factor IX Mm3'UTR miR-122 seed; mRNA sequence shown in SEQ ID NO: 5038; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-122 sequence without the seed sequence in the 3'UTR (Factor IX Mm3'UTR miR-122 seedless; mRNA sequence shown in SEQ ID NO: 5040; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001015] Each G-CSF or Factor IX mRNA sequence was tested at a concentration of 500 ng per well in 24 well plates. 24, 48 and 72 hours after transfection, the expression

of protein was measured by ELISA. The protein levels for G-CSF are shown in Table 33 and the protein levels for Factor IX are shown in Table 34.

Table 33. G-CSF Protein Expression

Description	Protein Expression (ng/ml)		
	24 Hours	48 Hours	72 Hours
G-CSF Hs3'UTR	43.9	18.8	5.7
G-CSF Hs3'UTR miR-122	6.9	0.7	0.12
G-CSF Hs3'UTR miR-122 seed	48.5	25.6	8.2
G-CSF Hs3'UTR miR-122 seedless	31.7	11.7	3.4
G-CSF Mm3'UTR	84.9	100.4	21.3
G-CSF Mm3'UTR miR-122	24.0	3.03	0.8
G-CSF Mm3'UTR miR-122 seed	115.8	96.4	19.2
G-CSF Mm3'UTR miR-122 seedless	113.1	92.9	18.9

Table 34. Factor IX Protein Expression

Description	Protein Expression (ng/ml)		
	24 Hours	48 Hours	72 Hours
Factor IX Hs3'UTR	63.2	124.8	44.3
Factor IX Hs3'UTR miR-122	15.9	4.4	0.4
Factor IX Hs3'UTR miR-122 seed	60.2	63.0	20.1
Factor IX Hs3'UTR miR-122 seedless	53.7	75.0	24.5
Factor IX Mm3'UTR	90.8	159.6	70.5
Factor IX Mm3'UTR miR-122	11.8	5.0	1.0
Factor IX Mm3'UTR miR-122 seed	77.2	115.0	41.7
Factor IX Mm3'UTR miR-122 seedless	69.3	123.8	49

Example 36. In Vitro Expression of modified nucleic acid with mir-142 or miR-146 binding sites

[001016] HeLa and RAW264 cells were seeded at a density of 17000 and 80000 per well respectively, in 100 ul cell culture medium (DMEM+10%FBS).

[001017] G-CSF mRNA (G-CSF; mRNA sequence shown in SEQ ID NO: 4258; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) was fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001018] G-CSF mRNA having a miR-142-3p sequence in the 3'UTR (G-CSF miR-142-3p; mRNA sequence shown in SEQ ID NO: 4992; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-142-3p seed sequence in the 3'UTR (G-CSF miR-142-3p seed; mRNA sequence shown in SEQ ID NO: 4994; polyA

tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-142-3p sequence without the seed sequence in the 3'UTR (G-CSF miR-142-3p seedless; mRNA sequence shown in SEQ ID NO: 4996; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001019] G-CSF mRNA having a miR-142-5p sequence in the 3'UTR (G-CSF miR-142-5p; mRNA sequence shown in SEQ ID NO: 4986; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-142-5p seed sequence in the 3'UTR (G-CSF miR-142-5p seed; mRNA sequence shown in SEQ ID NO: 4988; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-142-5p sequence without the seed sequence in the 3'UTR (G-CSF miR-142-5p seedless; mRNA sequence shown in SEQ ID NO: 4990; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001020] G-CSF mRNA having a miR-146a sequence in the 3'UTR (G-CSF miR-146a; mRNA sequence shown in SEQ ID NO: 4998; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-146a seed sequence in the 3'UTR (G-CSF miR-146a seed; mRNA sequence shown in SEQ ID NO: 5000; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-146a sequence without the seed sequence in the 3'UTR (G-CSF miR-146a seedless; mRNA sequence shown in SEQ ID NO: 5002; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001021] Each G-CSF mRNA sequence was tested at a concentration of 500 ng per well in 24 well plates for each cell type. 24 hours after transfection, the expression of protein was measured by ELISA and the protein levels are shown in Table 35. The G-CSF sequences with a miR-142-3p sequence in the 3'UTR down regulated G-CSF expression in RAW264 cells whereas the G-CSF sequences with a miR-142-5p or miR-146a sequence in the 3'UTR did not down regulate G-CSF expression.

Table 35. G-CSF Expression

Description	HeLa Cells	RAW264 Cells

	Protein Expression (ng/ml)	Protein Expression (ng/ml)
G-CSF	243.5	173.7
G-CSF miR-142-3p	309.1	67.6
G-CSF miR-142-3p seed	259.8	178.1
G-CSF miR-142-3p seedless	321.7	220.2
G-CSF miR-142-5p	291.8	223.3
G-CSF miR-142-5p seed	261.3	233.1
G-CSF miR-142-5p seedless	330.2	255.1
G-CSF miR- 146a	272.6	125.2
G-CSF miR-146a seed	219.4	138.3
G-CSF miR- 146a seedless	217.7	132.8

Example 37. Effect of Kozak sequence on expression of modified nucleic acids

[001022] HeLa cells were seeded at a density of 17000 per well in 100 ul cell culture medium (DMEM + **10%FBS**). G-CSF mRNA having an IRES sequence and Kozak sequence (G-CSF IRES Kozak; mRNA sequence shown in SEQ ID NO: 5004; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), G-CSF mRNA having an IRES sequence but not a Kozak sequence (G-CSF IRES; mRNA sequence shown in SEQ ID NO: 5006; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), G-CSF mRNA without an IRES or Kozak sequence (G-CSF no Kozak; mRNA sequence shown in SEQ ID NO: 5008; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a G-CSF sequence having a Kozak sequence (G-CSF Kozak; mRNA sequence shown in SEQ ID NO: 5720; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with fully modified with 5-methylcytidine and 1-methylpseudouridine and tested at a concentration of 75 ng per well in 24 well plates. 24 hours after transfection, the expression of G-CSF was measured by ELISA, and the results are shown in Table 36.

Table 36. G-CSF expression

Description	Protein Expression (ng/ml)
G-CSF IRES Kozak	2.01
G-CSF IRES	1.64
G-CSF no Kozak	795.53
G-CSF Kozak	606.28

Example 38. MALAT1 Constructs

[001023] Modified mRNA encoding G-CSF or mCherry with a human or mouse MALAT1 sequence and their corresponding cDNA sequences are shown below in Table 37. In Table 37, the start codon of each sequence is underlined and the MALAT1 sequences are bolded.

Table 37. MALAT1 Constructs

	Sequence	SEQ ID NO:
G-CSF with Mouse MALAT1 sequence	Optimized G-CSF cDNA sequence containing a T7 polymerase site, kozak sequence, and a Mouse MALAT1 sequence (bold): TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC <u>ATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTA</u> TGGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGG ACAGTCCAAGAAGCGACTCCTCTCGGACCTGCCTCAT CGTTGCCGCAGTCATTCCTTTTGAAGTGTCTGGAGCAG GTGCGAAAGATTCAGGGCGATGGAGCCGCACTCCAAG AGAAGCTCTGCGCGACATACAACTTTGCCATCCCGA GGAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCC TGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCCTTTCCCAGCTCCACTCCGGTTTGT TCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAG CTCGACGTGGCGGATTTGCAACAACCATCTGGCAGC AGATGGAGGAACTGGGGATGGCACCCGCGCTGCAGCC CACGCAGGGGGCAATGCCGGCCTTTGCGTCCGCGTTT CAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCACC TTCAATCATTTTTTGAAGTCTCGTACCGGGTGCTGAGA CATCTTGCGCAGCCG TGATAATAG GATTCGTCAGTAGGGTTGTAAAGGTTTTTCTTTTCC TGAGAAAACAACCTTTTGTTTTCTCAGGTTTTGCTT TTTGGCCTTTCCCTAGCTTTAAAAAAAAAAAAAGCAA AAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTCTA GA	5721
	mRNA sequence (transcribed): GGGAAUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAU AUAAGAGCCACC <u>AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU</u> AUGGCCUGCAGUUGCUGCUUUGGCACUCGGCCUC UGGACAGUCCAAGAAGCGACUCCUCUCGGACCUGCC UCAUCGUUGCCGCAGUCAUUCUUUGAAGUGUCUG GAGCAGGUGCGAAAGAUUCAGGGCGAUGGAGCCGCA	5722

	CUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUGC CAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUG GGGAUUCCCUGGGCUCUCUCUGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCCAGCUC CACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCAA GCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACG CUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCA ACAACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUG GCACCCGCGCUGCAGCCACGCAGGGGGCAAUGCCG GCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUUCAAUUUUUUGGAA GUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAG GAUUCGUCAGUAGGGUUGUAAAGGUUUUUCUUUU CCUGAGAAAACAACCUUUUGUUUUCUCAGGUUUUG CUUUUUGGCCUUUCCCUAGCUUUAAAAAAAAAAAAA GCAAAAGUGGUCUUUGAAUAAAGUCUGAGUGGGCG GC	
mCherry with Mouse MALAT1 sequence	Optimized mCherry cDNA sequence containing a T7 polymerase site, kozak sequence, and a Mouse MALAT1 sequence (bold): TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC <u>AT</u>GGTATCCAAGGGGGAGGAGGACAACATGGCGATC ATCAAGGAGTTCATGCGATTCAAGGTGCACATGGAAG GTTCGGTCAACGGACACGAATTTGAAATCGAAGGAGA GGGTGAAGGAAGGCCCTATGAAGGGACACAGACCGC GAAACTCAAGGTCACGAAAGGGGGACCACTTCCTTTC GCCTGGGACATTCTTTCGCCCCAGTTTATGTACGGGTC CAAAGCATATGTGAAGCATCCCGCCGATATTCCTGAC TATCTGAAACTCAGCTTTCCCGAGGGATTCAAGTGGG AGCGGGTCATGAACTTTGAGGACGGGGGTGTAGTCAC CGTAACCCAAGACTCAAGCCTCCAAGACGGCGAGTTC ATCTACAAGGTCAAAGTTCGCGGGGACTAACTTTCCGT CGGATGGGCCCGGTGATGCAGAAGAAAACGATGGGAT GGGAAGCGTCATCGGAGAGGATGTACCCAGAAGATG GTGCATTGAAGGGGGAGATCAAGCAGAGACTGAAGTT GAAAGATGGGGGACATTATGATGCCGAGGTGAAAAC GACATACAAAGCGAAAAAGCCGGTGCAGCTTCCCGGA GCGTATAATGTGAATATCAAGTTGGATATTACTTCACA CAATGAGGACTACACAATTGTGCGAACAGTACGAACGC GCTGAGGGTAGACACTCGACGGGAGGCATGGACGAG TTGTACAAA TGATAATAG GATTCGTCAGTAGGGTTGTAAAGGTTTTTCTTTTCC TGAGAAAACAACCTTTTGTCTTCAGGTTTTGCTT TTTGGCCTTTCCCTAGCTTTAAAAAAAAAAAAAGCAA	5723

	AAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTCTA GA mRNA sequence (transcribed): GGGAAUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAU AUAAGAGCCACC <u>AUGGUAUCCAAGGGGGAGGAGGACAACAUGGCGAUC</u> AUCAAGGAGUUCAUGCGAUUCAAGGUGCACAUGGAA GGUUCGGUCAACGGACACGAAUUUGAAAUCGAAGGA GAGGGUGAAGGAAGGCCCUAUGAAGGGACACAGACC GCGAAACUCAAGGUCACGAAAGGGGGACCACUCCU UUCGCCUGGGACAUUCUUUCGCCCCAGUUUAUGUAC GGGUCCAAAGCAUAUGUGAAGCAUCCCGCCGAUAUU CCUGACUAUCUGAAACUCAGCUUUCGAGGGGAUUC AAGUGGGAGCGGGUCAUGAACUUUGAGGACGGGGG UGUAGUCACCGUAACCCAAGACUCAAGCCUCCAAGA CGGCGAGUUCAUCUACAAGGUCAAACUGCGGGGGAC UAACUUUCCGUCGGAUGGGCCGGUGAUGCAGAAGAA AACGAUGGGAUGGGAAGCGUCAUCGGAGAGGAUGU ACCCAGAAGAUGGUGCAUUGAAGGGGGAGAUAAGC AGAGACUGAAGUUGAAAGAUGGGGGACAUUAUGAU GCCGAGGUGAAAACGACAUACAAAGCGAAAAAGCCG GUGCAGCUUCCCGGAGCGUAUAAUGUGAAUAUCAAG UUGGAUAUUACUUCACACAAUGAGGACUACACAAUU GUCGAACAGUACGAACGCGCUGAGGGUAGACACUCG ACGGGAGGCAUGGACGAGUUGUACAAA UGAUAAUAG GAUUCGUCAGUAGGGUUGUAAAGGUUUUUCUUUU CCUGAGAAAACAACCUUUUGUUUUCUCAGGUUUUG CUUUUUGGCCUUUCCCUAGCUUUAAAAAAAAAAAA GCAAAGUGGUCUUUGAAUAAAGUCUGAGUGGGCG GC	5724
G-CSF with Human MALAT1 sequence	Optimized G-CSF cDNA sequence containing a T7 polymerase site, kozak sequence, and a Human MALAT1 sequence (bold): TAATACGACTCACTATA GGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC <u>ATGGCCGGTCCCGCGACCCAAAGCCCCATGAACTTA</u> TGGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGG ACAGTCCAAGAAGCGACTCCTCTCGGACCTGCCTCAT CGTTGCCGCGAGTCATTCTTTTGAAGTGTCTGGAGCAG GTGCGAAAGATTGAGGGCGATGGAGCCGCACTCCAAG AGAAGCTCTGCGCGACATACAACTTTGCCATCCCGA GGAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCC TGGGCTCCTCTCTCGTCCTGTCCGTGCGAGGCTTTGCA GTTGGCAGGGTGCTTTCCAGCTCCACTCCGGTTTGT TCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCCGACGCTGGACACGTTGCAG	5725

	CTCGACGTGGCGGATTTTCGCAACAACCATCTGGCAGC AGATGGAGGAACTGGGGATGGCACCCGCGCTGCAGCC CACGCAGGGGGCAATGCCGGCCTTTGCGTCCGCGTTT CAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCACC TTCAATCATTTTTTGGAAGTCTCGTACCGGGTGCTGAGA CATCTTGCGCAGCCG TGATAATAG TGCTCTTCAGTAGGGTCATGAAGGTTTTTCTTTTCC TGAGAAAACAACACGTATTGTTTTCTCAGGTTTTGC TTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGC AAAAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTC TAGA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAU AUAAGAGCCACC <u>AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU</u> AUGGCCUGCAGUUGCUGCUUUGGCACUCGGCCCUC UGGACAGUCCAAGAAGCGACUCCUCUCGGACCUGCC UCAUCGUUGCCGCAGUCAUUCUUUUGAAGUGUCUG GAGCAGGUGCGAAAGAUUCAGGGCGAUGGAGCCGCA CUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUGC CAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUG GGGAUUCCUGGGCUCUCCUCUCUGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCAGCUC CACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCAA GCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACG CUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCA ACAACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUG GCACCCGCGCUGCAGCCCACGCAGGGGGCAAUGCCG GCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUUCAAUUUUUGGAA GUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAG UGCUCUUCAGUAGGGUCAUGAAGGUUUUUCUUUUC CUGAGAAAACAACACGUAAUUGUUUUCUCAGGUUUU GCUUUUUGGCCUUUUUCUAGCUUAAAAAAAAAAAA AGCAAAAGUGGUCUUUGAAUAAAGUCUGAGUGGGC GGC	5726
mCherry with Human MALAT1 sequence	Optimized mCherry cDNA sequence containing a T7 polymerase site, kozak sequence, and a Human MALAT1 sequence (bold): TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC <u>ATGGTATCCAAGGGGGAGGAGGACAACATGGCGATC</u> ATCAAGGAGTTCATGCGATTCAAGGTGCACATGGAAG GTTCCGTCAACGGACACGAATTTGAAATCGAAGGAGA GGGTGAAGGAAGGCCCTATGAAGGGACACAGACCCG	5727

	GAAACTCAAGGTCACGAAAGGGGGACCACTTCCTTTC GCCTGGGACATTCTTTCGCCCCAGTTTATGTACGGGTC CAAAGCATATGTGAAGCATCCCGCCGATATTCCTGAC TATCTGAAACTCAGCTTTCCCGAGGGATTCAAGTGGG AGCGGGTCATGAACTTTGAGGACGGGGGTGTAGTCAC CGTAACCCAAGACTCAAGCCTCCAAGACGGCGAGTTC ATCTACAAGGTCAAACCTGCGGGGGACTAACTTTCCGT CGGATGGGCCGGTGATGCAGAAGAAAACGATGGGAT GGGAAGCGTCATCGGAGAGGATGTACCCAGAAGATG GTGCATTGAAGGGGGAGATCAAGCAGAGACTGAAGTT GAAAGATGGGGGACATTATGATGCCGAGGTGAAAAC GACATACAAAGCGAAAAAGCCGGTGCAGCTTCCCGGA GCGTATAATGTGAATATCAAGTTGGATATTACTTCACA CAATGAGGACTACACAATTGTCGAACAGTACGAACGC GCTGAGGGTAGACACTCGACGGGAGGCATGGACGAG TTGTACAAA TGATAATAG TGCTCTTCAGTAGGGTCATGAAGGTTTTTCTTTTCC TGAGAAAACAACACGTATTGTTTTCTCAGGTTTTGC TTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGC AAAAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTC TAGA	
	mRNA sequence (transcribed): GGGAAAUAAAGAGAGAAAAAGAAGAGUAAGAAGAAAU AUAAGAGCCACC <u>AUG</u>GUAUCCAAGGGGGAGGAGGACAACAUGGCGAUC AUCAAGGAGUUCAUGCGAUUCAAGGUGCACAUGGAA GGUUCGGUCAACGGACACGAAUUUGAAAUCGAAGGA GAGGGUGAAGGAAGGCCCUAUGAAGGGACACAGACC GCGAAACUCAAGGUCACGAAAGGGGGACCACUCCU UUCGCCUGGGACAUUCUUUCGCCCCAGUUUAUGUAC GGGUCCAAAGCAUAUGUGAAGCAUCCCGCCGAUAUU CCUGACUAUCUGAAACUCAGCUUUCGGAGGGAUUC AAGUGGGAGCGGGUCAUGAACUUUGAGGACGGGGG UGUAGUCACCGUAACCCAAGACUCAAGCCUCCAAGA CGGCGAGUUCAUCUACAAGGUCAAACUGCGGGGGAC UAACUUUCCGUCGGAUGGGCCGGUGAUGCAGAAGAA AACGAUGGGAUGGGAAGCGUCAUCGGAGAGGAUGU ACCCAGAAGAUGGUGCAUUGAAGGGGGAGAUAAGC AGAGACUGAAGUUGAAAGAUGGGGGACAUAUAUGAU GCCGAGGUGAAAACGACAUACAAAGCGAAAAAGCCG GUGCAGCUUCCCGGAGCGUAUAUAUGUGAAUAUCAAG UUGGAUAUUACUUCACACAAUGAGGACUACACAAUU GUCGAACAGUACGAACGCGCUGAGGGUAGACACUCG ACGGGAGGCAUGGACGAGUUGUACAAA UGAUAAUAG	5728

	UGCUCUUCAGUAGGGUCAUGAAGGUUUUUCUUUUC CUGAGAAAACAACACGUAUUGUUUUCUCAGGUUUU GCUUUUUGGCCUUUUUCUAGCUAAAAAAAAAAAAA AGCAAAAGUGGUCUUUGAAUAAAGUCUGAGUGGGC I GGC I	

[001024] These modified mRNA sequences can include at least one chemical modification described herein. The G-CSF or mCherry modified mRNA sequence can be formulated, using methods described herein and/or known in the art, prior to transfection and/or administration.

[001025] The modified mRNA sequence encoding G-CSF or mCherry can be transfected *in vitro* to various cell types such as HEK293, HeLa, PBMC and BJ fibroblast and those described in Table 25 using methods disclosed herein and/or are known in the art. The cells are then analyzed using methods disclosed herein and/or are known in the art to determine the concentration of G-CSF or mCherry and/or the cell viability.

Example 39. Utilization of heterologous 5'UTRs

[001026] A 5' UTR may be provided as a flanking region to the nucleic acids, modified nucleic acids or mmRNA of the invention. 5'UTR may be homologous or heterologous to the coding region found in the nucleic acids, modified nucleic acids or mmRNA of the invention. Multiple 5' UTRs may be included in the flanking region and may be the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical modifications, before and/or after codon optimization.

[001027] Shown in Lengthy Table 21 in US Provisional Application No 61/775,509, filed March 9, 2013, entitled Heterologous Untranslated Regions for mRNA and in Lengthy Table 21 in US Provisional Application No 61/829,372, filed May 31, 2013, entitled Heterologous Untranslated Regions for mRNA is a listing of the start and stop site of the polynucleotides, primary constructs or mmRNAs of the invention. Each 5'UTR (5'UTR-005 to 5'UTR 6851 1) is identified by its start and stop site relative to its native or wild type (homologous) transcript (ENST; the identifier used in the ENSEMBL database).

[001028] To alter one or more properties of the nucleic acids, modified nucleic acids or mmRNA of the invention, 5'UTRs which are heterologous to the coding region of the

nucleic acids, modified nucleic acids or mmRNA of the invention are engineered into compounds of the invention. The nucleic acids, modified nucleic acids or mmRNA are then administered to cells, tissue or organisms and outcomes such as protein level, localization and/or half life are measured to evaluate the beneficial effects the heterologous 5'UTR may have on the nucleic acids, modified nucleic acids or mmRNA of the invention. Variants of the 5' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G. 5'UTRs may also be codon-optimized or modified in any manner described herein.

Example 40. Further Utilization of 5' Untranslated Regions

[001029] A 5' UTR may be provided as a flanking region to the nucleic acids, modified nucleic acids or mmRNA of the invention. 5'UTR may be homologous or heterologous to the coding region found in the nucleic acids, modified nucleic acids or mmRNA of the invention. Multiple 5' UTRs may be included in the flanking region and may be the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical modifications, before and/or after codon optimization.

[001030] Shown in Table 38 is a listing of 5'-untranslated regions which may be used with the nucleic acids, modified nucleic acids or mmRNA of the present invention.

[001031] To alter one or more properties of the nucleic acids, modified nucleic acids or mmRNA of the invention, 5'UTRs which are heterologous to the coding region of the nucleic acids, modified nucleic acids or mmRNA of the invention are engineered into compounds of the invention. The nucleic acids, modified nucleic acids or mmRNA are then administered to cells, tissue or organisms and outcomes such as protein level, localization and/or half life are measured to evaluate the beneficial effects the heterologous 5'UTR may have on the nucleic acids, modified nucleic acids or mmRNA of the invention. Variants of the 5' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G. 5'UTRs may also be codon-optimized or modified in any manner described herein.

Table 38. 5'-Untranslated Regions

5' UTR Identifier	Name/Description	Sequence	SEQ ID NO.

5UTR-68512	Upstream UTR	GGGAGATCAGAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC	5729
5UTR-68513	Upstream UTR	GGAATAAAAGTCTCAACACAACATATACAAAACAAAC GAATCTCAAGCAATCAAGCATTCTACTTCTATTGCAGC AATTTAAATCATTTCTTTTAAAGCAAAAGCAATTTTCT GAAAATTTTCACCATTACGAACGATAGCAAC	5730
5UTR-68514	Upstream UTR	GGGAGACAAGCUUGGCAUCCGGUACUGUUGGUA GCCACC	5731
5UTR-68515	Upstream UTR	GGGAATTAACAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC	5732
5UTR-68516	Upstream UTR	GGGAAATTAGACAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC	5733
5UTR-68517	Upstream UTR	GGGAAATAAGAGAGTAAAGAACAGTAAGAAGAAATA TAAGAGCCACC	5734
5UTR-68518	Upstream UTR	GGGAAAAAGAGAGAAAAGAAGACTAAGAAGAAATA TAAGAGCCACC	5735
5UTR-68519	Upstream UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGATATA TAAGAGCCACC	5736
5UTR-68520	Upstream UTR	GGGAAATAAGAGACAAAACAAGAGTAAGAAGAAATA TAAGAGCCACC	5737
5UTR-68521	Upstream UTR	GGGAAATTAGAGAGTAAAGAACAGTAAGTAGAATTAA AAGAGCCACC	5738
5UTR-68522	Upstream UTR	GGGAAATAAGAGAGAATAGAAGAGTAAGAAGAAATA TAAGAGCCACC	5739
5UTR-68523	Upstream UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAAAT TAAGAGCCACC	5740
5UTR-68524	Upstream UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATT TAAGAGCCACC	5741

Example 41. Protein Production Using Heterologous 5'UTRs

[001032] The day before transfection, 20,000 HeLa cells (ATCC no. CCL-2; Manassas, VA) were harvested by treatment with Trypsin-EDTA solution (LifeTechnologies, Grand Island, NY) and seeded in a total volume of 100 μ l EMEM medium (supplemented with 10%FCS and 1x Glutamax) per well in a 96-well cell culture plate (Corning, Manassas, VA). The cells were grown at 37°C in 5% CO₂ atmosphere overnight. The next day, 37.5 ng, 75 ng or 150 of G-CSF modified RNA comprising a nucleic acid sequence for 5UTR-001 (mRNA sequence shown in SEQ ID NO: 5; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF modified RNA comprising a nucleic acid sequence for 5UTR-68515 (mRNA sequence shown in SEQ ID NO: 5732; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF modified RNA comprising a nucleic acid sequence for 5UTR-68516 (mRNA sequence shown in SEQ ID NO: 5733;

polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF modified RNA comprising a nucleic acid sequence for 5UTR-68521 (mRNA sequence shown in SEQ ID NO: 5738; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF modified RNA comprising a nucleic acid sequence for 5UTR-68522 (mRNA sequence shown in SEQ ID NO: 5739; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) were diluted in 10ul final volume of OPTI-MEM (LifeTechnologies, Grand Island, NY). Lipofectamine 2000 (LifeTechnologies, Grand Island, NY) was used as transfection reagent and 0.2 ul were diluted in 10 ul final volume of OPTI-MEM. After 5 minutes of incubation at room temperature, both solutions were combined and incubated an additional 15 minute at room temperature. Then the 20ul combined solution was added to the 100ul cell culture medium containing the HeLa cells and incubated at room temperature.

[001033] After an incubation of 24 hours cells expressing G-CSF were lysed with 100 ul of Passive Lysis Buffer (Promega, Madison, WI) according to manufacturer instructions. G-CSF protein production was determined by ELISA.

[001034] These results, shown in Table 39, demonstrate that G-CSF mRNA comprising the 5UTR-68515 or 5UTR-68521 produced the most protein whereas G-CSF mRNA comprising 5UTR-68522 produced the least amount of protein.

Table 39. G-CSF Protein Production from Heterologous 5'UTRs

5'UTR	G-CSF Protein (ng/ml)		
	37.5 ng	75 ng	150 ng
5UTR-001	131.3	191.1	696.1
5UTR-68515	245.6	394.3	850.3
5UTR-68516	188.6	397.4	719.6
5UTR-68521	191.4	449.1	892.1
5UTR-68522	135.9	331.3	595.6

Example 42. Effect of the Kozak sequence in modified nucleic acids

[001035] HeLa cells were seeded at a density of 15,000 per well in 100 ul cell culture medium DMEM+FBS 10%. G-CSF mRNA having a Kozak sequence (G-CSF Kozak; mRNA sequence shown in SEQ ID NO: 5004; polyA tail of approximately 140

nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA not having a Kozak sequence (G-CSF no Kozak; mRNA sequence shown in SEQ ID NO: 5008; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) and transfected in triplicate at a concentration of 75 ng per well in 96 well plates. 24 hours, 48 hours and 72 hours after transfection, the supernatant was collected and expression of G-CSF was measured by ELISA, and the results are shown in Table 40.

Table 40. G-CSF Expression

Time point	G-CSF Kozak	G-CSF No Kozak
	Protein Expression (ng/ml)	Protein Expression (ng/ml)
24 hours	223.93	408.23
48 hours	604.76	1217.29
72 hours	365.48	703.93

Example 43. Effect of the Altering the 5'UTR in modified nucleic acids

[001036] mRNA encoding a polypeptide of interest and having a polyA sequence and a cap, is fully modified with at least one chemistry described herein and/or in co-pending International Publication No WO2013052523, the contents of which are herein incorporated by reference in its entirety and the mRNA comprises a 5'UTR from Table 41. HeLa cells are seeded in cell culture medium and are transfected with the mRNA at a pre-determined concentration (ng per well) in well plates. At pre-determined intervals (e.g., 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 36 hours, 48 hours, 60 hours and/or 72 hours) after transfection, the supernatant is collected and expression of protein is measured by ELISA.

Table 41. 5' UTR

5' UTR Identifier	Name/Description	Sequence	SEQ ID NO.
5UTR-001	Synthetic UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAG AAATATAAGAGCCACC	5
5UTR-68525	Synthetic UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAG AAATATAAGAGCCTCC	5742
5UTR-	Synthetic	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAG	5743

68526	UTR	AAATATATGA	
5UTR- 68527	Synthetic UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAG AAATATA	5744

Example 44. Effect of the PolyA tail length in modified nucleic acids

A. Bioanalyzer

[001037] Modified G-CSF mRNA (mRNA sequence shown in SEQ ID NO: 5745; 5'cap, Cap 1 fully modified with 5-methylcytidine and 1-methylpseudouridine (5mc/lmpU) or fully modified with 1-methylpseudouridine (ImpU)), modified Factor IX (FIX) mRNA (mRNA sequence shown in SEQ ID NO: 5746; 5'cap, Cap 1 fully modified with 5-methylcytidine and 1-methylpseudouridine (5mc/lmpU) or fully modified with 1-methylpseudouridine (ImpU)), modified erythropoietin (EPO) mRNA (mRNA sequence shown in SEQ ID NO: 5747; 5'cap, Cap 1 fully modified with 5-methylcytidine and 1-methylpseudouridine (5mc/lmpU) or fully modified with 1-methylpseudouridine (ImpU)) or modified mCherry mRNA (mRNA sequence shown in SEQ ID NO: 5748; 5'cap, Cap 1 fully modified with 5-methylcytidine and 1-methylpseudouridine (5mc/lmpU) or fully modified with 1-methylpseudouridine (ImpU)) having a polyA tail of 20, 40, 80, 100, 120, 140 or 160 nucleotides in length or no polyA tail were analyzed by bioanalyzer (Agilent 2100 bioanalyzer). All samples, maintained integrity of the mRNA as determined by bioanalyzer.

B. BJ Fibroblasts

[001038] Human primary foreskin fibroblasts (BJ fibroblasts) are obtained from American Type Culture Collection (ATCC) (catalog # CRL-2522) and grown in Eagle's Minimum Essential Medium (ATCC, cat# 11095-1 14) supplemented with 10% fetal bovine serum at 37°C, under 5% CO₂. BJ fibroblasts are seeded on a 24-well plate at a density of 100,000 cells per well in 0.5 ml of culture medium. 250 ng of modified G-CSF mRNA (mRNA sequence shown in SEQ ID NO: 5745; 5'cap, Cap 1 fully modified with 5-methylcytidine and 1-methylpseudouridine (5mc/lmpU) or fully modified with 1-methylpseudouridine (ImpU)), modified Factor IX (FIX) mRNA (mRNA sequence shown in SEQ ID NO: 5746; 5'cap, Cap 1 fully modified with 5-methylcytidine and 1-methylpseudouridine (5mc/lmpU) or fully modified with 1-methylpseudouridine (ImpU)), modified erythropoietin (EPO) mRNA (mRNA sequence shown in SEQ ID

NO: 5747; 5'cap, Cap 1 fully modified with 5-methylcytidine and 1-methylpseudouridine (5mc/lmpU) or fully modified with 1-methylpseudouridine (ImpU)) or modified mCherry mRNA (mRNA sequence shown in SEQ ID NO: 5748; 5'cap, Cap 1 fully modified with 5-methylcytidine and 1-methylpseudouridine (5mc/lmpU) or fully modified with 1-methylpseudouridine (ImpU)) having a polyA tail of 20, 40, 80, 100, 120, 140 or 160 nucleotides in length or no polyA tail were transfected using Lipofectamine 2000, following manufacturer's protocol. FIX, G-CSF and EPO were transfected in triplicate.

[001039] The supernatant was collected at 24 hours, 48 hours and 72 hours for FIX, G-CSF and EPO and at 24 hours for mCherry. The protein expression was analyzed by ELISA for FIX, G-CSF and EPO and fluorescence-activated cell sorting (FACS) for mCherry. The results for G-CSF are shown in Table 42, the results for EPO are shown in Table 43, the results for FIX are shown in Table 44 and the results for mCherry are shown in Table 45.

Table 42. G-CSF Protein Expression

Description	PolyA Tail Length	Time Point	Protein (ng/ml)
G-CSF 5mC/lmpU	0	24	1.13
		48	0.39
		72	0.2
G-CSF ImpU	0	24	2
		48	0.3
		72	0.16
G-CSF 5mC/lmpU	20	24	41.85
		48	32.75
		72	13.38
G-CSF ImpU	20	24	204.43
		48	138.71
		72	96.36
G-CSF 5mC/lmpU	40	24	102.75
		48	101.96
		72	48.97
G-CSF ImpU	40	24	451.71
		48	373.75
		72	217.62
G-CSF 5mC/lmpU	80	24	135.85
		48	167.21
		72	96.66
G-CSF ImpU	80	24	534.89
		48	352.39
		72	203.89
G-CSF 5mC/lmpU	100	24	168.31
		48	195.16
		72	127.8

G-CSF ImpU	100	24	561
		48	406.8
		72	265.64
G-CSF 5mC/ImpU	120	24	152.54
		48	187.06
		72	100.41
G-CSF ImpU	120	24	656.23
		48	511.01
		72	239.95
G-CSF 5mC/ImpU	140	24	146.24
		48	202.05
		72	121.89
G-CSF ImpU	140	24	724.58
		48	627.6
		72	341.61
G-CSF 5mC/ImpU	160	24	59.83
		48	101.30
		72	64.69
G-CSF ImpU	160	24	814.54
		48	579.65
		72	274.7

Table 43. EPO Protein Expression

Description	PolyA Tail Length	Time Point	Protein (ng/ml)
EPO 5mC/ImpU	0	24	3.12
		48	0.13
		72	0
EPO ImpU	0	24	0.77
		48	0.07
		72	0.007
EPO 5mC/ImpU	20	24	48.93
		48	21.72
		72	5.88
EPO ImpU	20	24	199.24
		48	42.9
		72	20.29
EPO 5mC/ImpU	40	24	400.66
		48	165.38
		72	63.36
EPO ImpU	40	24	210
		48	182.56
		72	54.31
EPO 5mC/ImpU	80	24	368.09
		48	303.05
		72	117.98
EPO ImpU	80	24	422.95
		48	229.53
		72	131.05
EPO 5mC/ImpU	100	24	619.59
		48	366.19
		72	199.63
EPO ImpU	100	24	374.88

		48	240.21
		72	128.08
		24	430.64
EPO 5mC/ImpU	120	48	354.6
		72	165.72
		24	358.02
EPO ImpU	120	48	193.77
		72	104.89
		24	53.1
EPO 5mC/ImpU	140	48	426.96
		72	164.3
		24	355.96
EPO ImpU	140	48	202.27
		72	99.88
		24	608.66
EPO 5mC/ImpU	160	48	324.31
		72	181.94
		24	351.01
EPO ImpU	160	48	197.76
		72	109.64
		24	

Table 44. FIX Protein Expression

Description	PolyA Tail Length	Time Point	Protein (ng/ml)
FIX 5mC/ImpU	0	24	0.51
		48	1.14
		72	0.47
FIX ImpU	0	24	0.61
		48	0.39
		72	0.36
FIX 5mC/ImpU	20	24	0.92
		48	0.46
		72	0.49
FIX ImpU	20	24	5.97
		48	14.99
		72	7.47
FIX 5mC/ImpU	40	24	2.27
		48	1.62
		72	0.5
FIX ImpU	40	24	15.32
		48	41.92
		72	21.05
FIX 5mC/ImpU	80	24	7.12
		48	10.14
		72	3.66
FIX ImpU	80	24	35.32
		48	74.18
		72	38.47
FIX 5mC/ImpU	100	24	8.47
		48	13.33
		72	6.73
FIX ImpU	100	24	40.5
		48	90.56

		72	54.85
FIX 5mC/1mpU	120	24	10.06
		48	15.89
		72	6.2
		72	6.2
FIX ImpU	120	24	47.5
		48	106.55
		72	59.35
		72	59.35
FIX 5mC/1mpU	140	24	11.16
		48	20.13
		72	8.85
		72	8.85
FIX ImpU	140	24	46.44
		48	109.03
		72	60.17
		72	60.17
FIX 5mC/1mpU	160	24	13.06
		48	22.3 1
		72	10.19
		72	10.19
FIX ImpU	160	24	45.35
		48	99.47
		72	60.48
		72	60.48

Table 45. mCherry Expression

Description	PolyA Tail Length	Time Point	Expression index
mCherry 5mC/1mpU	0	24	445946.66
mCherry 1mpU	0	24	509423.33
mCherry 5mC/1mpU	20	24	510846.66
mCherry 1mpU	20	24	1688910
mCherry 5mC/1mpU	40	24	1443583.33
mCherry 1mpU	40	24	3398540
mCherry 5mC/1mpU	80	24	1949826.66
mCherry 1mpU	80	24	5783383.33
mCherry 5mC/1mpU	100	24	4963426.66
mCherry 1mpU	100	24	4639580
mCherry 5mC/1mpU	120	24	5372706.66
mCherry 1mpU	120	24	9184466.66
mCherry 5mC/1mpU	140	24	5127563.33
mCherry 1mpU	140	24	5273213.33
mCherry 5mC/1mpU	160	24	5627163.33
mCherry 1mpU	160	24	4876160

Example 45. Modified nucleic acids with a mir-122 sequence**A. HeLa Cells**

[001040] HeLa cells were seeded at a density of 15,000 per well in 100 ul cell culture medium (DMEM + 10%FBS). G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mRNA sequence shown in SEQ ID NO: 5024; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap 1; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA having a miR-122

sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 5028; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) were transfected with 0.3ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates. The supernatant was collected between 16-18 hours after transfection and expression of G-CSF was measured by ELISA, and the results are shown in Table 46.

Table 46. G-CSF Expression in HeLa

Description	Protein Expression (ng/ml)
G-CSF miR122	292.1
G-CSF seedless	335.7

B. *Primary Human and Rat Hepatocytes*

[001041] Primary human or rat hepatocytes cells were seeded at a density of 350,000 cells per well in 500 ul cell culture medium (*InvitroGRO* CP and *InVitroGRO* HI Medium + 2.2% Torpedo Antibiotic Mix). G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mRNA sequence shown in SEQ ID NO: 5024; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 5028; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) were transfected with 1 ul per well of Lipofectamine 2000 at a concentration of 500 ng of mRNA per well in 24 well plates for the primary human hepatocytes and the primary rat hepatocytes. The supernatant was collected between 16-18 hours after transfection and expression of G-CSF was measured by ELISA, and the results are shown in Table 47. The mir-122 binding site sequence in the mRNA dampened the G-CSF protein expression in the primary hepatocytes.

Table 47. G-CSF Expression in Hepatocytes

Description	Primary Human	Primary Rat Hepatocytes

	Hepatocytes	
	Protein Expression (ng/ml)	Protein Expression (ng/ml)
G-CSF miR122	116	26
G-CSF seedless	463	85

Example 46. Time Course of Modified nucleic acids with a mir-122 sequence

A. HeLa Cells

[001042] HeLa cells were seeded at a density of 17,000 per well in 100 ul cell culture medium (DMEM + 10%FBS). G-CSF mRNA without a miR-122 sequence in the 3'UTR (G-CSF; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5717; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5024; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5018; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 seed sequence in the 3'UTR (G-CSF seed; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5026; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5020; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5028; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5022; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI;

fully modified with 5-methylcytidine and 1-methylpseudouridine), Factor IX mRNA without a miR-122 sequence in the 3'UTR (FIX; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5719; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5718; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine), Factor IX mRNA having a miR-122 sequence in the 3'UTR (FIX miR122; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5036; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5030; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine), Factor IX mRNA having a miR-122 seed sequence in the 3'UTR (FIX seed; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5038; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5032; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) or Factor IX mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (FIX seedless; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5040; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5034; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) were transfected with 0.3 ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates. The supernatant was collected between 16-18 hours after transfection, expression of G-CSF or Factor IX was measured by ELISA, and the results are shown in Table 48.

Table 48. Expression in HeLa

Description	Protein Expression Mm 3'UTR (ng/ml)	Protein Expression Hs 3'UTR (ng/ml)

G-CSF	271.72	69.4
G-CSF miR122	305.36	68.8
G-CSF seed	209.5	98.0
G-CSF seedless	243.2	80.9
FIX	249.8	131.6
FIX mirl22	204.6	55.4
FIX seed	290.05	127.6
FIX seedless	180.9	31.6

B. *Primary Human and Rat Hepatocytes*

[001043] Primary human or rat hepatocytes cells were seeded at a density of 350,000 cells per well in 500 ul cell culture medium (*InvitroGRO* CP and *InVitroGRO* HI Medium + 2.2% Torpedo Antibiotic). G-CSF mRNA without a miR-122 sequence in the 3'UTR (G-CSF; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5717; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5024; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5018; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 seed sequence in the 3'UTR (G-CSF seed; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5026; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5020; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5028; polyA tail of approximately 140

nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5022; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine), Factor IX mRNA without a miR-122 sequence in the 3'UTR (FIX; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5719; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5718; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine), Factor IX mRNA having a miR-122 sequence in the 3'UTR (FIX miR122; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5036; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5030; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine), Factor IX mRNA having a miR-122 seed sequence in the 3'UTR (FIX seed; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5038; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5032; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) or Factor IX mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (FIX seedless; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 5040; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 5034; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) were transfected with 1 ul per well of Lipofectamine 2000 at a concentration of 500 ng per well in 24 well plates for the primary human hepatocytes and the primary rat hepatocytes. The supernatant was collected at 24 hours, 48 hours and 72 hours after transfection, expression of G-CSF and Factor IX was measured by ELISA, and the results

are shown in Table 49. The mir-122 binding site sequence in the mRNA dampened the G-CSF and Factor IX protein expression in the primary hepatocytes.

Table 49. G-CSF Expression in Hepatocytes

Description	Time Point	Primary Human Hepatocytes	Primary Human Hepatocytes
		Protein Expression (ng/ml) Mm 3'UTR	Protein Expression (ng/ml) Hs 3'UTR
G-CSF	24 hours	43.9	84.9
	48 hours	18.8	100.4
	72 hours	5.7	21.3
G-CSF miR122	24 hours	6.9	24.0
	48 hours	.7	3.03
	72 hours	.12	.88
G-CSF seed	24 hours	48.5	115.8
	48 hours	25.6	96.4
	72 hours	8.2	19.2
G-CSF seedless	24 hours	31.7	113.1
	48 hours	11.7	92.9
	72 hours	3.4	18.9
FIX	24 hours	90.8	63.2
	48 hours	159.6	124.8
	72 hours	70.5	44.3
FIX mir122	24 hours	11.8	15.9
	48 hours	5.0	4.4
	72 hours	1.0	.4
FIX seed	24 hours	77.2	60.2
	48 hours	115.0	63.0
	72 hours	41.7	20.1
FIX seedless	24 hours	69.3	53.7
	48 hours	123.8	75.0
	72 hours	49.0	24.5

Example 47. Time Course of Modified nucleic acids with a mir-122 sequence in cancer cells

A. Base Level of miR-122

[001044] The base level of mir-122 in Human hepatocytes, rat hepatocytes, human hepatocellular carcinoma cells (Hep3B) and HeLa cells were determined by TAQMAN® analysis using the manufacturers protocol. The levels were normalized to U6 and the results are shown in Table 50.

Table 50. miR-122 Levels in Various Cell Types

Cell Type	miR-122 level (normalized to U6)
Human Hepatocytes	16.8
Rat Hepatocytes	10.9
Hep3B	0
HeLa	0

B. Primary Human Hepatocytes and Hep3B Cells

[001045] Primary human hepatocytes were seeded at a density of 50,000 cells per well in 100 ul cell culture medium (*InvitroGRO* CP and *InVitroGRO* HI Medium + 2.2% Torpedo Antibiotic Mix) and Hep3B cells were seeded at a density of 20,000 cells per well in 100 ul cell culture medium MEM + 10%FBS. G-CSF mRNA without a miR-122 sequence in the 3'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 5745; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mRNA sequence shown in SEQ ID NO: 5018; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 seed sequence in the 3'UTR (G-CSF seed; mRNA sequence shown in SEQ ID NO: 5020; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 5022; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) were transfected with 0.3 ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates for the primary human hepatocytes and the Hep3B cells. The supernatant was collected at 24 hours, 48 hours and 72 hours after transfection, expression of G-CSF was measured by ELISA, and the results are shown in Table 51. The mir-122 binding site sequence in the mRNA dampened the G-CSF protein expression in the primary human hepatocytes but not in the Hep3B cells.

Table 51. G-CSF Expression

Description	Time Point	Primary Human Hepatocytes	Hep3B
		Protein Expression (ng/ml) Hs 3'UTR	Protein Expression (ng/ml) Hs 3'UTR
G-CSF	24 hours	76	55
	48 hours	12	33
	72 hours	6	10
G-CSF miR122	24 hours	32	37
	48 hours	1	27
	72 hours	0	6
G-CSF seed	24 hours	75	39
	48 hours	11	28
	72 hours	4	6
G-CSF seedless	24 hours	79	49
	48 hours	15	35
	72 hours	6	9

Example 48. Time Course of Modified nucleic acids with a mir-142 3p sequence**A. Base Level of miR-143 3p**

[001046] The base level of miR-142 3p in RAW264.7 cells and HeLa cells were determined by TAQMAN® analysis using the manufacturer's protocol. The levels were normalized to U6 and the results are shown in Table 52.

Table 52. miR-142 3p Levels in Various Cell Types

Cell Type	miR-122 level (normalized to U6)
Human Hepatocytes	16.8
Rat Hepatocytes	10.9
Hep3B	0
HeLa	0

B. HeLa and RAW264.7 Cells

[001047] HeLa cells were seeded at a density of 17,000 per well in 100 ul cell culture medium DMEM + 10%FBS and RAW264.7 cells were seeded at a density of 200,000 per well in 100 ul cell culture medium DMEM + 10%FBS. G-CSF mRNA without a miR-142 3p sequence in the 3'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 5749; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1;

fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p sequence in the 3'UTR (G-CSF miR142 3p; mRNA sequence shown in SEQ ID NO: 5750; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p seed sequence in the 3'UTR (G-CSF seed; mRNA sequence shown in SEQ ID NO: 5751; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capI; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA having a miR-142 3p sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 5752; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) were transfected with 0.3ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates for HeLa or with 1 ul per well of Lipofectamine 2000 at a concentration of 250 ng of mRNA per well in 24 well plates for RAW264.7 cells. The supernatant was collected 16-18 hours after transfection, expression of G-CSF was measured by ELISA, and the results are shown in Table 53. miR-142 3p sites in G-CSF were shown to down-regulate G-CSF expression in RAW264.7 cells.

Table 53. Expression

Description	HeLa Protein Expression (ng/ml)	RAW264.7 Protein Expression (ng/ml)
G-CSF	243.5	124.8
G-CSF miR142 3p	309.1	42.8
G-CSF seed	259.8	148.1
G-CSF seedless	321.7	185.2

C. Time Course in RAW264.7 Cells

[001048] RAW264.7 cells were seeded at a density of 60,000 cells per well in 100 ul cell culture medium (DMEM + 10%FBS). G-CSF mRNA without a miR-142 3p sequence in the 3'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 5749; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p sequence in the 3'UTR (G-CSF miR142 3p; mRNA sequence shown in SEQ ID NO:

5750; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p seed sequence in the 3'UTR (G-CSF seed; mRNA sequence shown in SEQ ID NO: 5751; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA having a miR-142 3p sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 5752; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) were transfected with 0.3 ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates. The supernatant was collected at 24 hours, 48 hours and 72 hours after transfection, expression of G-CSF was measured by ELISA, and the results are shown in Table 54. The mir-142 3p binding site sequence in the mRNA showed a strong suppression of G-CSF expression in RAW264.7 cells over time.

Table 54. G-CSF Expression

Description	Time Point	RAW264.7 Cells
		Protein Expression (ng/ml)
G-CSF	24 hours	133.5
	48 hours	69.7
	72 hours	2.1
G-CSF miR142 3p	24 hours	60.1
	48 hours	9.2
	72 hours	.3
G-CSF seed	24 hours	244.9
	48 hours	68.9
	72 hours	2.3
G-CSF seedless	24 hours	250.2
	48 hours	95.9
	72 hours	3.0

D. *miR-142 3D in PBMC*

[001049] Peripheral blood mononuclear cells (PBMCs) were seeded at a density of 150,000 cells per well in 100 ul cell culture medium (Opti-MEM and after transfection add 10%FBS). G-CSF mRNA having a miR-142 3p sequence in the 3'UTR (G-CSF miR142 3p; mRNA sequence shown in SEQ ID NO: 5750; polyA tail of approximately

140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p seed sequence in the 3'UTR (G-CSF seed; mRNA sequence shown in SEQ ID NO: 5751; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA having a miR-142 3p sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 5752; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) were transfected in triplicate with 0.4 ul per well of Lipofectamine 2000 at a concentration of 500 ng of mRNA per well in 96 well plates for 2 or 3 donors. The supernatant was collected at 24 hours after transfection and the expression of G-CSF was measured by ELISA. The results for the 2 donors are shown in Table 55 and the results for the 3 donors are shown in Table 56. The mir-142 3p binding site sequence in the mRNA was shown to down regulate G-CSF expression in human PBMC.

Table 55. Expression PBMC (2 donors)

Description	Protein Expression (ng/ml)
G-CSF miR142 3p	5.09
G-CSF seed	10.06
G-CSF seedless	9.38

Table 56. Expression PBMC (3 donors)

Description	Protein Expression (ng/ml)
G-CSF miR142 3p	7.48
G-CSF seed	13.40
G-CSF seedless	13.98

Example 49. In Vivo Expression of Modified mRNA

A. BALB/C nude mice

[001050] BALB/c nude mice were injected intravenously with 0.1 mg/kg luciferase modified mRNA without a miR-122 binding site ("non-targeted mRNA"; mRNA sequence shown in SEQ ID NO: 5753; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine) formulated in a lipid nanoparticle described in Table 57 or luciferase modified mRNA with a miR-122 binding site in the 3'UTR ("miR-122 targeted

mRNA"; mRNA sequence shown in SEQ ID NO: 5754; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) formulated in a lipid nanoparticle described in Table 58.

Table 57. Lipid Nanoparticle for Non-targeted mRNA

LNP	Luciferase: non-targeted mRNA
Lipid	DLin-KC2-DMA
Lipid/RNA wt/wt	20
Mean size	73.3 nm PDI: 0.06

Table 58. Lipid Nanoparticle for Targeted mRNA

LNP	Luciferase: targeted mRNA
Lipid	DLin-KC2-DMA
Lipid/RNA wt/wt	20
Mean size	70.6 nm PDI: 0.08

[001051] 24 hours post-treatment, animals were anesthetized, injected with the luciferase substrate D-luciferin and the bioluminescence imaging (BLI) from living animals was evaluated in an IVIS imager 15 minutes later. Signals were obtained from animals injected with non-targeted mRNA and from miR-122 targeted mRNA, and presented in Table 59. The total light signal produced from livers of animals treated with miR 122 targeted mRNA is 29x lower than non-targeted mRNA, showing that the engineered element in the 3'UTR may inhibit protein expression in normal tissue.

Table 59. *In vivo* expression of modified mRNA modulated by an engineered miR122 binding site

Description	Luciferase signal from liver (photons/sec)
Non-targeted mRNA	7.9×10^7
miR-122 targted mRNA	2.7×10^6

B. BALB/c nude mice with hepatocellular carcinoma Hep3B cells

[001052] BALB/c nude mice were intrahepatically implanted with 2×10^6 hepatocellular carcinoma Hep3B cells and resulting orthotopic tumors allowed to grow for 24 days. Tumor-bearing mice were then intravenously injected with 0.1 mg/kg luciferase modified

mRNA without a miR-122 binding site ("non-targeted mRNA"; mRNA sequence shown in SEQ ID NO: 5753; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) or luciferase modified mRNA with a miR-122 binding site in the 3'UTR ("miR-122 targeted mRNA"; mRNA sequence shown in SEQ ID NO: 5754; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) formulated in a lipid nanoparticle described in Table 57 and 58 (above). 24 hr post-treatment animals were anesthetized, injected with the luciferase substrate D-luciferin and bioluminescence imaging (BLI) from living animals was evaluated in an IVIS imager 20 minutes later. Signal from orthotopic tumors compared to adjacent normal liver was quantified, and miR-122-targeted mRNA systemically delivered via lipid nanoparticles achieved over 2-fold enrichment in tumor compared to normal liver.

Example 50. Effect of the Kozak sequence in modified nucleic acids

[001053] HeLa cells were seeded at a density of 15,000 per well in 100 ul cell culture medium DMEM+FBS 10%. G-CSF mRNA having a Kozak sequence (G-CSF Kozak; mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA not having a Kozak sequence (G-CSF no Kozak; mRNA sequence shown in SEQ ID NO: 5008; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA where the -3 position A, upstream of the start codon, was converted to a T, (G-CSF 3t5'; mRNA sequence shown in SEQ ID NO: 5755 (Table 60); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine), G-CSF mRNA where the -9 position A, upstream of the start codon, was converted to a T, (G-CSF 9t5'; mRNA sequence shown in SEQ ID NO: 5756 (Table 60); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA where 9 nucleotides upstream of the start codon (AGAGCCACC) were deleted (G-CSF 9del5'; mRNA sequence shown in SEQ ID NO: 5757 (Table 60); polyA tail of approximately 140

nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) and transfected in triplicate at a concentration of 37.5 ng per well in 96 well plates. 24 hours, 48 hours and 72 hours after transfection, the supernatant was collected and expression of G-CSF was measured by ELISA, and the results are shown in Table 61. In Table 60, the start codon in each sequence is underlined. In Table 60, for G-CSF 3t5' the -3 position A, upstream of the start codon is in bold and underlined and for G-CSF 9t5' the -9 position A upstream of the start codon is in bold and underlined.

Table 60. G-CSF Sequences

Description	Sequence	SEQ ID NO
G-CSF 3t5'	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAA UAUAAGAGCC <u>UCCAUGG</u> CCGGUCCCGCGACCCAAA GCCCCAUGAAACUUAUGGCCCUUGCAGUUGCUGCU UUGGCACUCGGCCCUUGGACAGUCCAAGAAGCG ACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUC AUUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGC UCUGCGCGACAUAACAAACUUUGCCAUCCCGAGGA GCUCGUACUGCUCGGGCACAGCUUGGGGAUUCCC UGGGCUCCUCUCUGUCCUGUCCGUCGCAGGCUUU GCAGUUGGCAGGGUGCCUUUCCCAGCUCCACUCCG GUUUGUUCUUGUAUCAGGGACUGCUGCAAGCCCU UGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAA CAACCAUCUGGCAGCAGAUGGAGGAACUGGGGAU GGCACCCGCGCUGCAGCCACGCAGGGGGGCAAUGC CGGCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGG UGGAGUCCUCGUAGCGAGCCACCUUCAAUCAUUU UUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUG CGCAGCCGUGAUAAUAGGCUGGAGCCUCGGUGGC CAUGCUCUUGCCCCUUGGGCCUCCCCCAGCCCC UCCUCCCCUUCUGCACCCGUACCCCGUGGUCUU UGAAUAAAGUCUGAGUGGGCGGC	5755
G-CSF 9t5'	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAA UAUA <u>UGAGCCACCAUGG</u> CCGGUCCCGCGACCCAAA GCCCCAUGAAACUUAUGGCCCUUGCAGUUGCUGCU UUGGCACUCGGCCCUUGGACAGUCCAAGAAGCG ACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUC AUUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAG AUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGC	5756

	UCUGCGCGACAUACAAACUUUGCCAUCCCGAGGA GCUCGUACUGCUCGGGCACAGCUUGGGGAUUCCC UGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUU GCAGUUGGCAGGGUGCCUUUCCCAGCUCCACUCCG GUUUGUUCUUGUAUCAGGGACUGCUGCAAGCCCU UGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUG GACACGUUGCAGCUCGACGUGGCGGAUUUCGCAA CAACCAUCUGGCAGCAGAUGGAGGAACUGGGGAU GGCACCCGCGCUGCAGCCACGCAGGGGGCAAUGC CGGCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGG UGGAGUCCUCGUAGCGAGCCACCUUCAUAUUU UUUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUG CGCAGCCGUGAUAAUAGGCUGGAGCCUCGGUGGC CAUGCUCUUGCCCCUUGGGCCUCCCCCAGCCCC UCCUCCCCUCCUGCACCCGUACCCCGUGGUCUU UGAAUAAAGUCUGAGUGGGCGGC	
G-CSF 9del5'	GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAA UAUA <u>AUG</u> GGCCGGUCCCGCGACCCAAAGCCCCAUGA AACUUAUGGCCCUGCAGUUGCUGCUUUGGCACUC GGCCCUCUGGACAGUCCAAGAAGCGACUCCUCUCG GACCUGCCUCAUCGUUGCCGCAGUCAUUCCUUUU GAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGC GAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGA CAUACAAACUUUGCCAUCCCGAGGAGCUCGUACU GCUCGGGCACAGCUUGGGGAUUCCCUGGGCUCCU CUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUGG CAGGGUGCCUUUCCCAGCUCCACUCCGGUUUGUUC UUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGAA UCUCGCCAGAAUUGGGCCCGACGCUGGACACGUU GCAGCUCGACGUGGCGGAUUUCGCAACAACCAUC UGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCG CGCUGCAGCCCACGCAGGGGGCAAUGCCGGCCUUU GCGUCCGCGUUUCAGCGCAGGGCGGGUGGAGUCC UCGUAGCGAGCCACCUUCAAUCAUUUUUGGAAGU CUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAGGCUGGAGCCUCGGUGGCAUGCUUC UUGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCCC UUCUGCACCCGUACCCCGUGGUCUUUGAAUAA AGUCUGAGUGGGCGGC	5757

Table 61. G-CSF Expression

	24 hours	48 hours	72 hours
	Protein Expression (ng/ml)	Protein Expression (ng/ml)	Protein Expression (ng/ml)

G-CSF Kozak	239.08	339.89	283.43
G-CSF No Kozak	399.83	544.08	437.23
G-CSF 3t5'	157.39	239.67	195.20
G-CSF 9t5'	171.84	263.1 1	195.22
G-CSF 9del5'	308.16	563.64	397.20

Example 51. Effect of modification of 5'UTR in modified nucleic acids

[001054] BJ Fibroblast cells were seeded at a density of 100,000 per well in 500 ul cell culture medium EMEM+FBS 10%. G-CSF mRNA having a synthetic 5'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA containing a 5'UTR with five tandem repeats of an 18 nucleotide sequence from the IRES of the GTX gene (GTX G-CSF; mRNA sequence shown in SEQ ID NO: 5758 (Table 62); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) and transfected in triplicate at a concentration of 250 ng per well in 24 well plates. 24 hours, 48 hours and 72 hours after transfection, the supernatant was collected and expression of G-CSF was measured by ELISA, and the results are shown in Table 63. In Table 62, the start codon is underlined and the five tandem repeats of an 18 nucleotide sequence from the IRES of the GTX gene is bolded and the first, third and fifth tandem repeat of the 18 nucleotide sequence is also underlined.

Table 62. GTX G-CSF Sequence

Description	Sequence	SEQ ID NO
GTX G-CSF	GGGA <u>AAUUCUGACA</u> UCCGGCGGA <u>AAUUCUGACA</u> UCCGGCGGA <u>AAUUCUGACA</u> UCCGGCGGA <u>AAUUCUGACA</u> UCCGGCGGAAGACUCACAACCCAGAAACAGACAUUAAGAGAGAAAA GAAGAGUAAGAAGAAUAUAAGAGCCACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAAGAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUUCCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUAACAAACU	5758

	UUGCCAUCCCGAGGAGCUCGUACUGCUCGGGCAC AGCUUGGGGAUUCCUGGGCUCCUCUCUCGUCCU GUCCGUCGCAGGCUUUGCAGUUGGCAGGGUGCCU UUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCAG GGACUGCUGCAAGCCCUUGAGGGAAUCUCGCCAG AAUUGGGCCCGACGCUGGACACGUUGCAGCUCGA CGUGGCGGAUUUCGCAACAACCAUCUGGCAGCAG AUGGAGGAACUGGGGAUGGCACCCGCGCUGCAGC CCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCG UUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGA GCCACCUUCAUAUUAUUUGGAAGUCUCGUACCG GGUGCUGAGACAUCUUGCGCAGCCGUGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCU GGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCAC CCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAGU GGGCGGC	

Table 63. 5' UTR

Time point	G-CSF	Gtx G-CSF
	Protein Expression (ng/ml)	Protein Expression (ng/ml)
24 hours	26.13	79.65
48 hours	138.75	444.81
72 hours	55.37	198.14

Example 53. Effect of modification of 5'UTR in modified nucleic acids

[001055] BJ Fibroblast cells were seeded at a density of 100,000 per well in 500 ul cell culture medium EMEM+FBS 10%. G-CSF mRNA having a synthetic 5'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 1-methylpseudouridine) or G-CSF mRNA containing a 5'UTR with five tandem repeats of an 18 nucleotide sequence from the IRES of Gtx gene (Gtx G-CSF; mRNA sequence shown in SEQ ID NO: 5758 (Table 62); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 1-methylpseudouridine) and transfected in triplicate at a concentration of 250 ng per well in 24 well plates. 24 hours, 48 hours and 72 hours after transfection, the supernatant was collected and expression of G-CSF was measured by ELISA, and the results are shown in Table 64.

Table 64. 5' UTR

Time point	G-CSF	Gtx G-CSF
	Protein Expression (ng/ml)	Protein Expression (ng/ml)
24 hours	129.10	178.68
48 hours	569.97	1067.62
72 hours	325.16	738.30

Example 54. *In vivo* effect of the modification of 5'UTR in nucleic acids modified with 5-methylcytidine and 1-methylpseudouridine

[001056] To study the effect of the modification of the 5'UTR in modified nucleic acids female Balb/c mice (n=3; 12 weeks old; Harlan Laboratories (South Easton, MA)) were treated with lipoplexed mRNA.

[001057] 8 ug of G-CSF mRNA having a synthetic 5'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA where 9 nucleotides upstream of the start codon were deleted (G-CSF 9del5'; mRNA sequence shown in SEQ ID NO: 5757 (Table 60); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, CapI; fully modified with 5-methylcytidine and 1-methylpseudouridine) for treatment of 3 mice is diluted with sterile and serum-free DMEM (Life Technologies) to obtain a total volume of 200ul. A total of 8 ul of Lipofectamine2000 (LifeTechnologies, 11668019) for the treatment of 3 mice was diluted with sterile and serum-free DMEM (LifeTechnologies, Grand Island, NY; 11965-118) to obtain a total volume of 200 ul. After 5 minutes of incubation, the two solutions were combined and carefully mixed with a pipette. After 20 minutes the formation of mRNA-Lipofectamine2000 lipoplexes was completed. The lipoplex solution was transferred to a sterile 1ml syringe (BD Falcon) carrying a 27gauge injection needle (0.3mL BD SafetyGlide insulin syringe with 29 G x ½ in BD permanently attached needle (Catalog #305935)). The Balb/C mice were placed under a heat lamp for 5 minutes prior to the 100ul intravenous tail vein injection containing 2 ug of lipoplexed mRNA. 6 hours after injection the mice were anesthetized and bleed for serum collection by cardiac puncture. The serum samples were then run on a G-CSF ELISA (R&D

systems catalog #SCS50) and the results are shown in Table 65. The G-CSF mRNA having the 9 nucleotides upstream of the start codon deleted had a higher G-CSF expression level at 6 hours as compared to the G-CSF having a synthetic UTR.

Table 65. G-CSF Kozak Expression *In Vivo*

Time Point	G-CSF Expression (ng/ml)	G-CSF 9del5' Expression (ng/ml)
6 hours	256.2	752.4

Example 55. *In vivo* effect of the GTX modification of 5'UTR in G-CSF nucleic acids modified with 5-methylcytidine and 1-methylpseudouridine

[001058] To study the effect of the modification of the 5'UTR in modified nucleic acids female Balb/c mice (n=3; 12 weeks old; Harlan Laboratories (South Easton, MA)) were treated with lipoplexed mRNA.

[001059] 8 ug of G-CSF mRNA having a synthetic 5'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) or G-CSF mRNA containing a 5'UTR with five tandem repeats of an 18 nucleotide sequence from the IRES of GTX gene (GTX G-CSF; mRNA sequence shown in SEQ ID NO: 5758 (Table 62); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 5-methylcytidine and 1-methylpseudouridine) for treatment of 3 mice is diluted with sterile and serum-free DMEM (Life Technologies) to obtain a total volume of 200ul. A total of 8 ul of Lipofectamine2000 (LifeTechnologies, 11668019) for the treatment of 3 mice was diluted with sterile and serum-free DMEM (LifeTechnologies, Grand Island, NY; 11965-1 18) to obtain a total volume of 200 ul. After 5 minutes of incubation, the two solutions were combined and carefully mixed with a pipette. After 20 minutes the formation of mRNA-Lipofectamine2000 lipoplexes was completed. The lipoplex solution was transferred to a sterile 1ml syringe (BD Falcon) carrying a 27gauge injection needle (0.3mL BD SafetyGlide insulin syringe with 29 G x ½ in BD permanently attached needle (Catalog #305935)). The Balb/C mice were placed under a heat lamp for 5 minutes prior to the 100ul intravenous tail vein injection containing 2 ug of lipoplexed mRNA. 6 hours after injection the mice were anesthetized and bleed for serum collection by cardiac puncture.

The serum samples were then run on a G-CSF ELISA (R&D systems catalog #SCS50) and the results are shown in Table 66. The G-CSF mRNA having five tandem repeats of an 18 nucleotide sequence from the IRES of GTX gene had a higher G-CSF expression level at 6 hours as compared to the G-CSF having a synthetic UTR.

Table 66. GTX G-CSF Expression *In Vivo*

Time Point	G-CSF Expression (ng/ml)	GTX G-CSF Expression (ng/ml)
6 hours	266.4	1284.4

Example 56. *In vivo* effect of the GTX modification of 5'UTR in G-CSF nucleic acids modified with 1-methylpseudouridine

[001060] To study the effect of the modification of the 5'UTR in modified nucleic acids female Balb/c mice (n=3; 12 weeks old; Harlan Laboratories (South Easton, MA)) were treated with lipoplexed mRNA.

[001061] 8 ug of G-CSF mRNA having a synthetic 5'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 5716; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 1-methylpseudouridine) or G-CSF mRNA containing a 5'UTR with five tandem repeats of an 18 nucleotide sequence from the IRES of GTX gene (GTX G-CSF; mRNA sequence shown in SEQ ID NO: 5758 (Table 62); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Cap1; fully modified with 1-methylpseudouridine) for treatment of 3 mice is diluted with sterile and serum-free DMEM (Life Technologies) to obtain a total volume of 200ul. A total of 8 ul of Lipofectamine2000 (LifeTechnologies, 11668019) for the treatment of 3 mice was diluted with sterile and serum-free DMEM (LifeTechnologies, Grand Island, NY; 11965-118) to obtain a total volume of 200 ul. After 5 minutes of incubation, the two solutions were combined and carefully mixed with a pipette. After 20 minutes the formation of mRNA-Lipofectamine2000 lipoplexes was completed. The lipoplex solution was transferred to a sterile 1ml syringe (BD Falcon) carrying a 27gauge injection needle (0.3mL BD SafetyGlide insulin syringe with 29 G x ½ in BD permanently attached needle (Catalog #305935)). The Balb/C mice were placed under a heat lamp for 5 minutes prior to the 100ul intravenous tail vein injection containing 2 ug of lipoplexed mRNA. 6 hours after injection the mice were anesthetized and bleed for serum collection

by cardiac puncture. The serum samples were then run on a G-CSF ELISA (R&D systems catalog #SCS50) and the results are shown in Table 67. The G-CSF mRNA having five tandem repeats of an 18 nucleotide sequence from the IRES of GTX gene had a higher G-CSF expression level at 6 hours as compared to the G-CSF having a synthetic UTR.

Table 67. GTX G-CSF Expression *In Vivo*

Time Point	G-CSF Expression (ng/ml)	GTX G-CSF Expression (ng/ml)
6 hours	5638.2	6281.1

OTHER EMBODIMENTS

[001062] It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the invention in its broader aspects.

[001063] While the present invention has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

All publications, patent applications, patents, databases, database entries, other references and art mentioned herein are incorporated by reference in their entirety, even if not expressly stated in the citation. In case of conflict, the present specification, including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

Claims

1. A synthetic isolated terminally optimized mRNA comprising
 - (a) a first region of linked nucleosides encoding a polypeptide of interest;
 - (b) a first terminal region located 5' relative to said first region comprising at least one translation enhancer element (TEE);
 - (c) a second terminal region located 3' relative to said first region; and
 - (d) a 3' tailing region of linked nucleosides.
2. The synthetic isolated terminally optimized mRNA of claim 1, wherein any of the regions (a) - (d) comprise at least one modified nucleoside
3. The synthetic isolated terminally optimized mRNA of claim 2, wherein the at least one modified nucleoside is selected from the group consisting of pseudouridine analogs.
4. The synthetic isolated terminally optimized mRNA of claim 3, wherein the pseudouridine analog is 1-methylpseudouridine.
5. The synthetic isolated terminally optimized mRNA of claim 4, further comprising the modified nucleoside 5-methylcytidine.
6. The synthetic isolated terminally optimized mRNA of claim 1, wherein at least one region of the synthetic isolated terminally optimized mRNA is codon optimized.
7. The synthetic isolated terminally optimized mRNA of claim 6, wherein the first region of linked nucleosides is codon optimized.
8. The synthetic isolated terminally optimized mRNA of claim 1, wherein the first terminal region comprises a 5' untranslated region (UTR).
9. The synthetic isolated terminally optimized mRNA of claim 8, wherein the 5' UTR is the native 5'UTR of the encoded polypeptide of interest.
10. The synthetic isolated terminally optimized mRNA of claim 9, wherein the 5'UTR comprises a translation initiation sequence selected from the group consisting of Kozak sequence and an internal ribosome entry site (IRES).
11. The synthetic isolated terminally optimized mRNA of claim 9, wherein the 5'UTR is a structured UTR.

12. The synthetic isolated terminally optimized mRNA of claim 1, wherein the first terminal region comprises at least one 5' cap structure.
13. The synthetic isolated terminally optimized mRNA of claim 12, wherein the at least one 5' cap structure is selected from the group consisting of CapO, Cap1, ARCA, inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azido-guanosine, Cap2, Cap4, and CAP-003 - CAP-225.
14. The synthetic isolated terminally optimized mRNA of claim 1, wherein the TEE is selected from the group of TEE-001 - TEE-705.
15. The synthetic isolated terminally optimized mRNA of claim 1, wherein the second terminal region comprises at least one microRNA binding site or seed of said microRNA binding site
16. The synthetic isolated terminally optimized mRNA of claim 15, wherein the at least one microRNA binding site or seed of said microRNA binding site is for an immune cell specific microRNA.
17. The synthetic isolated terminally optimized mRNA of claim 16, wherein the immune cell specific microRNA is selected from the group consisting of mir-122, miR-142-3p, miR-142-5p, miR-146a and miR-146b.
18. The synthetic isolated terminally optimized mRNA of claim 1, wherein the 3' tailing region of linked nucleosides further comprises a chain terminating nucleoside.
19. The synthetic isolated terminally optimized mRNA of claim 18, wherein the chain terminating nucleoside is selected from the group consisting of 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, 2',3'- dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'- dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, and -O- methylnucleoside.
20. The synthetic isolated terminally optimized mRNA of claim 1, wherein the 3' tailing region comprises a stem loop sequence.
21. A synthetic isolated terminally optimized mRNA comprising
 - (a) a first region of linked nucleosides encoding a polypeptide of interest;

- (b) a first terminal region located 5' relative to said first region;
 - (c) a second terminal region located 3' relative to said first region;
 - (d) a 3' tailing region of linked nucleosides; and
 - (e) at least one chain terminating nucleoside located 3' relative to said synthetic isolated terminally optimized mRNA.
22. The synthetic isolated terminally optimized mRNA of claim 21, wherein any of the regions (a) - (e) comprise at least one modified nucleoside
23. The synthetic isolated terminally optimized mRNA of claim 22, wherein the at least one modified nucleoside is selected from the group consisting of pseudouridine analogs.
24. The synthetic isolated terminally optimized mRNA of claim 23, wherein the pseudouridine analog is 1-methylpseudouridine.
25. The synthetic isolated terminally optimized mRNA of claim 24, further comprising the modified nucleoside 5-methylcytidine.
26. The synthetic isolated terminally optimized mRNA of claim 21, wherein at least one region of the synthetic isolated terminally optimized mRNA is codon optimized.
27. The synthetic isolated terminally optimized mRNA of claim 26, wherein the first region of linked nucleosides is codon optimized.
28. The synthetic isolated terminally optimized mRNA of claim 21, wherein the first terminal region comprises a 5' untranslated region (UTR).
29. The synthetic isolated terminally optimized mRNA of claim 28, wherein the 5' UTR is the native 5'UTR of the encoded polypeptide of interest.
30. The synthetic isolated terminally optimized mRNA of claim 28, wherein the 5'UTR is a structured UTR.
31. The synthetic isolated terminally optimized mRNA of claim 21, wherein the first terminal region comprises at least one 5' cap structure.
32. The synthetic isolated terminally optimized mRNA of claim 31, wherein the at least one 5' cap structure is selected from the group consisting of CapO, CapI, ARCA, inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-

- oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azido-guanosine, Cap2, Cap4, and CAP-003 - CAP-225.
33. The synthetic isolated terminally optimized mRNA of claim 21, wherein the 5'UTR comprises a translation initiation sequence selected from the group consisting of Kozak sequence and an internal ribosome entry site (IRES).
34. The synthetic isolated terminally optimized mRNA of claim 21, wherein the at least one chain terminating nucleoside is selected from the group consisting of 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, 2',3'-dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'-dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, and -O-methylnucleoside.
35. The synthetic isolated terminally optimized mRNA of claim 21, wherein the 3' tailing region comprises a stem loop sequence.
36. A method of reducing antigen-mediated immune response in an organism comprising contacting the organism with a synthetic isolated terminally optimized mRNA, said synthetic isolated terminally optimized mRNA comprising
- (a) a first region of linked nucleosides encoding said antigen;
 - (b) a first terminal region located 5' relative to said first region;
 - (c) a second terminal region located 3' relative to said first region comprising at least one microRNA binding site or seed of said microRNA binding site; and
 - (d) a 3' tailing region of linked nucleosides.
37. The method of claim 36, wherein any of the regions (a) - (d) comprise at least one modified nucleoside.
38. The method of claim 37, wherein the at least one modified nucleoside is selected from the group consisting of pseudouridine analogs.
39. The method of claim 38, wherein the pseudouridine analog is 1-methylpseudouridine.
40. The method of claim 39, wherein the synthetic isolated terminally optimized mRNA further comprises the modified nucleoside 5-methylcytidine.

41. The method of claim 36, wherein any of the regions (a) - (d) are codon optimized.
42. The method of claim 41, wherein the first region of linked nucleosides is codon optimized.
43. The method of claim 36, wherein the first terminal region comprises a 5' untranslated region (UTR).
44. The method of claim 42, wherein the 5' UTR is the native 5'UTR of the encoded antigen.
45. The method of claim 43, wherein the 5'UTR is a structured UTR.
46. The method of claim 43, wherein the 5'UTR comprises at least one translation initiation sequence selected from the group consisting of Kozak sequence, an internal ribosome entry site (IRES) or a fragment thereof.
47. The method of claim 46, wherein the 5'UTR comprises at least one fragment of an IRES.
48. The method of claim 47, wherein the 5'UTR comprises five fragments of an IRES.
49. The method of claim 36, wherein the first terminal region comprises at least one 5' cap structure.
50. The method of claim 49, wherein the at least one 5' cap structure is selected from the group consisting of CapO, Cap1, ARCA, inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azido-guanosine, Cap2, Cap4, and CAP-003 - CAP-225.
51. The method of claim 36, wherein the at least one microRNA binding site or seed of said microRNA binding site is an immune cell specific microRNA.
52. The method of claim 50, wherein the immune cell specific microRNA is selected from the group consisting of mir-122, miR-142-3p, miR-142-5p, miR-146a and miR-146b.
53. The method of claim 36, wherein first terminal region comprises at least one translation enhancer element (TEE) sequence.
54. The method of claim 36, wherein the 3'tailing region comprises a chain terminating nucleoside.

55. The method of claim 54, wherein the chain terminating nucleoside is selected from the group consisting of 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, 2',3'- dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'-dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, and -O-methylnucleoside.
56. The method of claim 36, wherein the 3' tailing region comprises a stem loop sequence.

FIGURE 1

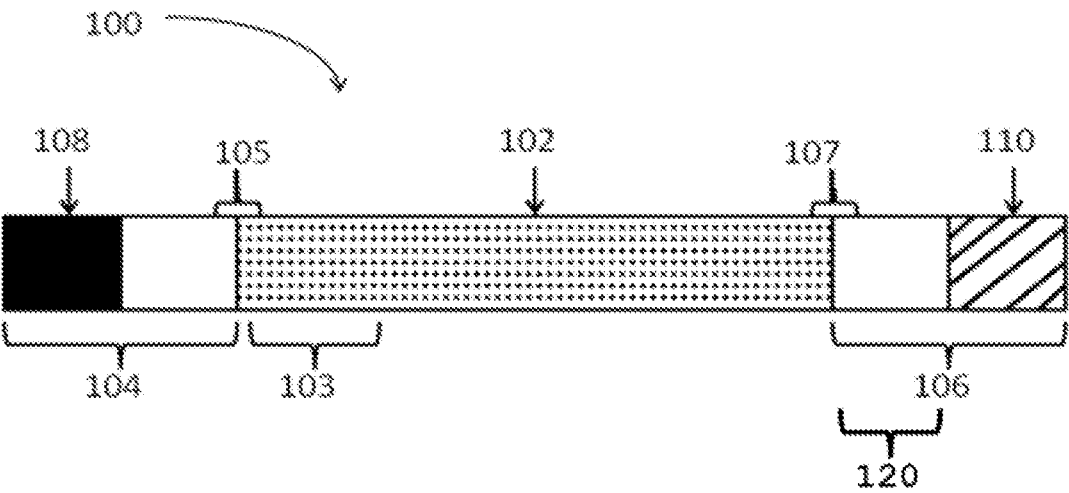


FIGURE 2

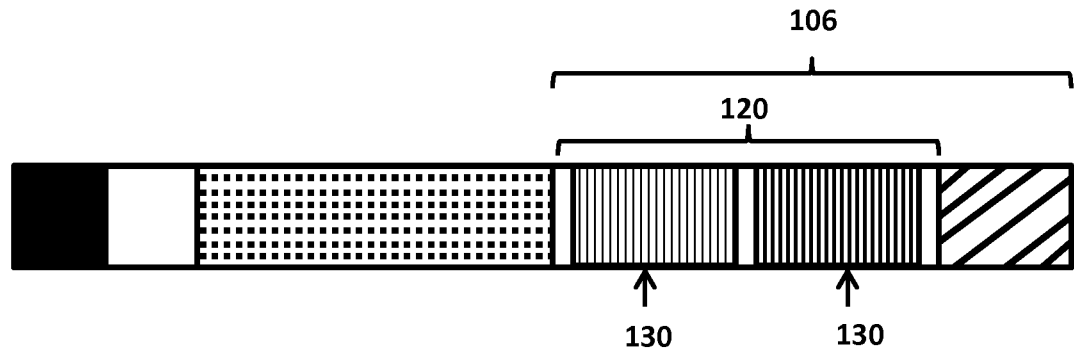
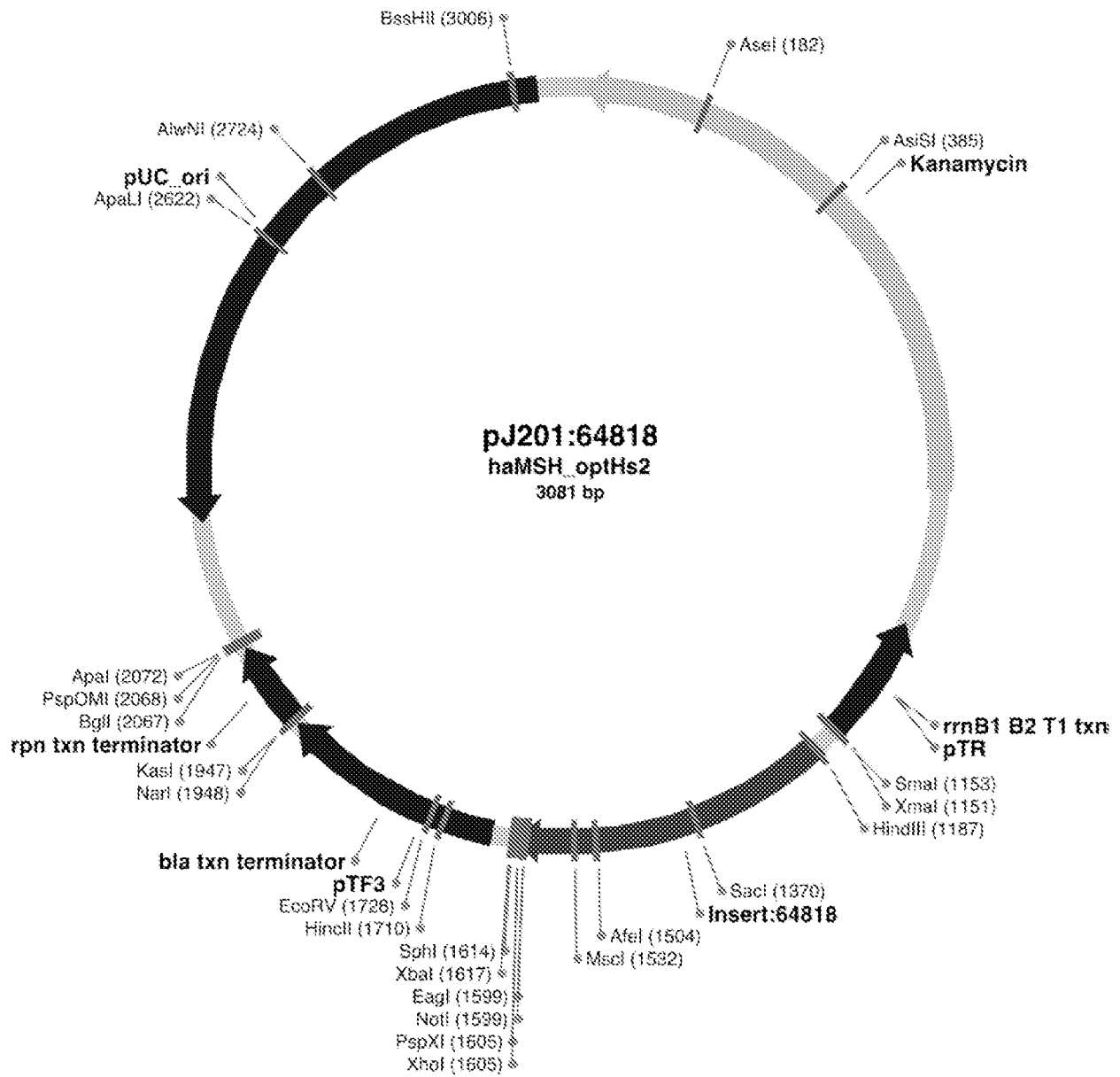


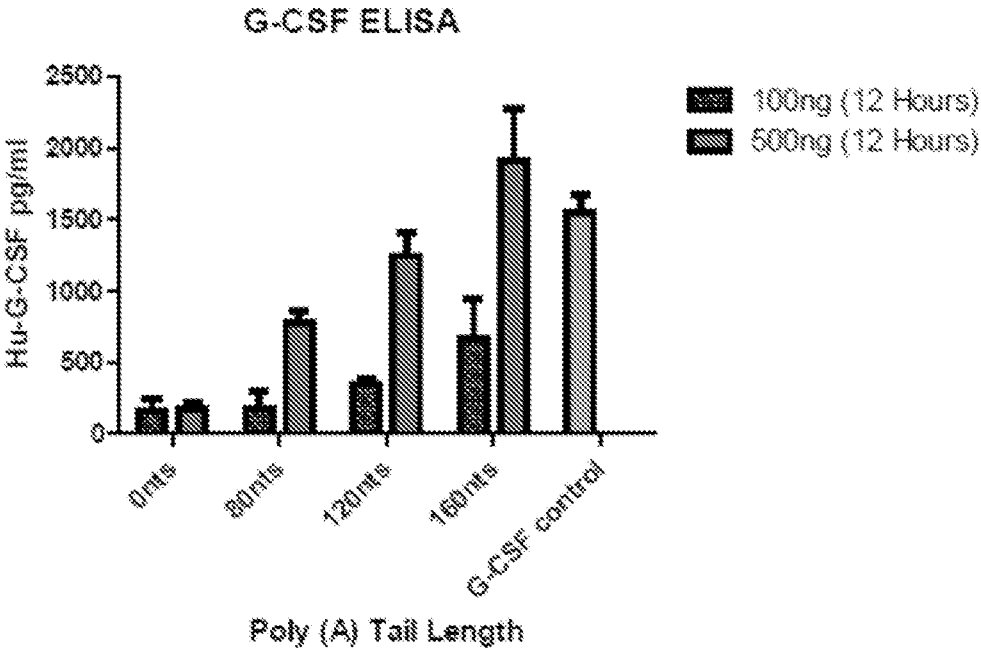
FIGURE 3

Only single cutters are shown in the map



PRIOR ART

FIGURE 4



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/062943

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7088 A61K31/7115 C12N15/85 C12N15/87
ADD.

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)
A61K C12N

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)

EPO-Internal , WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with Indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	wo 2013/090648 AI (MODERNA THERAPEUTICS [US]] 20 June 2013 (2013-06-20) paragraph [0004] - paragraph [0101] ; examples 1-94 -----	1-5, 8-13, 15-17 , 21-25 , 28-33 , 36-40, 43-47 , 50-52
X	US 2012/251618 AI (SCHRUM JASON P [US] ET AL) 4 October 2012 (2012-10-04)	1-13, 18, 21-33 , 36-50, 53,54
Y	paragraph [0099] - paragraph [0102] ; claims 1-26; examples 1-36 paragraph [0159] ----- -/--	14, 19 , 20,34, 35,55 ,56



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

16 December 2013

Date of mailing of the international search report

07/01/2014

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/062943

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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