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(54) **Title**: CELL-BASED BIOPROCESSING

(57) **Abstract**: The invention provides compositions and methods for producing an immunogenic agent from a host cell. In various embodiments, the immunogenic agent is a polypeptide, an antigen, a virus particle, or a vaccine In one aspect, the invention provides for a method for producing an immunogenic agent from a host cell. The method generally comprises contacting the cell with a RNA effector molecule, a portion of which is complementary to a target gene, maintaining the cell in a large-scale bioreactor for a time sufficient to modulate expression of the target gene, wherein the modulation enhances production of the immunogenic agent from the cell, and isolating the immunogenic agent from the cell.

CELL-BASED BIOPROCESSING

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/319,578, filed March 31, 2010, entitled CELL-BASED BIOPROCESSING, by Rossomando et al.; U.S. Provisional Patent Application No. 61/223,370, filed July 6, 2009, entitled COMPOSITIONS AND METHODS FOR ENHANCING PRODUCTION OF A BIOLOGICAL PRODUCT, by Maraganore et al.; U.S. Provisional Patent Application No. 61/244,868, filed September 22, 2009, entitled COMPOSITIONS AND METHODS FOR ENHANCING PRODUCTION OF A BIOLOGICAL PRODUCT, by Maraganore et al.; U.S. Provisional Patent Application No. 61/267,419, filed December 7, 2009, entitled NOVEL LIPIDS AND COMPOSITIONS FOR THE DELIVERY OF THERAPEUTICS, by Manoharan et al., filed ; U.S. Provisional Patent Application No. 61/334,398, filed May 13, 2010, entitled CHARGED LIPIDS AND COMPOSITIONS FOR NUCLEIC ACID DELIVERY, by Manoharan et al.; U.S. Provisional Patent Application No. 61/293,980, filed January 11, 2010, entitled COMPOSITIONS AND METHODS FOR ENHANCING PRODUCTION OF A BIOLOGICAL PRODUCT, by Rossomando et al.; U.S. Provisional Patent Application No. 61/319,589, filed March 31, 2010, entitled CELL-BASED BIOPROCESSING, by Rossomando et al.; and U.S. Provisional Patent Application No. 61/354,932, filed June 15, 2010, entitled CHINESE HAMSTER OVARY (CHO) CELL TRANSCRIPTOME, CORRESPONDING siRNAs AND USES THEREOF, by Rossomando et al.; each of which is incorporated fully herein by reference.

REFERENCES TO TABLES AND SEQUENCES

[0002] The specification includes a Sequence Listing as part of the originally filed subject matter. The sequence listing for SEQ ID NOs 1 to 3,290,939 is provided herein in an electronic format on 4 compact discs (CD-R), labeled “CRF,” “COPY 1,” “COPY 2,” and “COPY 3,” as file name “51058077.TXT,” and is incorporated herein by reference in their entirety in to the present specification.

[0003] The instant application contains a "lengthy" Sequence Listing which has been submitted via CD-R in lieu of a printed paper copy, and is hereby incorporated by reference in its entirety. Said CD-R, recorded on July 1, 2010, are labeled CRF, "Copy 1," "Copy 2" and "Copy 3", respectively, and each contains only one identical 774,635 KB file (51058077.TXT).

FIELD OF THE INVENTION

[0004] The invention relates generally to the field of bioprocessing and more particularly to methods for producing an immunogenic agent in a host cell by contacting the cell with a RNA effector molecule capable of modulating expression of a target gene, wherein the modulation enhances production of the immunogenic agent. The invention also relates generally to transcriptomes, organized transcriptomes, and systems and methods using the transcriptomes for designing targeted modulation of immunogenic agent production in cells. The invention further relates to engineering cells and cell lines for more effective and efficient production of immunogenic agents. The invention also relates to molecules, compositions, cells, and kits useful for carrying out the methods and immunogenic agent produced by the methods.

BACKGROUND

[0005] Cell culture techniques are used to manufacture a wide range of biological products, including biopharmaceuticals, biofuels, metabolites, vitamins, nutraceuticals, immunogenic agents and vaccines. A number of strategies have been developed to enhance productivity, yield, efficiency, and other aspects of cell culture bioprocesses in order to facilitate industrial scale production and meet applicable standards for product quality and consistency. Traditional strategies for optimizing cell culture bioprocesses involve adjusting physical and biochemical parameters, such as culture media (e.g., pH, nutrients) and conditions (e.g., temperature, duration), and selecting host cells having desirable phenotypes. Genetic approaches have also been developed for optimizing cell culture bioprocesses by introducing recombinant DNA into host cells, where the DNA encodes an exogenous protein that influences the production of an immunogenic agent, or regulates expression of an endogenous protein that influences production of the immunological agent. Such methods require costly and time-consuming laboratory manipulations, however, and can be incompatible with certain genes, proteins, host cells, and biological products including immunogenic agents. Accordingly, there is a need in the art for new genetic approaches for optimizing cell culture bioprocesses involving a wide range of host cells and biological products, such as immunogenic agents.

[0006] More recently, host cells for biological production have been modified to incorporate into their genome genes that express shRNAs for the silencing of genes that influence production of the biological product. In these cases, product yield has proven difficult to regulate, however, because of uncontrolled, unintended, expression of the shRNAs which compromises host cell viability. The process of incorporating shRNAs also requires cell engineering, which is time-consuming. Furthermore, uncontrolled expression ultimately leads to

phenotypic changes and overtime the host cells carrying the genes for expressed shRNA lose their ability to produce biological product at any significant yield.

[0007] For example, Chinese hamster (*Cricetulus griseus*) ovary cells (CHO cells) have been used widely in various bioprocesses, yet relatively little is known about gene expression s in these cells; thus, targeted and intelligent modulation of bioprocesses in these cells cannot be done or designed readily. Accordingly, there is a need in the art for new genetic approaches for optimizing cell culture bioprocesses involving a wide range of host cells, including CHO cells, and immunogenic agents produced in these cells.

SUMMARY

[0008] The invention is based at least in part on the surprising discovery that RNA effector molecules can be applied at low concentrations to cells in culture to effect potent, durable modulation of gene expression, such that the quality and quantity of an immunogenic agent produced by a host cell can be improved without the need for extensive cell line engineering. As such, in a first aspect, the invention provides compositions and methods for producing an immunogenic agent from a host cell. In various embodiments, the immunogenic agent is a polypeptide, a viral product, a virus particle, or a vaccine.

[0009] In one aspect, the invention provides for a method for producing an immunogenic agent from a host cell. The method generally comprises contacting the cell with a RNA effector molecule, a portion of which is complementary to a target gene, maintaining the cell in a large-scale bioreactor for a time sufficient to modulate expression of the target gene, wherein the modulation enhances production of the immunogenic agent from the cell, and isolating the immunogenic agent from the cell.

[0010] In one embodiment, the RNA effector molecule transiently modulates expression of the target gene. In another embodiment, the RNA effector molecule transiently inhibits expression of the target gene. In one embodiment, the RNA effector molecule can activate the target gene. In another embodiment, the RNA effector can inhibit the target gene.

[0011] In further embodiments, the host cell is an animal cell, a plant cell, an insect cell, or a fungal cell. In one embodiment, the animal cell is a mammalian cell. In a further embodiment, the mammalian cell is a human cell, a rodent cell, a canine cell, or a non-human primate cell. In a particular embodiment, the host cell is a cell derived from a CHO cell. In another embodiment, a host cell contains a transgene that encodes an immunogenic agent.

[0012] In one embodiment, the cell is contacted with a plurality of different RNA effector molecules. The plurality of RNA effector molecules can be used to modulate expression of a single target gene or multiple target genes.

[0013] In another embodiment, the composition is formulated for administration to cells according to a dosage regimen described herein, e.g., at a frequency of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 84 hr, 96 hr, 108 hr, or more. In another embodiment, the administration of the composition can be maintained during one or more cell growth phases, e.g., lag phase, early log phase, mid-log phase, late-log phase, stationary phase, or death phase. In some of the embodiments, contacting a host cell with a RNA effector molecule (e.g., a dsRNA) occurs prior to, during or after the viral infection or vector inoculation to inhibit cellular and/or anti-viral processes that compromise the yield and quality of the immunogenic agent harvest.

[0014] In another embodiment, a composition containing two or more RNA effector molecules directed against separate target genes is used to enhance production of a immunogenic agent in cell culture by modulating expression of a first target gene and at least a second target gene in the cultured cells. In another embodiment, a composition containing two or more RNA effector molecules directed against the same target gene is used to enhance production of an immunogenic agent in cell culture by modulating expression of the target gene in cultured cells.

[0015] In another embodiment, a first RNA effector molecule is administered to a cultured cell, and then a second RNA effector molecule is administered to the cell (or vice versa). In a further embodiment, the first and second RNA effector molecules are administered to a cultured cell substantially simultaneously.

[0016] In one embodiment, the RNA effector molecule is added to the cell culture medium used to maintain the cells under conditions that permit production of an immunogenic agent. The RNA effector molecule can be added at different times or simultaneously. In one embodiment, one or more of the different RNA effector molecules are added by continuous infusion into the cell culture medium, for example, to maintain a continuous average percent inhibition or RNA effector molecule concentration. In another embodiment, one or more of the different RNA effector molecules are added by continuous infusion into the cell culture medium, for example, to maintain a minimum average percent inhibition or RNA effector molecule concentration. In one embodiment, the continuous infusion is administered at a rate to achieve a desired average percent inhibition for at least one target gene. In one embodiment, the continuous infusion is performed for a distinct period of time (which can be repeated), e.g., for 1 hr, 2 hr, 3 hr, 4 hr, 8 hr, 16 hr, 18 hr, 24 hr, 48 hr, 72 hr, or longer. When applying a

plurality of differen RNA effector molecules, each of the different RNA effector molecules can be added at the same frequency or different frequencies. Each of the different RNA effector molecules is added at the same concentration or at different concentrations. In some embodiments, the last contact of cells with a RNA effector molecule is at least 24 hr, 48 hr, 72 hr, 120 hr, or later, before isolation of the immunogenic agent or harvesting the supernatant.

[0017] Generally, the RNA effector molecule is added at a given concentration of less than or equal to 200 nM (e.g., 100 nM, 80 nM, 50 nM, 20 nM, 10 nM, 1 nM, or less). As described herein, low concentrations of RNA effector molecules can be used in large scale bioprocessing to efficiently modulate target genes. There are significant economic and commercial advantages (e.g., lower costs and easier removal) of using low concentrations of RNA effector molecules. Thus, in one embodiment, cells are contacted with a RNA effector molecule at a concentration of 100 nM or less , 50 nM or less, 20 nM or less, 10 nM or less, 5 nM or less, or 1 nM or less. In a particular embodiment, the one or more RNA effector molecules is administered into the cell culture medium at a final concentration of 1 nM at least once (e.g., at least two times, at least three times, at least four times, or more) during the growth phase and/or production phase.

[0018] In still another embodiment, the RNA effector molecule is added at a given starting concentration of each of the different RNA effector molecules (e.g., at 1 nM each), and further supplemented with continuous infusion of the RNA effector molecule.

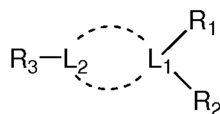
[0019] In one embodiment, the RNA effector composition comprises a reagent that facilitates RNA effector molecule uptake, for example, an emulsion, a cationic lipid, a non-cationic lipid, a charged lipid, a liposome, an anionic lipid, a penetration enhancer, a transfection reagent or a modification to the RNA effector molecule for attachment, e.g., a ligand, a targeting moiety, a peptide, a lipophilic group, etc.

[0020] The RNA effector molecule to be contacted with the cell can be incorporated into a formulation that facilitates uptake and delivery into the cell. The one or more of the different RNA effector molecules can be added by contacting the cells with the RNA effector molecule and a reagent that facilitates RNA effector molecule uptake, for example, an emulsion, a cationic lipid, a non-cationic lipid, a charged lipid, a liposome, an anionic lipid, a penetration enhancer, a transfection reagent or a modification to the RNA effector molecule for attachment, e.g., a ligand, a targeting moiety, a peptide, a lipophilic group, etc.

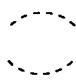
[0021] In certain embodiments, a lipid formulation is used in a RNA effector molecule composition as a reagent that facilitates RNA effector molecule uptake. In certain embodiments, the lipid formulation can be a LNP formulation, a LNP01 formulation, a XTC-SNALP

formulation, or a SNALP formulation as described herein. In related embodiments, the XTC-SNALP formulation is as follows: using 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC) with XTC/DPPC/Cholesterol/PEG-cDMA in a ratio of 57.1/7.1/34.4/1.4 and a lipid:siRNA ratio of about 7. In still other related embodiments, the RNA effector molecule is a dsRNA and is formulated in a XTC-SNALP formulation as follows: using 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC) with a XTC/DPPC/Cholesterol/PEG-cDMA in a ratio of 57.1/7.1/34.4/1.4 and a lipid:siRNA ratio of about 7. Alternatively, a RNA effector molecule such as those described herein can be formulated in a LNP09 formulation as follows: using XTC/DSPC/Chol/PEG2000-C14 in a ratio of 50/10/38.5/1.5 mol% and a lipid:siRNA ratio of about 11:1. In some embodiments, the RNA effector molecule is formulated in a LNP11 formulation as follows: using MC3/DSPC/Chol/PEG2000-C14 in a ratio of 50/10/38.5/1.5 mol% and a lipid:siRNA ratio of about 11:1. In still another embodiment, the RNA effector molecule is formulated in a LNP09 formulation or a LNP11 formulation and reduces the target gene mRNA levels by about 85 to 90% at a dose of 0.3mg/kg, relative to a PBS control group. In yet another embodiment, the RNA effector molecule is formulated in a LNP09 formulation or a LNP11 formulation and reduces the target gene mRNA levels by about 50% at a dose of 0.1 mg/kg, relative to a PBS control group. In yet another embodiment, the RNA effector molecule is formulated in a LNP09 formulation or a LNP11 formulation and reduces the target gene protein levels in a dose-dependent manner relative to a PBS control group as measured by a western blot. In yet another embodiment, the RNA effector molecule is formulated in a SNALP formulation as follows: using DlinDMA with a DLinDMA/DPPC/Cholesterol/PEG2000-cDMA in a ratio of 57.1/7.1/34.4/1.4 and a lipid:siRNA ratio of about 7.

[0022] In some embodiments, the lipid formulation comprises a lipid having the following formula:



where R_1 and R_2 are each independently for each occurrence optionally substituted C_{10}-C_{30} alkyl, optionally substituted C_{10}-C_{30} alkoxy, optionally substituted C_{10}-C_{30} alkenyl, optionally substituted C_{10}-C_{30} alkenyloxy, optionally substituted C_{10}-C_{30} alkynyl, optionally substituted C_{10}-C_{30} alkynyloxy, or optionally substituted C_{10}-C_{30} acyl;

 represents a connection between L_2 and L_1 which is:

(1) a single bond between one atom of L_2 and one atom of L_1, wherein

L_1 is $C(R_x)$, O, S or N(Q);

L_2 is $-CR_5R_6-$, -O-, -S-, -N(Q)-, $=C(R_5)-$, -C(O)N(Q)-, -C(O)O-, -N(Q)C(O)-, -OC(O)-, or -C(O)-;

(2) a double bond between one atom of L_2 and one atom of L_1; wherein

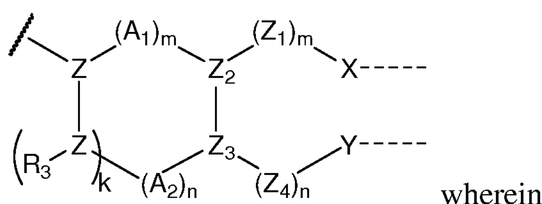
L_1 is C;

L_2 is $-CR_5=$, -N(Q)=, -N-, -O-N=, -N(Q)-N=, or -C(O)N(Q)-N=;

(3) a single bond between a first atom of L_2 and a first atom of L_1, and a single bond between a second atom of L_2 and the first atom of L_1, wherein

L_1 is C;

L_2 has the formula



wherein

X is the first atom of L_2, Y is the second atom of L_2, - - - - - represents a single bond to the first atom of L_1, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, $-OS(O)(Q_2)O-$, and $-OP(O)(Q_2)O-$;

Z_1 and Z_4 are each, independently, -O-, -S-, $-CH_2-$, $-CHR^5-$, or $-CR^5R^5-$;

Z_2 is CH or N;

Z_3 is CH or N;

or Z_2 and Z_3, taken together, are a single C atom;

A_1 and A_2 are each, independently, -O-, -S-, $-CH_2-$, $-CHR^5-$, or $-CR^5R^5-$;

each Z is N, $C(R_5)$, or $C(R_3)$;

k is 0, 1, or 2;

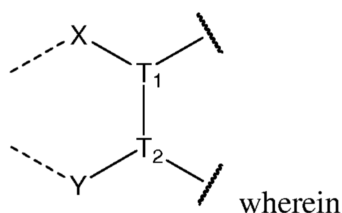
each m, independently, is 0 to 5;

each n, independently, is 0 to 5;

where m and n taken together result in a 3, 4, 5, 6, 7 or 8 member ring;

(4) a single bond between a first atom of L_2 and a first atom of L_1, and a single bond between the first atom of L_2 and a second atom of L_1, wherein

(A) L_1 has the formula:



wherein

X is the first atom of L_1, Y is the second atom of L_1, - - - - - represents a single bond to the first atom of L_2, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, -OS(O)(Q_2)O-, and -OP(O)(Q_2)O-;

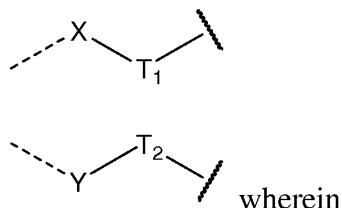
T_1 is CH or N;

T_2 is CH or N;

or T_1 and T_2 taken together are C=C;

L_2 is CR_5; or

(B) L_1 has the formula:



wherein

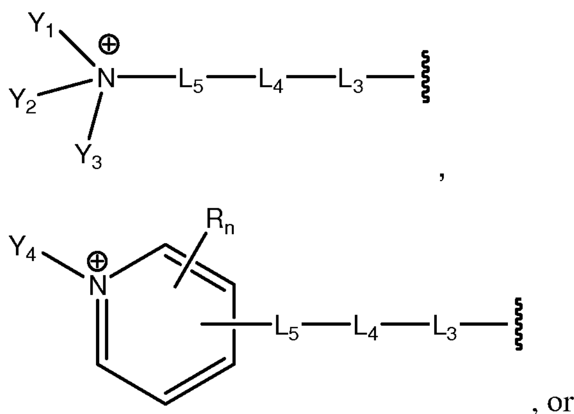
X is the first atom of L_1, Y is the second atom of L_1, - - - - - represents a single bond to the first atom of L_2, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, -OS(O)(Q_2)O-, and -OP(O)(Q_2)O-;

T_1 is -CR_5R_5-, -N(Q)-, -O-, or -S-;

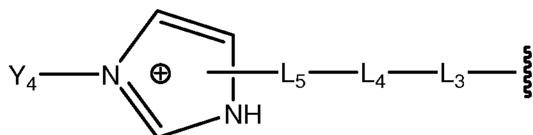
T_2 is -CR_5R_5-, -N(Q)-, -O-, or -S-;

L_2 is CR_5 or N;

R_3 has the formula:



, or



wherein

each of Y_1, Y_2, Y_3, and Y_4, independently, is alkyl, cycloalkyl, aryl, aralkyl, or alkynyl; or

any two of Y_1, Y_2, and Y_3 are taken together with the N atom to which they are attached to form a 3- to 8- member heterocycle; or

Y_1, Y_2, and Y_3 are all be taken together with the N atom to which they are attached to form a bicyclic 5- to 12- member heterocycle;

each R_n, independently, is H, halo, cyano, hydroxy, amino, alkyl, alkoxy, cycloalkyl, aryl, heteroaryl, or heterocyclyl;

L_3 is a bond, -N(Q)-, -O-, -S-, $-(CR_5R_6)_a-$, -C(O)-, or a combination of any two of these;

L_4 is a bond, -N(Q)-, -O-, -S-, $-(CR_5R_6)_a-$, -C(O)-, or a combination of any two of these;

L_5 is a bond, -N(Q)-, -O-, -S-, $-(CR_5R_6)_a-$, -C(O)-, or a combination of any two of these;

each occurrence of R_5 and R_6 is, independently, H, halo, cyano, hydroxy, amino, alkyl, alkoxy, cycloalkyl, aryl, heteroaryl, or heterocyclyl; or two R_5 groups on adjacent carbon atoms are taken together to form a double bond between their respective carbon atoms; or two R_5 groups on adjacent carbon atoms and two R_6 groups on the same adjacent carbon atoms are taken together to form a triple bond between their respective carbon atoms;

each a, independently, is 0, 1, 2, or 3;

wherein

an R_5 or R_6 substituent from any of L_3, L_4, or L_5 is optionally taken with an R_5 or R_6 substituent from any of L_3, L_4, or L_5 to form a 3- to 8- member cycloalkyl, heterocyclyl, aryl, or heteroaryl group; and

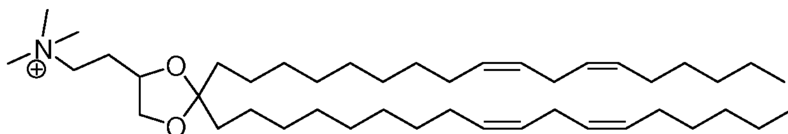
any one of Y_1, Y_2, or Y_3, is optionally taken together with an R_5 or R_6 group from any of L_3, L_4, and L_5, and atoms to which they are attached, to form a 3- to 8- member heterocyclyl group;

each Q, independently, is H, alkyl, acyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl or heterocyclyl; and

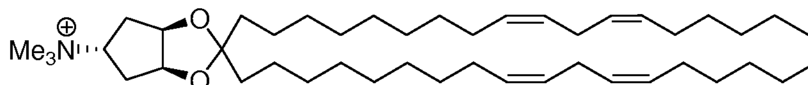
each Q_2, independently, is O, S, N(Q)(Q), alkyl or alkoxy.

[0023] In a particular embodiment, the formulation comprises a lipid containing a quaternary amine, such as those described herein (for example, Lipid H, Lipid K, Lipid L, Lipid M, Lipid P, and Lipid R). Thus, in some embodiments, the RNA effector molecule composition comprises a reagent that facilitates RNA effector molecule uptake which comprises “Lipid H”,

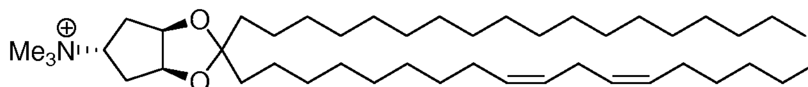
"Lipid K", "Lipid L", "Lipid M", "Lipid P", or "Lipid R", whose formulae are indicated as follows:



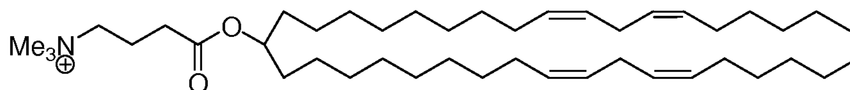
Lipid H (Lipid No. 200)
Formula I



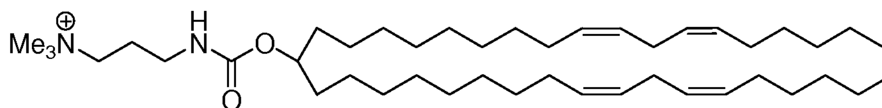
Lipid K (Lipid No. 201)
Formula II



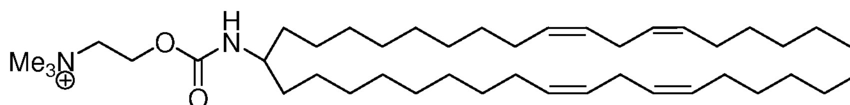
Lipid L (Lipid No. 202)
Formula III



Lipid M (Lipid No. 203)
Formula IV



Lipid P (Lipid No. 204
Formula V



Lipid R (Lipid No. 205)
Formula VI

[0024] In embodiments in which the RNA effector molecule composition is formulated with a delivery facilitating agent, the composition can be in solution (e.g., a sterile solution, for example, packaged in a unit dosage form), or as a sterile lyophilized composition (pre-dosed, for example, in units for use in 1 L of cell culture media).

[0025] In another embodiment, the RNA effector molecule composition further comprises a growth medium (e.g., chemically defined media such as Biowhittaker® POWERCHO® medium (Lonza), HYCLONE PF CHO™ medium (Thermo Scientific), GIBCO® CD DG44 MEDIUM (Invitrogen, Carlsbad, CA), Medium M199 (Sigma-Aldrich), OPTIPRO™ SFM medium (Gibco), etc.). The RNA effector can be present in a concentration such that, when reconstituted in a medium, provides the desired concentration.

[0026] In still another embodiment, the RNA effector molecule composition further comprises an agent selected from the group consisting of essential amino acids (e.g., glutamine), 2-mercapto-ethanol, bovine serum albumin (BSA), lipid concentrate, cholesterol, catalase, insulin, human transferrin, superoxide dismutase, biotin, DL α-tocopherol acetate, DL α-tocopherol, vitamins (e.g., Vitamin A), choline chloride, D-calcium pantothenate, folic acid, Nicotinamide, pyridoxal hydrochloride, riboflavin, thiamine hydrochloride, i-Inositol, corticosterone, D-galactose, ethanolamine HCl, glutathione (reduced), L-carnitine HCl , linoleic acid, linolenic acid, progesterone, putrescine 2HCl, sodium selenite, T3 (triodo-I-thyronine), growth factors (e.g., EGF), iron, L-glutamine, L-alanyl-L-glutamine, sodium hypoxanthine, aminopterin and thymidine, arachidonic acid , ethyl alcohol 100%, myristic acid, oleic acid, palmitic acid, palmitoleic acid, PLURONIC F68® (Invitrogen), stearic acid 10, TWEEN 80® nonionic surfactant (Invitrogen), sodium pyruvate, and glucose.

[0027] In various embodiments, the RNA effector molecule can comprise siRNA, miRNA, dsRNA, saRNA, shRNA, piRNA, tkRNAi, eiRNA, pdRNA, a gapmer, an antagomir, or a ribozyme. In one embodiment the RNA effector molecule is not shRNA. In one embodiment the RNA effector molecule is a dsRNA.

[0028] In some embodiments, the RNA effector molecule is selected from a group of siRNAs, wherein the RNA effector molecule comprises sense strand and an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides, etc.). In one embodiment, the antisense strand comprises at least 16 contiguous nucleotides. In one embodiment, the antisense strand comprises at least 17 contiguous nucleotides. In one embodiment, the antisense strand comprises at least 18 contiguous nucleotides. In one embodiment, the antisense strand comprises at least 19 contiguous nucleotides. In one embodiment, the antisense strand further comprises at least one deoxyribonucleotide. In one embodiment, the antisense strand further comprises at least two deoxyribonucleotides. In one embodiment, the antisense strand further comprises two deoxythymidine residues.

[0029] In some embodiments, the RNA effector molecule comprises an antisense strand of a double-stranded oligonucleotide in which the antisense strand comprises at least 16 contiguous nucleotides (e.g., 17, nucleotides, 18 nucleotides, or 19 nucleotides). In one embodiment, the antisense strand comprises at least 16 contiguous nucleotides. In one embodiment, the antisense strand comprises at least 17 contiguous nucleotides. In one embodiment, the antisense strand comprises at least 18 contiguous nucleotides. In one embodiment, the antisense strand comprises at least 19 contiguous nucleotides. In one embodiment, the antisense strand further comprises at least one deoxyribonucleotide. In one embodiment, the antisense strand further comprises at least two deoxyribonucleotides. In one embodiment, the antisense strand further comprises two deoxythymidine residues.

[0030] In some embodiments, the maintaining step further comprises monitoring at least one measurable parameter selected from the group consisting of cell density, medium pH, oxygen levels, glucose levels, lactic acid levels, temperature, and protein production.

[0031] In some embodiments, at least one measurable parameter can be monitored during production of an immunogenic agent, including any one of cell density, medium pH, oxygen levels, glucose levels, lactic acid levels, temperature, and protein production.

[0032] In further embodiments, the method further comprises administering to the host cell a second agent. The second agent can be a growth factor; an apoptosis inhibitor; a kinase inhibitor; a phosphatase inhibitor; a protease inhibitor; an inhibitor of pathogens (e.g., where a virus is the immunogenic agent, an agent that inhibits growth and/or propagation of other viruses or fungal or bacterial pathogens); or a histone demethylating agent. Where the virus being propagated is influenza, the second agent can be a protease that cleaves influenza hemagglutinin, such as pronase, thermolysin, subtilisin A, or a recombinant protease.

[0033] In another embodiment, a composition containing a RNA effector molecule described herein, e.g., a dsRNA directed against a host cell target gene, is administered to a cultured cell with a non-RNA agent useful for enhancing the production of an immunogenic agent by the cell. The non-RNA agent can be selected from the group consisting of: an antibiotic, an antimycotic, an antimetabolite (e.g., methotrexate), an antibody; a growth factor (e.g., insulin); an apoptosis inhibitor; a kinase inhibitor, such as a MAP kinase inhibitor, a CDK inhibitor, and/or a K252a; a phosphatase inhibitor, such as sodium vanadate and okadaic acid; a protease inhibitor; and a histone demethylating agent, such as 5-azacytidine.

[0034] In some embodiments, the immunogenic agent is a polypeptide and the target gene encodes a protein that affects post-translational modification in the host cell. In various embodiments, the post-translational modification can be protein glycosylation, protein

deamidation, protein disulfide bond formation, methionine oxidation, protein pyroglutamation, protein folding, or protein secretion.

[0035] In additional embodiments, the target gene encodes a protein that affects a physiological process of the host cell. In various embodiments, the physiological process is apoptosis, cell cycle progression, cellular immune response, carbon metabolism or transport, lactate formation, RNAi uptake and/or efficacy, or actin dynamics.

[0036] In further embodiments, the target gene encodes a pro-oxidant enzyme, or a protein that affects cellular pH.

[0037] In another aspect, the invention provides a cultured eukaryotic cell containing at least one RNA effector molecule provided herein. The cell is a mammalian cell, such as a rodent cell, a canine cell, a non-human primate cell, or a human cell.

[0038] In another aspect, the invention provides a composition for enhancing production of an immunogenic agent in cell culture by modulating the expression of a target gene in a host cell. The composition typically includes one or more RNA effector molecules described herein and a suitable carrier or delivery vehicle, e.g., an acceptable carrier and/or a reagent that facilitates RNA effector molecule uptake. The RNA effector molecule composition can be formulated as suspension in aqueous, non-aqueous, or mixed media and can be formulated in a lipd or non-lipid formulation. The RNA effector molecule composition can be provided in a sterile solution or lyophilized (e.g., provided in discrete units by concentration and/or volume).

[0039] In another embodiment, a composition containing a RNA effector molecule described herein, e.g., a dsRNA directed against a host cell target gene, is administered to a cultured cell with a non-RNA agent useful for enhancing the production of an immunogenic agent by the cell.

[0040] In one embodiment, a vector is provided for modulating the expression of a target gene in a cultured cell, where the target gene encodes a protein that affects production of an immunogenic agent by the cell. In one embodiment, the vector includes at least one regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a RNA effector molecule. In one embodiment, the RNA effector molecule is not encoded by a vector.

[0041] In another embodiment, the invention provides a cell containing a vector for inhibiting the expression of a target gene in a cell. The vector includes a regulatory sequence operably linked to a polynucleotide encoding at least one strand of a RNA effector molecule.

[0042] Still another aspect of the invention encompasses kits comprising RNA effector molecules described herein. In one embodiment, the kits comprise a RNA effector molecule that modulates expression of a target gene encoding a protein that affects production of the

immunogenic agent. In another embodiment, the kits further comprise a modified cell line which expresses a RNA effector molecule which modulates expression of a protein that affects production of the immunogenic agent. The kits can also comprise instructions for carrying out methods provided herein.

[0043] In one embodiment, the kit further comprises suitable culture media for growing host cells and/or constructs (e.g., plasmid, viral, etc.) for introducing a nucleic acid sequence encoding a RNA effector molecule into host cells. In still another embodiment, the kits can further comprise reagents for detecting and/or purifying the immunogenic agent. Non-limiting examples of suitable reagents include PCR primers, polyclonal antibodies, monoclonal antibodies, affinity chromatography media, and the like.

[0044] In one embodiment, a kit comprises a RNA effector molecule that modulates expression of a target gene to inhibit expression of a latent, adventitious, or endogenous virus and thus affect production of the desired immunogenic agent. In another embodiment, a kit comprises a host cell that expresses a RNA effector molecule that modulates expression of latent, adventitious, or endogenous virus that affects production of the desired immunogenic agent. Such kits can also comprise instructions for carrying out methods provided herein. The kits can also include at least one reagent that facilitates RNA effector molecule-uptake, comprising a charged lipid, an emulsion, a liposome, a cationic or non-cationic lipid, an anionic lipid, a transfection reagent or a penetration enhancer. In a particular embodiment, the reagent that facilitates RNA effector molecule-uptake comprises a charged lipid.

[0045] Some embodiments of the present invention relate to initiating RNA interference in a host cell, during or after microbial inoculation or vector transduction, to inhibit expression of endogenous, latent or adventitious virus that can compromise the yield and/or quality of the harvested immunogenic agent. For example, an embodiment administers a siRNA, or, e.g., a shRNA in naked, conjugated or formulated form (e.g., lipid nanoparticle), that targets an endogenous, latent or adventitious virus pathway (e.g., *ev* loci of endogenous avian leukosis virus (ALV-E) in avian cells; endogenous type C retrovirus-like particle genomes in CHO cells; or the *rep* gene of porcine circovirus type 1 (PCV-1) in Vero cells), and thereby increases quality and/or yield of the desired immunogenic agent.

[0046] In some embodiments of the invention, simple naked (i.e., unconjugated) RNA effector molecules, or conjugated (e.g., directly conjugated to cholesterol or other targeting ligands) RNA effector molecules can be used. In another embodiment, plasmid- or viral vector-encoded RNA effector molecules for shRNA can be used.

[0047] In some embodiments of the invention, LNP or alternate polymer formulations are used. In some embodiments, the formulation includes an agent that facilitates RNA effector molecule-uptake, e.g., a charged lipid, an emulsion, a liposome, a cationic or non-cationic lipid, an anionic lipid, a transfection reagent or a penetration enhancer. In a particular embodiment, the reagent that facilitates RNA effector molecule-uptake comprises a charged lipid. In addition, the formulations can be co-formulated or incorporated into the infective seed or vectors themselves to facilitate delivery or stabilize RNAi materials to the relevant cell where the agent/vector can produce the desired immunogenic agent.

[0048] In particular embodiments, the target gene is associated with endogenous, adventitious or latent herpesviruses, polyomaviruses, hepadnaviruses, papillomaviruses, adenoviruses, poxviruses, bornaviruses, retroviruses, arenaviruses, orthomyxoviruses, paramyxoviruses, reoviruses, picornaviruses, flaviviruses, rabdoviruses, hantaviruses, circoviruses, or vesiviruses.

[0049] Particular endogenous and latent viruses that can be targeted by the methods of the present invention include Minute Virus of Mice (MVM), Murine leukemia/sarcoma (MLV), Circoviruses including porcine circovirus (PCV-1, PCV-2), Human herpesvirus 8 (HHV-8), arenavirus Lymphocytic choriomeningitis virus (LCMV), Lactate dehydrogenase virus (LDH or LDV), human species C adenoviruses, avian adeno-associated virus (AAV), primate endogenous retrovirus family K (ERV-K), and human endogenous retrovirus K (HERV-K).

[0050] Further regarding ERVs, in embodiments of the present invention the target genes of ERVs can be those of primate/human Class I Gamma ERVs pt01-Chr10r-17119458, pt01-Chr5-53871501, BaEV, GaLV, HERV-T, ERV-3, HERV-E, HERV-ADP, HERV-I, MER4like, HERV-FRD, HERV-W, HERVH-RTVLH2, HERVH-RGH2, HERV-Hconsensus, HERV-Fc1; primate/human Epsilon ERV hg15-chr3-152465283; primate/human Intermediate (epsilon-like) HERVL66; primate/human Class III Spuma-like ERVs HSRV, HFV, HERV-S, HERV-L, HERVL40, HERVL74; primate/human Delta ERVs HTLV-1, HTLV-2; primate/human Lenti ERVs HIV-1, HIV-2; primate/human Class II, Beta ERV MPMV, MMTV, HML1, HML2, HML3, HML4, HML7, HML8, HML5, HML10, HML6, or HML9.

[0051] In other embodiments of the present invention, the ERV is selected from rodent Class II, Beta ERV MMTV; rodent Class I Gamma ERV MLV; feline Class I Gamma ERV FLV; ungulate Class I Gamma ERV PERV; ungulate Delta ERV BLV; ungulate lentivirus Visna, EIAV; ungulate Class II, Beta ERV JSRV; avian Class III, Spuma-like ERVs gg01-chr7-7163462; gg01-chrU-52190725, gg01-Chr4-48130894; avian Alpha ERVs ALV, gg01-chr1-15168845; avian Intermediate Beta-like ERVs gg01-chr4-77338201;

gg01-ChrU-163504869, gg01-chr7-5733782; Reptilian Intermediate Beta-like ERV Python-molurus; Fish Epsilon ERV WDSV; fish Intermediate (epsilon-like) ERV SnRV; Amphibian Epsilon ERV Xen1; Insect Errantivirus ERV Gypsy.

[0052] Other embodiments of the present invention target adventitious viruses of animal-origin, such as vesivirus, circovirus, hantaan virus, Marburg virus, SV40, SV20, Semliki Forest virus (SFV), simian virus 5 (sv5), lymphocytic choriomeningitis virus, feline sarcoma virus, porcine parvovirus, adenoassociated viruses (AAV), mouse hepatitis virus (MHV), murine leukemia virus (MuLV), pneumonia virus of mice (PVM), Theiler's encephalomyelitis virus (THEMV), murine minute virus (MMV or MVM), mouse adenovirus (MAV), mouse cytomegalovirus (MCMV), mouse rotavirus (EDIM), Kilham rat virus (KRV), Toolan's H-1 virus, Sendai virus (SeV, also know as murine parainfluenza virus type 1 or hemagglutinating virus of Japan (HVJ)), Parker's rat coronavirus (RCV or SDA), pseudorabies virus (PRV), reoviruses, Cache Valley virus, bovine viral diarrhoea virus, bovine parainfluenza virus type 3, bovine respiratory syncytial virus, bovine adenoviruses, bovine parvoviruses, bovine herpesvirus 1 (infectious bovine rhinotracheitis virus), other bovine herpesviruses, bovine reovirus, rabies virus, bluetongue viruses, bovine polyoma virus, bovine circovirus, and orthopoxviruses other than vaccinia, pseudocowpox virus (a widespread parapoxvirus that can infect humans), papillomavirus, herpesviruses, or leporipoxviruses.

[0053] Other embodiments target human-origin adventitious agents including HIV-1 and HIV-2; human T cell lymphotropic virus type I (HTLV-I) and HTLV-II; human hepatitis A, B, and C viruses; human cytomegalovirus; Epstein Barr virus (EBV or HHV-4); human herpesviruses 6, 7, and 8; human parvovirus B19; reoviruses; polyoma (JC/BK) viruses; SV40 virus; human coronaviruses; human papillomaviruses; influenza A, B, and C viruses; human enteroviruses; human parainfluenza viruses; and human respiratory syncytial virus.

[0054] Yet other embodiments of the present invention target host cell surface receptors or intracellular proteins to which endogenous, latent, or adventitious virus bind or which are required for viral replication. For example, in a particular embodiment, the target gene is a CHO cell MVM receptor gene, such as a gene associated with cellular sialic acid production.

[0055] In addition to the target genes associated with sialic acid, as described herein, yield and/or qualities of an immunogenic agent can be optimized by targeting genes associated with glycosylation in the host cell.

[0056] The hamster *Gale* gene encodes UDP-galactose-4-epimerase, e.g., CHO *Gale* transcript SEQ ID NO:5564, and can be targeted a RNA effector molecule comprising a sense strand and an antisense strand, one of which comprises at least 16 contiguous nucleotides

(e.g., 17 nucleotides, 18 nucleotides, or 19 nucleotides) of the nucleotide sequence selected from the group consisting of SEQ ID NOs:1888656-1889007. In one embodiment, the antisense strand comprises at least 16 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs:1888656-1889007. In another embodiment, one strand comprises at least 17 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs:1888656-1889007. In another embodiment, one strand comprises at least 18 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs:1888656-1889007. In another embodiment, one strand comprises at least 19 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs:1888656-1889007. In a particular embodiment, the antisense strand comprises sequence of SEQ ID NOs:1888656-1889007, and further comprises at least one deoxyribonucleotide. In another particular embodiment, the antisense strand comprises sequence of SEQ ID NOs:1888656-1889007, and further comprises at least two deoxyribonucleotides. In another particular embodiment, the antisense strand comprises sequence of SEQ ID NOs:1888656-1889007, and further comprises at least two deoxythymidine residues. This enzyme enables the cell to process galactose by converting it to glucose, and vice versa.

[0057] UDP-galactose is used to build galactose-containing proteins and fats, which play critical roles in chemical signaling, building cellular structures, transporting molecules, and producing energy. Hamster GDP-mannose 4,6-dehydratase (GMDS) and can be targeted a RNA effector molecule comprising a sense strand and an antisense strand, one of which comprises at least 16 contiguous nucleotides (e.g., 17 nucleotides, 18 nucleotides, or 19 nucleotides) of the nucleotide sequence selected from the group consisting of SEQ ID NOs: 3152754-3152793. In one embodiment, the antisense strand comprises at least 16 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs: 3152754-3152793. In another embodiment, one strand comprises at least 17 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs: 3152754-3152793. In another embodiment, one strand comprises at least 18 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs: 3152754-3152793. In another embodiment, one strand comprises at least 19 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs: 3152754-3152793. In a particular embodiment, the antisense strand comprises sequence of SEQ ID NOs: 3152754-3152793, and further comprises at least one deoxyribonucleotide. In another particular embodiment, the antisense strand comprises sequence of SEQ ID NOs: 3152754-3152793, and further comprises at least two deoxyribonucleotides. In another particular embodiment, the antisense strand comprises

sequence of SEQ ID NOs:3152754-3152793, and further comprises at least two deoxythymidine residues.

[0058] In various embodiments, the immunogenic agent is a polypeptide. The polypeptide can be a recombinant polypeptide or a polypeptide endogenous to the host cell. In some embodiments, the polypeptide is an antigen, a glycoprotein, a receptor, membrane protein, immune effector, binding protein, oncoprotein or proto-oncoprotein, or structural protein. In some embodiments, the polypeptide immunogenic agent is a vaccine or the immunogenic agent can be used in a vaccine.

[0059] The method of the invention also can include the steps of monitoring the growth, production and activation levels of the host cell culture, and as well as for varying the conditions of the host cell culture to maximize the growth, production and activation levels of the host cells and desired product, and for harvesting the immunogenic agent from the cell or culture, preparing a formulation with the harvested immunogenic agent, and for the treatment and/or the prevention of a disease by administering to a subject in need thereof a formulation obtained by the method.

[0060] In one embodiment, the host cell is administered a plurality of different RNA effector molecules to modulate expression of multiple target genes. The RNA effector molecules can be administered at different times or simultaneously, at the same frequency or different frequencies, at the same concentration or at different concentrations.

[0061] In another embodiment, the invention provides a composition for enhancing production of an immunogenic agent in a host cell by modulating the expression of a target gene in the cell. The composition typically includes one or more oligonuceotides, such as RNA effector molecules described herein, and a suitable carrier or delivery vehicle.

[0062] In additional embodiments, the target gene encodes a protein that affects a physiological process of the host cell. In various embodiments, the physiological process is apoptosis, cellular immunity, cell cycle progression, carbon metabolism or transport, lactate formation, or RNAi uptake and/or efficacy.

[0063] More specifically, in some embodiments the second target gene is a gene associated with host cell immune response, and the target gene encodes the host cell target selected from the group consisting of TLR3, TLR7, TLR21, RIG-1, LPGP2, RIG 1-like receptors, TRIM25, IFN-α, IFN-β, IFN-γ, MAVS, IFNAR1, IFNR2, STAT-1, STAT-2, STAT-3, STAT-4, JAK-1, JAK-2, JAK-3, IRF1, IRF2, IRF3, IRF4, IRF5, IRF6 IRF7, IRF8, IRF 9, IRF10, 2′,5′ oligoadenylate synthetase, RNaseL, dsRNA-dPKR, Mx, IFITM1, IFITM2,

IFITM3, Proinflammatory cytokines, MYD88, TRIF, PKR, and a regulatory region of any of the foregoing.

[0064] In other specific embodiments, the second target gene is a gene associated with host cell viability, growth or cell cycle, and the target gene encodes the host cell target selected from the group consisting of Bax, Bak, LDHA, LDHB, BIK, BAD, BIM, HRK, BCLG, HR, NOXA, PUMA, BOK, BOO, BCLB, CASP2, CASP3, CASP6, CASP7, CASP8, CASP9, CASP10, BCL2, p53, APAF1, HSP70, TRAIL, BCL2L1, BCL2L13, BCL2L14, FASLG, DPF2, AIFM2, AIFM3, STK17A, APITD1, SIVA1, FAS, TGFβ2, TGFBR1, LOC378902, or BCL2A1, PUSL1, TPST1, WDR33, Nod2, MCT4, ACRC, AMELY, ATCAY, ANP32B, DEFA3, DHRS10, DOCK4, FAM106A, FKBP1B, IRF3, KBTBD8, KIAA0753, LPGAT1, MSMB, NFS1, NPIP, NPM3, SCGB2A1, SERPINB7, SLC16A4, SPTBN4, TMEM146, CDKN1B, CDKN2A, FOXO1, PTEN, FN1, CSKN2B, a miRNA antagonist, host sialidase, NEU2 sialidase 2, NEU3 sialidase 3, Dicer, ISRE, B4GalT1, B4GalT6, Cmas, Gne, SLC35A1, and a regulatory region of any of the foregoing.

[0065] In one aspect, the methods described herein relate to a method for improving the viability of a mammalian cell in culture, comprising: (a) contacting the cell with a plurality of different RNA effector molecules that permit inhibition of expression of Bax, Bak, and LDH; and (b) maintaining the cell for a time sufficient to inhibit expression of Bax, Bak, and LDH; wherein the inhibition of expression improves viability of the mammalian cell. In one embodiment of this aspect, the RNA effector molecule targeting BAX comprises a sense strand, and wherein at least one strand comprises at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides etc) of an oligonucleotide having a sequence selected from the group consisting of SEQ ID NOs:3152412-3152539, NOs:3152794-3152803, NOs:3023234-3023515, NOs:3154393-3154413, NOs:3154414-3154434, NOs:3154923-3154970, and NOs:3154971-3155018. In another embodiment of this aspect, the RNA effector molecule targeting BAK comprises a sense strand, and wherein at least one strand comprises at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides etc) of an oligonucleotide having a sequence selected from the group consisting of SEQ ID NOs:3152412-3152475, NOs:3152804-3152813, NOs:2259855-2260161, NOs:3154393-3154413, NOs:3154414-3154434, NOs:3154827-3154874, NOs:3154875-3154922 and sequences listed in Table 22. In another embodiment of this aspect, the RNA effector molecule targeting LDH comprises a sense strand, and wherein at least one strand comprises at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides etc) of an oligonucleotide having a sequence selected from the group consisting of SEQ ID NOs:3152540-3152603,

NOs:3152814-3152823, NOs:1297283-1297604, NOs:3154553-3154578, NOs:3154579-3154604, NOs:3155589-3155635, and NOs:3155636-3155682.

[0066] In one aspect, the methods described herein provide a method for producing an immunogenic agent in a large scale host cell culture, comprising: (a) contacting a host cell in a large scale host cell culture with at least a first RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell, (b) maintaining the host cell culture for a time sufficient to modulate expression of the at least one first target gene, wherein the modulation of expression improves production of an immunogenic agent in the host cell; (c) isolating theimmunogenic agent from the host cell; wherein the large scale host cell culture is at least 1 Liter in size, and wherein the host cell is contacted with at least a first RNA effector molecule by addition of the RNA effector molecule to a culture medium of the large scale host cell culture such that the target gene expression is inhibited transiently.

[0067] Also provided herein in another aspect, are methods for producing an immunogenic agent in a large scale host cell culture, comprising: (a) contacting a host cell in a large scale host cell culture with at least a first RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell, (b) maintaining the host cell culture for a time sufficient to modulate expression of the at least one first target gene, wherein the modulation of expression improves production of an immunogenic agent in the host cell; and (c) isolating the immunogenic agent from the host cell; wherein the host cell is contacted with at least a first RNA effector molecule by addition of the RNA effector molecule to a culture medium of the large scale host cell culture multiple times throughout production of the immunogenic agent such that the target gene expression is inhibited transiently.

[0068] In one embodiment of the aspects described herein, the host cell is contacted with the plurality of RNA effector molecules by addition of the RNA effector molecule to a culture medium of the large scale host cell culture such that the target gene expression is inhibited transiently.

[0069] In one embodiment of the aspects described herein, the host cell in the large scale host cell culture is contacted with a plurality of RNA effector molecules, wherein the plurality of RNA effector molecules modulate expression of at least one target gene, at least two target genes, or a plurality of target genes.

[0070] In another embodiment of the aspects described herein, the RNA effector molecule, or plurality of RNA effector molecules, comprises a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a

second sequence comprising a region of complementarity which is substantially complementary to at least part of a target gene, and wherein said region of complementarity is 10 to 30 nucleotides in length.

[0071] In another embodiment of the aspects described herein, the contacting step is performed by continuous infusion of the RNA effector molecule, or plurality of RNA effector molecules, into the culture medium used for maintaining the host cell culture to produce the immunogenic agent.

[0072] In another embodiment of the aspects described herein, the modulation of expression is inhibition of expression, and wherein the inhibition is a partial inhibition.

[0073] In another embodiment of the aspects described herien, the partial inhibition is no greater than a percent inhibition selected from the group consisting of 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, and 85%.

[0074] In another embodiment of the aspects described herein, the RNA effector molecule is contacted at a concentration of less than 100 nM.

[0075] In another embodiment of the aspects described herein, the RNA effector molecule is contacted at a concentration of less than 50 nM.

[0076] In some embodiments, at least one RNA effector molecule, a portion of which is complementary to the target gene, is a corresponding siRNA that comprises an antisense strand comprising at least 16 contiguous nucleotides of a nucleotide sequence, wherein the nucleotide sequence (SEQ ID NO) is referred to herein.

[0077] Also provided herein are compositions useful for enhancing production of an immunogenic agent. In one aspect, a composition is provided that comprises at least one RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell, and a cell medium suitable for culturing the host cell, wherein the RNA effector molecule is capable of modulating expression of the target gene and the modulation of expression enhances production of an immunogenic agent, wherein the at least one RNA effector molecule is an siRNA that comprises an antisense strand comprising at least 16 contiguous nucleotides of a nucleotide sequence (SEQ ID NO) referred to herein.

[0078] Another aspect described herein provides a kit for enhancing production of an immunogenic agent by a cultured cell, comprising: (a) a substrate comprising one or more assay surfaces suitable for culturing the cell under conditions in which the immunogenic agent is produced; (b) one or more RNA effector molecules, wherein at least a portion of each RNA effector molecule is complementary to a target gene; and (c) a reagent for detecting the immunogenic agent or production thereof by the cell, wherein the one or more RNA effector

molecules is an siRNA comprising an antisense strand that comprises at least 16 contiguous nucleotides of the nucleotide sequence (SEQ ID NO) referred to herein.

[0079] Also provided herein is a kit for optimizing production of an immunogenic agent by cultured cells, comprising: (a) a microarray substrate comprising a plurality of assay surfaces, the assay surfaces being suitable for culturing the cells under conditions in which the immunogenic agent is produced; (b) one or more RNA effector molecules, wherein at least a portion of each RNA effector molecule is complementary to a target gene; and (c) a reagent for detecting the effect of the one or more RNA effector molecules on production of the immunogenic agent, wherein the one or more RNA effector molecules is an siRNA comprising an antisense strand that comprises at least 16 contiguous nucleotides of a nucleotide sequence (SEQ ID NO) referred to herein.

[0080] In one embodiment, the invention provides for a host cell that contains at least one RNA effector molecule provided herein. The host cell can be derived from an insect, amphibian, fish, reptile, bird, mammal, or human, or can be a hybridoma cell. For example, the cell can be a human Namalwa Burkitt lymphoma cell (BLcl-kar-Namalwa), baby hamster kidney fibroblast (BHK), CHO cell, Murine myeloma cell (e.g., NS0, SP2/0), hybridoma cell, human embryonic kidney cell (293 HEK), human retina-derived cell (PER.C6® cells), insect cell line (Sf9, derived from pupal ovarian tissue of *Spodoptera frugiperda*; or Hi-5, derived from *Trichoplusia ni* egg cell homogenates), Madin-Darby canine kidney cell (MDCK), primary mouse brain cells or tissue, primary calf lymph cells or tissue, primary monkey kidney cell, embryonated chicken egg, primary chicken embryo fibroblast (CEF), Rhesus fetal lung cell (FRhL-2), Human fetal lung cell (WI-38, MRC-5), African green monkey kidney epithelial cell (e.g., Vero, CV-1), Rhesus monkey kidney cell (LLC-MK2), or yeast cell. In a particular embodiment, the cell is a MDCK cell.

[0081] Embodiments also provide compositions and methods for producing an immunogenic agent from a host cell, particularly from CHO cell, the methods comprising contacting the cell with a RNA effector molecule, such as one or more siRNA molecules targeting the CHO transcriptome transcripts, a portion of which is complementary to a target transcript, maintaining the cell in a bioreactor for a time sufficient to modulate expression of the target gene, wherein the modulation enhances production of the immunogenic agent from the cell, and isolating the immunogenic agent from the cell.

[0082] An advantage of the present invention is the ability to substantially increase the yield and/or purity of the immunogenic agents produced by the host cells, and thereby reduce production costs, or to significantly reduce development times. Improved manufacturing

logistics have the follow-on effect of enhancing quality, as well as expanding immunogenic agent product supply.

[0083] The details of one or more 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 claim.

DESCRIPTION OF THE DRAWINGS

[0084] Figures 1A and 1B: Figure 1A is am immunoblot labeling the Bax protein in day 2 CHO-S cells. The expression of Bax correlates with the decrease in viability over time in CHO-S cell cultures. The expression of Bax correlates with the decrease in viability over time in CHO-S cell cultures. Figure 1B is a graph depicting the growth curve for CHO-S cells showing cell viability, total cell number, and proportion of viable cells as a function of days in cell culture. Viability decreases sharply around day 6.

[0085] Figures 2A and 2B are graphs depicting concentration-dependent inhibition of expression of Bak (Figure 2B) and Bax (Figure 2A) in CHO cells by RNA effector molecules against hamster Bak and Bax genes (Tables 3 and 4, respectively). Each of the tested RNA effector molecules inhibited expression with an IC50 in the sub-nanomolar range, except for RNA effector molecule B2 against Bax, which inhibited expression with an IC50 in the low nanomolar range.

[0086] Figure 3 is a graph showing concentration-dependent inhibition of expression of LDH (measured as LDH activity) in CHO cells by RNA effector molecules against the hamster lactate dehydrogenase (LDH) gene. Each of the tested RNA effector molecules inhibited expression with an IC50 in the sub-nanomolar range.

[0087] Figures 4A to 4D: RNA effector molecules against hamster lactate dehydrogenase (LDH) decrease levels of LDH-A mRNA (Figure 4A), protein (Figure 4B), and activity (Figure 4C) in C2, C16 and C36 CHO cell lines relative to control cells. Inhibition of LDH significantly enhances productivity of the CHO cell lines (Figure 4D).

[0088] Figure 5A to 5B: Figure 5A is a bar graph and Figure 5B is a line graph, each showing the effect of RNA effector molecules against Bax/Bak and LDH on the viability of cultured CHO cells. siRNA (1 nM) were added to cultured cells at 0-hr, 48-hr and 96-hr timepoints (arrows on curve) and cell viability was measured as the integral cell area (ICA) at day 5 (graph) and over time (curve). Control cells were treated with Stealth siRNA (scrambled control). Cells treated with siRNA against Bax/Bak and LDH exhibited enhanced viability relative to control cells at all time points measured.

[0089] Figure 6 is a graph depicting that the addition of Bax/Bak/LDH siRNAs increases viable CHO cell density by at least 90%. Control cell (■) and treated cell (▲) densities were measured daily until cell viability reached 50%. Integral cell areas (IGA) were determined (inset; control vs. Bax/Bak/LDH siRNA-treated). Arrows on x-axis indicate siRNA dosing days or nutrient feed days.

[0090] Figure 7 is a graph depicting that the addition of Bax/Bak/LDH siRNAs increases percent viability of CHO by at least 50%. Percent viability of control cells (■) and cells treated with Bax/Bak/LDH siRNAs (▲) were determined using Trypan Blue. The rate of apoptotic cell death was determined by measuring the slopes of each sample from day-5 until day-12 (inset; control vs. Bax/Bak/LDH siRNA-treated). Arrows on x-axis indicate siRNA dosing days.

[0091] Figure 8 is a graph depicting that LDH enzyme activity is decreased in Bax/Bak/LDH siRNA-treated cells. Daily LDH activities were monitored in control-treated (■) and Bax/Bak/LDH siRNA-treated cells (▲). Arrows on x-axis indicate siRNA dosing days.

[0092] Figure 9 is a graph showing that lactate levels are lower in Bax/Bak/LDH siRNA-treated cell culture media compared to the control-treated cell media. Lactate levels in culture media were monitored daily in control siRNA-treated (■) and Bax/Bak/LDH siRNA-treated (▲) cell cultures. Arrows on x-axis indicate siRNA dosing days.

[0093] Figure 10 is a graph showing that glucose consumption in control siRNA-treated cells decreases following day 7 of the growth curve. Glucose levels from the Bax/Bak/LDH siRNA-treated cell media (▲) is significantly lower than the control siRNA-treated cell media (■). Arrows along x-axis indicate nutrient feed days.

[0094] Figure 11 is a graph showing that Bax/Bak/LDH siRNA-treated CHO cells have decreased Caspase 3 activity following log phase growth compared to control. Bax/Bak/LDH siRNA-treated cells demonstrate similar Caspase 3 activity to the control-siRNA-treated cells prior to day 6 but the following time points show higher Caspase activity in the control cells. A ratio (▲) between Caspase 3 activity in the Bax/Bak/LDH siRNA-treated cells and in control-treated cells shows a biphasic activity response.

[0095] Figure 12 is a graph showing the percent inhibition of mRNA level following Bax, Bak, and LDH siRNA addition.

[0096] Figure 13 is a graph depicting that Bax/ Bak/ LDH siRNA decreases CHO cell apoptosis death rate by ~300%.

[0097] Figure 14 is a graph depicting the viability and cell density of cell treated with Bax/Bak siRNA (1nM each) compared to a control FITC-siRNA (1nM).

[0098] Figures 15A and 15B: Figure 15A is a graph depicting the cell density and viability ratio of cells treated with siRNA targeting Bax/Bak/LDH compared to control treated cells. Figure 15B shows that Bax/Bak/LDH siRNA improves both CHO cell density and viability in a large scale, 1 L bioreactor.

[0099] Figure 16 shows a diagrammatic view of a computer system according to one embodiment of the invention.

[00100] Figure 17 shows a diagrammatic view of a computer system according to an laternative embodiment of the invention.

[00101] Figure 18 presents a diagram of the data structures according to one embodiment of the invention.

[00102] Figure 19 shows a flow diagram of a method according to one embodiment of the invention.

[00103] Figure 20 is a graph showing expression levels (fluorometric units, y-axis) of GFP over time in days (X-axis) in control DG44 CHO cells treated with lipid RNAiMax and no siRNAs, at temperatures of 37°C and 28°C, i.e. lipid treated control.

[00104] Figure 21 is a graph showing expression levels (fluorometric units, y-axis) of GFP over time in days (X-axis) in control DG44 CHO cells not treated with lipid RNAiMax or siRNAs, at temperatures of 37°C and 28°C, i.e untreated controls.

[00105] Figures 22A-22C are graphs showing the % inhibition of GFP expression (y-axis) in DG44 CHO cells by transiently transfected siRNAs against GFP at 37°C and 28 °C over time in days (x-axis). Fig. 22A, 0.1 nM siRNA. Fig. 22B, 1.0 nM siRNA. Fig. 22C, 10 nM siRNA.

[00106] Figure 23 is a bar graph showing relative % GFP signal knockdown (y-axis) using 9 uptake enhancing formulations compared to Lipofectamine RNAiMax, see Table 19, for the 9 formulations depicted on the x-axis.

[00107] Figure 24 is a bar graph showing LDH activity (y axis) using K8 (formulation 4) at various concentrations was effective as an uptake enhancer of siRNA against LDH in DG44 cells in a 250 mL shake flask.

[00108] Figure 25 is a bar graph showing LDH activity (y axis) using K8 (formulation 4), L8, and P8 formulations at various concentrations were effective as uptake enhancers of siRNA against LDH in DG44 in suspension.

[00109] Figures 26A-26B are graph showing cell density (Fig.26A) or % cell viability (Fig.26B) over time in suspension CHO cell 50 mL shake flasks using P8 formulation or commercial formulation RNAiMax at the recommended concentration. Lipid formulations were dosed onto cells at day 0.

[00110] Figure 27 is a graph that shows when sing the P8 NDL an siRNA directed against Lactate Dehydrogenase (LDH) achieves 80%-90% knockdown of LDH activity for 6 days with a single 1 nM dose in a 1 L bioreactor.

[00111] Figure 28 is a graph that shows the results of a single dose of a 1 nM LDH siRNA formulated with P8 lipid on viable cell density and % LDH activity over an elapsed time of 6 days in 3 L and 40 L cultures.

[00112] Figure 29 is a graph showing viable cell density and % viability (y-axis) over time in days after transfection of 40L of DG44 cell culture using P8 as the transfection reagent.

[00113] Figure 30 is a graph showing reduction in % LDH activity over time in 40L of DG44 cell culture and a single dose of siRNA at day 0.

[00114] Figures 31A and 31B are bar graphs of antibodies prepared from control cells of cells contacted with dsRNA targeting the fucosyltransferase (FUT8) and GDP-mannose 4,6-dehydratase (GMDS) genes. Fig. 31A is a graph that shows the concentration of antibody produced by these cells; Fig. 31B is a graph that shows that antibodies produced from the FUT8 and GMDS dsRNA treated cells have >85% reduced binding to fucose-specific lectin.

DETAILED DESCRIPTION

[00115] The present invention is not limited to the particular methodology, protocols, and compositions, etc., described herein, as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[00116] As used herein and in the claims, the singular forms include the plural reference and vice versa unless the context clearly indicates otherwise. Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about."

[00117] All patents, oligonucleotide sequences identified by gene identification numbers, and other publications identified herein are expressly incorporated by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the

applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00118] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood to one of ordinary skill in the art to which this invention pertains. Although human gene symbols are typically designated by upper-case letters, in the present specification the use of either upper-case or lower-case gene symbols may be used interchangeably and include both human or non-human species. Thus, for example, a reference in the specification to the gene or gene target "lactate dehydrogenase A" as "LDHA" ("Ldha" or "LdhA"), includes human and/or non-human (e.g., avian, rodent, canine) genes and gene targets. In other words, the upper-case or lower-case letters in a particular gene symbol do not limit the scope of the gene or gene target to human or non-human species. All gene identification numbers provided herein (GeneID) are those of the National Center for Biotechnology Information "Entrez Gene" web site unless identified otherwise.

[00119] The invention provides methods for producing an immunogenic agent in a host cell, the methods including the steps of contacting the cell with at least one RNA effector molecule, a portion of which is complementary to at least a portion of a target gene, maintaining the cell for a time sufficient to modulate expression of the target gene, wherein the modulation enhances production of the immunogenic agent, and recovering the immunogenic agent from the cell. The description provided herein discloses how to make and use RNA effector molecules to produce a immunogenic agent in a host cell according to methods provided herein. Also disclosed are cell culture reagents and compositions comprising the RNA effector molecules and kits for carrying out the disclosed methods.

I. Definitions

[00120] As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the invention, yet open to the inclusion of unspecified elements, whether essential or not.

[00121] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[00122] The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[00123] As used herein, "***immunogenic agent***" refers to an agent used to stimulate the immune system of a subject, so that one or more functions of the immune system are increased and directed towards the immunogenic agent. An antigen or immunogen is intended to mean a molecule containing one or more epitopes that can stimulate a host immune system to make a secretory, humoral and/or cellular immune response specific to that antigen. Immunogenic agents can be used in the production of antibodies, both isolated polyclonal antibodies and monoclonal antibodies, using techniques known in the art. Immunogenic agents include vaccines.

[00124] As used herein, "***vaccine***" refers to an agent used to stimulate the immune system of a subject so that protection is provided against an antigen not recognized as a self-antigen by the subject's immune system. Immunization refers to the process of inducing a high level of antibody and/or cellular immune response in a subject, that is directed against a pathogen or antigen to which the organism has been exposed. Vaccines and immunogenic agents as used herein, refer to a subject's immune system: the anatomical features and mechanisms by which a subject produces antibodies and/or cellular immune responses against an antigenic material that invades the subject's cells or extra-cellular fluids. In the case of antibody production, the antibody so produced can belong to any of the immunological classes, such as immunoglobulins, A, D, E, G, or M. Vaccines that stimulate production of immunoglobulin A (IgA) are of interest, because IgA is the principal immunoglobulinof the secretory system in warm-blooded animals. Vaccines are likely to produce a broad range of other immune responses in addition to IgA formation, for example cellular and humoral immunity. Immune responses to antigens are well-studied and reported widely. *See, e.g.*, Elgert, IMMUNOL. (Wiley Liss, Inc., 1996); Stites et al., BASIC & CLIN. IMMUNOL., (7th Ed., Appleton & Lange, 1991). By contrast, the phrase "immune response of the host cell" refers to the responses of unicellular host organisms to the presence of foreign bodies.

[00125] In the context of this invention, the term "***oligonucleotide***" or "nucleic acid molecule" encompasses not only nucleic acid molecules as expressed or found in nature, but also analogs and derivatives of nucleic acids comprising one or more ribo- or deoxyribo-nucleotide/nucleoside analogs or derivatives as described herein or as known in the art.. Such modified or substituted oligonucleotides are often used over native forms because of properties such as, for example, enhanced cellular uptake, increased stability in the presence of nucleases, and the like, discussed further herein. A "nucleoside" includes a nucleoside base and a ribose sugar, and a "nucleotide" is a nucleoside with one, two or three phosphate moieties. The terms "nucleoside" and "nucleotide" can be considered to be equivalent as used herein. An

oligonucleotide can be modified in the nucleobase structure or in the ribose-phosphate backbone structure, e.g., as described herein, including the modification of a RNA nucleotide into a DNA nucleotide. The molecules comprising nucleoside analogs or derivatives must retain the ability to form a duplex.

[00126] As non-limiting examples, an oligonucleotide can also include at least one modified nucleoside including but not limited to a 2'-O-methyl modified nucleoside, a nucleoside comprising a 5' phosphorothioate group, a terminal nucleoside linked to a cholesterol derivative or dodecanoic acid bisdecylamide group, a locked nucleoside, an abasic nucleoside, a 2'-deoxy-2'-fluoro modified nucleoside, a 2'-amino-modified nucleoside, 2'-alkyl-modified nucleoside, morpholino nucleoside, a phosphoramidate or a non-natural base comprising nucleoside, or any combination thereof. Alternatively, an oligonucleotide can comprise at least two modified nucleosides, 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 15, at least 20, or more, up to the entire length of the oligonucleotide. The modifications need not be the same for each of such a plurality of modified nucleosides in an oligonucleotide. When RNA effector molecule is double stranded, each strand can be independently modified as to number, type and/or location of the modified nucleosides. In one embodiment, modified oligonucleotides contemplated for use in methods and compositions described herein are peptide nucleic acids (PNAs) that have the ability to form the required duplex structure and that permit or mediate the specific degradation of a target RNA via a RISC pathway.

[00127] The terms “ribonucleoside”, “ribonucleotide”, “nucleotide”, or “deoxyribonucleotide” can also refer to a modified nucleotide, as further detailed herein, or a surrogate replacement moiety. A ribonucleotide comprising a thymine base is also referred to as 5-methyl uridine and a deoxyribonucleotide comprising a uracil base is also referred to as deoxy-Uridine in the art. Guanine, cytosine, adenine, thymine and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

[00128] Similarly, the skilled artisan will recognize that the term "***RNA molecule***" or "ribonucleic acid molecule" encompasses not only RNA molecules as expressed or found in nature, but also analogs and derivatives of RNA comprising one or more ribonucleotide or ribonucleoside analogs or derivatives as described herein or as known in the art. The terms "ribonucleoside" and "ribonucleotide" can be considered to be equivalent as used herein. The RNA can be modified in the nucleobase structure or in the ribose-phosphate backbone structure, e.g., as described herein.

[00129] In one aspect, a RNA effector molecule can include a deoxyribonucleoside residue. In such an instance, a RNA effector molecule agent can comprise one or more deoxynucleosides, including, for example, a deoxynucleoside overhang(s), or one or more deoxynucleosides within the double stranded portion of a dsRNA.

[00130] In some embodiments, a plurality of RNA effector molecules is used to modulate expression of one or more target genes. A "***plurality***" refers to at least 2 or more RNA effector molecules e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 80, 100 RNA effector molecules or more. "Plurality" can also refer to at least 2 or more target genes, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100 target genes or more.

[00131] As used herein the term "***contacting a host cell***" refers to the treatment of a host cell with an agent such that the agent is introduced into the cell. Typically the host cell is in culture, e.g., using at least one RNA effector molecule (e.g., a siRNA), often prepared in a composition comprising a delivery agent that facilitates RNA effector uptake into the cell e.g., to contact the cell in culture by adding the composition to the culture medium. In one embodiment the host cell is contacted with a vector that encodes a RNA effector molecule, e.g. an integrating or non-integrating vector. In one embodiment the cell is contacted with a vector that encodes a RNA effector molecule prior to culturing the host cell for immunogenic agent production, e.g., by transfection or transduction.

[00132] In one embodiment contacting a host cell does not include contacting the host cell with a vector that encodes a RNA effector molecule. In one embodiment, contacting a host cell does not include contacting a host cell with a vector the encodes a RNA effector molecule prior to culturing the host cell for immunogenic agent production, i.e., the cell is contacted with a RNA effector molecule only in cell growth culture, e.g., added to the host cell culture during the process of producing an immunogenic agent. For example, some embodiments of the present invention provide for contacting a host cell with a RNA effector molecule (e.g., a dsRNA) occurs prior to, during or after the viral infection or vector inoculation to inhibit cellular and

anti-viral processes that compromise the yield and quality of the immunogenic agent harvest. The step of contacting a host cell in culture with a RNA effector molecule(s) can be repeated more than once (e.g., twice, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 11x, 12x, 13x, 14x, 15x, 16x, 17x, 18x, 19x, 20x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, 100x or more). In one embodiment, the cell is contacted such that the target gene is modulated only transiently, e.g., by addition of a RNA effector molecule composition to the cell culture medium used for the production of an immunogenic agent where the presence of the RNA effector molecules dissipates over time, i.e., the RNA effector molecule is not constitutively expressed in the cell.

[00133] "***Introducing into a cell***", when referring to a RNA effector molecule, means facilitating or effecting uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of a RNA effector molecule can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. For example, introducing into a cell means contacting a host cell with at least one RNA effector molecule, or means the treatment of a cell with at least one RNA effector molecule and an agent that facilitates or effects uptake or absorption into the cell, often prepared in a composition comprising the RNA effector molecule and delivery agent that facilitates RNA effector molecule uptake (e.g., a transfection reagent, an emulsion, a cationic lipid, a non-cationic lipid, a charged lipid, a liposome, an anionic lipid, a penetration enhancer, or a modification to the RNA effector molecule to attach, e.g., a ligand, a targeting moiety, a peptide, a lipophillic group etc.). *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or known in the art.

[00134] As used herein, a "***RNA effector composition***" includes an effective amount of a RNA effector molecule and an acceptable carrier. As used herein, "effective amount" refers to that amount of a RNA effector molecule effective to produce an effect (e.g., modulatory effect) on a bioprocess for the production of an immunogenic agent. In one embodiment, the RNA effector composition comprises a reagent that facilitates RNA effector molecule uptake (e.g., a transfection reagent, an emulsion, a cationic lipid, a non-cationic lipid, a charged lipid, a liposome, an anionic lipid, a penetration enhancer, or a modification to the RNA effector molecule to attach e.g., a ligand, a targeting moiety, a peptide, a lipophillic group, etc.)

[00135] The term "***acceptable carrier***" refers to a carrier for administration of a RNA effector molecule to cultured cells. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. In one embodiment the term "acceptable carrier" specifically excludes cell culture medium.

[00136] The term "***expression***" as used herein is intended to mean the transcription to a RNA and/or translation to one or more polypeptides from a target gene coding for the sequence of the RNA and/or the polypeptide.

[00137] As used herein, "***target gene***" refers to a gene that encodes a protein that affects one or more aspects of the production of an immunogenic agent by a host cell, such that modulating expression of the gene enhances production of an immunogenic agent. Target genes can be derived from the host cell, endogenous to the host cell (present in the host cell genome), transgenes (gene constructs inserted at ectopic sites in the host cell genome), or derived from a pathogen (e.g., a virus, fungus or bacterium) that is capable of infecting the host cell or the subject who will use the immunogenic agent or derivatives thereof (e.g., humans). Additionally, in some embodiments, a "target gene"refers to a gene that regulates expression of a nucleic acid (i.e., non-encoding genes) that affects one or more aspects of the production of an immunogenic agent by a cell, such that modulating expression of the gene enhances production of the immunogenic agent.

[00138] By "target gene RNA" or "target RNA" is meant RNA transcribed from the target gene. Hence, a target gene can be a coding region, a promoter region, a 3' untranslated region (3'-UTR), and/or a 5'-UTR of the target gene.

[00139] A target gene RNA that encodes a polypeptide is more commonly known as messenger RNA (mRNA). Target genes can be derived from the host cell, latent in the host cell, endogenous to the host cell (present in the host cell genome), transgenes (gene constructs inserted at ectopic sites in the host cell genome), or derived from a pathogen (e.g., a virus, fungus or bacterium) which is capable of infecting either the host cell or the subject who will use the an immunogenic agent or derivatives or products thereof. In some embodiments, the target gene encodes a protein that affects one or more aspects of post-translational modification, e.g., peptide glycosylation, by a host cell. For example, modulating expression of a gene encoding a protein involved in post-translational processing enhances production of a polypeptide comprising at least one terminal mannose.

[00140] In some embodiments, the ***target gene*** encodes a non-coding RNA (***ncRNA***), such as an untranslated region. As used herein, a ncRNA refers to a target gene RNA that is not translated into a protein. The ncRNA can also be referred to as non-protein-coding RNA (npcRNA), non-messenger RNA (nmRNA), small non-messenger RNA (snmRNA), and functional RNA (fRNA) in the art. The target gene from which a ncRNA is transcribed as the end product is also referred to as a RNA gene or ncRNA gene. ncRNA genes include highly abundant and functionally important RNAs such as transfer RNA (tRNA) and ribosomal RNA

(rRNA), as well as RNAs such as snoRNAs, microRNAs, siRNAs, and piRNAs. As used herein, a RNA effector molecule is said to target within a particular site of a RNA transcript if the RNA effector molecule promotes cleavage of the transcript anywhere within that particular site.

[00141] In some embodiments, the target gene is an endogenous gene of the host cell. For example, the target gene can encode the immunogenic agent or a portion thereof when the immunogenic agent is a polypeptide. The target gene can also encode a host cell protein that directly or indirectly affects one or more aspects of the production of the immunogenic agent. Examples of target genes that affect the production of polypeptides include genes encoding proteins involved in the secretion, folding or post-translational modification of polypeptides (e.g., glycosylation, deamidation, disulfide bond formation, methionine oxidation, or pyroglutamation); genes encoding proteins that influence a property or phenotype of the host cell (e.g., growth, viability, cellular pH, cell cycle progression, apoptosis, carbon metabolism or transport, lactate formation, cytoskeletal structure (e.g., actin dynamics), susceptibility to viral infection or RNAi uptake, activity, or efficacy); and genes encoding proteins that impair the production of an immunogenic agent by the host cell (e.g., a protein that binds or co-purifies with the immunogenic agent).

[00142] In some embodiments, the target gene encodes a host cell protein that indirectly affects the production of the immunogenic agent such that inhibiting expression of the target gene enhances production of the immunogenic agent. For example, the target gene can encode an abundantly expressed host cell protein that does not directly influence production of the immunogenic agent, but indirectly decreases its production, for example by utilizing cellular resources that could otherwise enhance production of the immunogenic agent. Target genes are discussed in more detail herein.

[00143] The term "***modulates expression of***" and the like, in so far as it refers to a target gene, herein refers to the modulation of expression of a target gene, as manifested by a change (e.g., an increase or a decrease) in the amount of target gene mRNA that can be isolated from or detected in a first cell or group of cells in which a target gene is transcribed and that has or have been treated such that the expression of a target gene is modulated, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but that has or have not been so treated (control cells). The degree of modulation can be expressed in terms of:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

[00144] Alternatively, the degree of modulation can be given in terms of a parameter that is functionally linked to target gene expression, e.g., the amount of protein encoded by a target

gene, or the number of cells displaying a certain phenotype, e.g., stabilization of microtubules. In principle, target gene modulation can be determined in any host cell expressing the target gene, either constitutively or by genomic engineering, and by any appropriate assay known in the art.

[00145] For example, in certain instances, expression of a target gene is inhibited. For example, expression of a target gene is inhibited by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of a RNA effector molecule provided herein. In some embodiments, a target gene is inhibited by at least about 60%, 70%, or 80% by administration of a RNA effector molecule. In some embodiments, a target gene is inhibited by at least about 85%, 90%, or 95% or more by administration of a RNA effector molecule as described herein. In other instances, expression of a target gene is activated by at least about 10%, 20%, 25%, 50%, 100%, 200%, 400% or more by administration of a RNA effector molecule provided herein. In some embodiments, the modulation of expression is a partial inhibition. In some aspects, the partial inhibition is no greater than a percent inhibition selected from the group consisting of: 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, and 85%.

[00146] As used herein, the term "***RNA effector molecule***" refers to an oligonucleotide agent capable of modulating the expression of a target gene, as defined herein, within a host cell, or a oligonucleotide agent capable of forming such an oligonucleotide, optionally, within a host cell (i.e., upon being introduced into a host cell). A portion of a RNA effector molecule is substantially complementary to at least a portion of the target gene, such as the coding region, the promoter region, the 3' untranslated region (3'-UTR), and/or the 5'-UTR of the target gene.

[00147] The RNA effector molecules described herein generally have a first strand and a second strand, one of which is substantially complementary to at least a portion of the target gene and modulate expression of target genes by one or more of a variety of mechanisms, including but not limited to, Argonaute-mediated post-transcriptional cleavage of target gene mRNA transcripts (sometimes referred to in the art as RNAi) and/or other pre-transcriptional and pre-translational mechanisms.

[00148] RNA effector molecules can comprise a single strand or more than one strand, and can include, e.g., double stranded RNA (dsRNA), microRNA (miRNA), antisense RNA, promoter-directed RNA (pdRNA), Piwi-interacting RNA (piRNA), expressed interfering RNA (eiRNA), short hairpin RNA (shRNA), antagomirs, decoy RNA, DNA, plasmids, and aptamers. The RNA effector molecule can be single-stranded or double-stranded. A single-stranded RNA

effector molecule can have double-stranded regions and a double-stranded RNA effector can have single-stranded regions.

[00149] The term "***portion***", when used in reference to an oligonucleotide (e.g., a RNA effector molecule), refers to a portion of a RNA effector molecule having a desired length to effect complementary binding to a region of a target gene, or a desired length of a duplex region. For example, a "portion" or "region" refers to a nucleic acid sequence of at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 or more nucleotides up to one nucleotide shorter than the entire RNA effector molecule. In some embodiments, the "region" or "portion" when used in reference to a RNA effector molecule includes nucleic acid sequence one nucleotide shorter than the entire nucleic acid sequence of a strand of a RNA effector molecule. One of skill in the art can vary the length of the "portion" that is complementary to the target gene or arranged in a duplex, such that a RNA effector molecule having desired characteristics (e.g., inhibition of a target gene or stability) is produced. Although not bound by theory, RNA effector molecules provided herein can modulate expression of target genes by one or more of a variety of mechanisms, including but not limited to, Argonaute-mediated post-transcriptional cleavage of target gene mRNA transcripts (sometimes referred to in the art as RNAi) and/or other pre-transcriptional and/or pre-translational mechanisms.

[00150] RNA effector molecules disclosed herein include a RNA strand (the antisense strand) having a region which is 30 nucleotides or less in length, e.g., 10 to 30 nucleotides in length, or 19 to 24 nucleotides in length, which region is substantially complementary to at least a portion of a target gene that affects one or more aspects of the production of an immunogenic agent, such as the yield, purity, homogeneity, biological activity, or stability of the immunogenic agent. The RNA effector molecules interact with RNA transcripts of target genes and mediate their selective degradation or otherwise prevent their translation.

[00151] The term "***antisense strand***" refers to the strand of a RNA effector molecule, e.g., a dsRNA, which includes a region that is substantially complementary to a target sequence. The term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, e.g., within 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

[00152] The term "***sense strand***" refers to the strand of a RNA effector molecule that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

[00153] As used herein, and unless otherwise indicated, the term "***complementary***", when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as understood by the skilled artisan. "Complementary" sequences can also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs includes, but are not limited to, G:U Wobble or Hoogstein base pairing. Hybridization conditions can, for example, be stringent conditions, where stringent conditions can include 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C, for 12 to 16 hours followed by washing. Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled artisan will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

[00154] The terms "complementary," "fully complementary" and "substantially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a RNA effector molecule agent and a target sequence, as will be understood from the context of use. As used herein, an oligonucleotide that is "substantially complementary to at least part of" a target gene refers to an oligonucleotide that is substantially complementary to a contiguous portion of a target gene of interest (e.g., a mRNA encoded by a target gene, the target gene's promoter region or 3' UTR, or ERV LTR). For example, an oligonucleotide is complementary to at least a part of a target mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoded by a target gene.

[00155] Complementary sequences within a RNA effector molecule, e.g., within a dsRNA (a double-stranded ribonucleic acid) as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. Where a first sequence is referred to as "substantially complementary" with

respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3 or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, e.g., inhibition of gene expression via a RISC pathway. Where two oligonucleotides are designed to form, upon hybridization, one or more single-stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as “fully complementary” for the purposes described herein.

[00156] In some embodiments, the RNA effector molecule comprises a ***single-stranded*** oligonucleotide that interacts with and directs the cleavage of RNA transcripts of a target gene. For example, single stranded RNA effector molecules comprise a 5’ modification including one or more phosphate groups or analogs thereof to protect the effector molecule from nuclease degradation. The RNA effector molecule can be a single-stranded antisense nucleic acid having a nucleotide sequence that is complementary to at least a portion of a “sense” nucleic acid of a target gene, e.g., the coding strand of a double-stranded cDNA molecule or a RNA sequence, e.g., a pre-mRNA, mRNA, miRNA, or pre-miRNA. Accordingly, an antisense nucleic acid can form hydrogen bonds with a sense nucleic acid target. In an alternative embodiment, the RNA effector molecule comprises a duplex region of at least nine nucleotides in length.

[00157] Given a coding strand sequence (e.g., the sequence of a sense strand of a cDNA molecule), antisense nucleic acids can be designed according to the rules of Watson-Crick base pairing. The antisense nucleic acid can be complementary to a portion of the coding or noncoding region of a RNA, e.g., the region surrounding the translation start site of a pre-mRNA or mRNA, e.g., the 5’ UTR. An antisense oligonucleotide can be, for example, about 10 to 25 nucleotides in length (e.g., 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, or 24 nucleotides in length). In some embodiments, the antisense oligonucleotide comprises one or more modified nucleotides, e.g., phosphorothioate derivatives and/or acridine substituted nucleotides, designed to increase its biological stability of the molecule and/or the physical stability of the duplexes formed between the antisense and target nucleic acids. Antisense oligonucleotides can comprise ribonucleotides only, deoxyribonucleotides only (e.g., oligodeoxynucleotides), or both deoxyribonucleotides and ribonucleotides. For example, an antisense agent consisting only of

ribonucleotides can hybridize to a complementary RNA and prevent access of the translation machinery to the target RNA transcript, thereby preventing protein synthesis. An antisense molecule including only deoxyribonucleotides, or deoxyribonucleotides and ribonucleotides, can hybridize to a complementary RNA and the RNA target can be subsequently cleaved by an enzyme, e.g., RNAse H, to prevent translation. The flanking RNA sequences can include 2'-O-methylated nucleotides, and phosphorothioate linkages, and the internal DNA sequence can include phosphorothioate internucleotide linkages. The internal DNA sequence is preferably at least five nucleotides in length when targeting by RNAseH activity is desired.

[00158] In some embodiments, RNA effector molecule is a ***double-stranded*** oligonucleotide. The term "double-stranded RNA" or "***dsRNA***", as used herein, refers to an oligonulceotide molecule or complex of molecules having a hybridized duplex region that comprises two anti-parallel and substantially complementary nucleic acid strands, which will be referred to as having "sense" and "antisense" orientations with respect to a target RNA. Typically, region of complementarity is 30 nucleotides or less in length, generally, for example, 10 to 26 nucleotides in length, 18 to 25 nucleotides in length, or 19 to 24 nucleotides in length, inclusive. Upon contact with a cell expressing the target gene, the RNA effector molecule inhibits the expression of the target gene by at least 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by protein immunoblot. Expression of a target gene in cell culture can be assayed by measuring target gene mRNA levels, e.g., by bDNA or TAQMAN® assay, or by measuring protein levels, e.g., by immunofluorescence analysis.

[00159] The duplex region can be of any length that permits specific degradation of a desired target RNA through a RISC pathway, but will typically range from 9 to 36 base pairs in length, e.g., 15 to 30 base pairs in length. More specifically, the duplex region can be of any length that permits specific degradation of a desired target RNA through a RISC pathway, but will typically range from 9 to 36 base pairs in length, e.g., 15 to 30 base pairs in length. Considering a duplex between 9 and 36 base pairs, the duplex can be any length in this range, for example, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 and any sub-range there between, including, but not limited to 15 to 30 base pairs, 15 to 26 base pairs, 15 to 23 base pairs, 15 to 22 base pairs, 15 to 21 base pairs, 15 to 20 base pairs, 15 to 19 base pairs, 15 to 18 base pairs, 15 to 17 base pairs, 18 to 30 base pairs, 18 to 26 base pairs, 18 to 23 base pairs, 18 to 22 base pairs, 18 to 21 base pairs, 18 to 20 base pairs, 19 to 30 base pairs, 19 to 26 base pairs, 19 to 23 base pairs, 19 to 22 base pairs, 19 to 21 base pairs, 19 to 20 base pairs, 20 to 30 base pairs, 20 to 26 base pairs, 20 to 25 base pairs, 20

to 24 base pairs, 20 to 23 base pairs, 20 to 22 base pairs, 20 to 21 base pairs, 21 to 30 base pairs, 21 to 26 base pairs, 21 to 25 base pairs, 21 to 24 base pairs, 21 to 23 base pairs, or 21 to 22 base pairs, inclusive.

[00160] dsRNAs generated in the cell by processing with Dicer and similar enzymes are generally in the range of 19 to 22 base pairs in length. One strand of the duplex region of a dsDNA comprises a sequence that is substantially complementary to a region of a target RNA. The two strands forming the duplex structure can be from a single RNA molecule having at least one self-complementary region, or can be formed from two or more separate RNA molecules. Where the duplex region is formed from two strands of a single molecule, the molecule can have a duplex region separated by a single stranded chain of nucleotides (a "***hairpin loop***") between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure. The hairpin loop can comprise at least one unpaired nucleotide; in some embodiments the hairpin loop can comprise 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 20, at least 23 or more unpaired nucleotides. Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than a hairpin loop, the connecting structure is referred to as a "linker." The term "sRNA effector molecule" is also used herein to refer to a dsRNA.

[00161] Described herein are RNA effector molecules that modulate expression of a target gene. In one embodiment, the RNA effector molecule agent includes double-stranded ribonucleic acid (***dsRNA***) molecules for inhibiting the expression of a target gene in a cell, where the dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of a target gene formed in the expression of a target gene, and where the region of complementarity is 30 nucleotides or less in length, generally 10 to 24 nucleotides in length, and where the dsRNA, upon contact with an cell expressing the target gene, inhibits the expression of the target gene by at least 10% as assayed by, for example, a PCR, PERT, or bDNA-based method, or by a protein-based method, such as a protein immunoblot (e.g., a western blot). Expression of a target gene in an cell can be assayed by measuring target gene mRNA levels, e.g., by PERT, bDNA or TAQMAN® gene expression assay, or by measuring protein levels, e.g., by immunofluorescence analysis or quantitative protein immunoblot.

[00162] A dsRNA includes two RNA strands that are sufficiently complementary to hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is

substantially complementary, and generally fully complementary, to a target sequence, derived, for example, from the sequence of an mRNA formed during the expression of a target gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Generally, the duplex structure is, for example between 9 and 36, between 10 to 30 base pairs, between 18 and 25, between 19 and 24, or between 19 and 21 base pairs in length, inclusive. Similarly, the region of complementarity to the target sequence is, for example, between 10 and 30, between 18 and 25, between 19 and 24, or between 19 and 21 nucleotides in length, inclusive. In some embodiments, the dsRNA is between 10 and 20 nucleotides in length, inclusive, and in other embodiments, the dsRNA is between 25 and 30 nucleotides in length, inclusive. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex of e.g., 15 to 30 base pairs that targets a desired RNA for cleavage, a RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. As the ordinarily skilled person will recognize, the targeted region of a RNA targeted for cleavage will most often be part of a larger RNA molecule, often a mRNA molecule.

[00163] Where relevant, a "part" of a mRNA target is a contiguous sequence of a mRNA target of sufficient length to be a substrate for RNAi-directed cleavage (i.e., cleavage through a RISC pathway). dsRNAs having duplexes as short as 9 base pairs can, under some circumstances, mediate RNAi-directed RNA cleavage. Most often a target will be at least 10 nucleotides in length, such as from 15 to 30 nucleotides in length, inclusive.

[00164] The skilled person is well aware that dsRNAs having a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference. Elbashir et al., 20 EMBO 6877-88 (2001). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences, dsRNAs described herein can include at least one strand of a length of 21 nucloetides. It can be reasonably expected that shorter duplexes having one of the sequences minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described in detail. Hence, dsRNAs having a partial sequence of at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from a given sequence, and differing in their ability to inhibit the expression of a target gene by not more than 5%, 10%, 15%, 20%, 25%, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated according to the invention.

[00165] The dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch Technologies (Novato, CA). In one embodiment, a target

gene is a human target gene. In specific embodiments, the first sequence is a sense strand of a dsRNA that includes a sense sequence and the second sequence is a strand of a ds RNA that includes an antisense sequence. Alternative dsRNA agents that target elsewhere in the target sequence can readily be determined using the target sequence and the flanking target sequence. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a target gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand and the second oligonucleotide is described as the antisense strand. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

[00166] A double-stranded oligonucleotide can include one or more single-stranded nucleotide ***overhangs***. As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the terminus of a duplex structure of a double-stranded oligonucleotide, e.g., a dsRNA. For example, when a 3'-end of one strand of double-stranded oligonucleotide extends beyond the 5'-end of the other strand, or vice versa, there is a nucleotide overhang. A double-stranded oligonucleotide can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5' end, 3' end, or both ends of either an antisense or sense strand of a dsRNA.

[00167] In one embodiment, at least one end of a dsRNA has a single-stranded nucleotide ***overhang*** of 1 to 4, generally 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties relative to their blunt-ended counterparts. Moreover, the presence of a nucleotide overhang on only one strand, at one end of a dsRNA, strengthens the interference activity of the dsRNA, without affecting its overall stability. Such an overhang need not be a single nucleotide overhang; a dinucleotide overhang can also be present.

[00168] The antisense strand of a double-stranded oligonucleotide has a 1 to 10 nucleotide overhang at the 3' end and/or the 5' end, such as a double-stranded oligonucleotide having a 1 to 10 nucleotide overhang at the 3' end and/or the 5' end. One or more of the

internucloside linkages in the overhang can be replaced with a phosphorothioate. In some embodiments, the overhang comprises one or more deoxyribonucleoside or the overhang comprises one or more dT, e.g. the sequence 5'-dTdT-3' or 5'-dTdTdT-3'. In some embodiments, overhang comprises the sequence 5'-dT*dT-3, wherein * is a phosphorothioate internucleoside linkage.

[00169] Without being bound theory, double-stranded oligonucleotides having at least one nucleotide overhang have unexpectedly superior inhibitory properties relative to their blunt-ended counterparts. Moreover, the presence of a nucleotide overhang on only one strand, at one end of a dsRNA, strengthens the interference activity of the double-stranded oligonucleotide, without affecting its overall stability.

[00170] dsRNA having only one overhang has proven particularly stable and effective *in vivo*, as well as in a variety of cells, cell culture media, blood, and serum. Generally, the single-stranded overhang is located at the 3'-terminal end of an antisense strand or, alternatively, at the 3'-terminal end of a sense strand. The dsRNA having an overhang on only one end will also have one blunt end, generally located at the 5'-end of the antisense strand. Such dsRNAs have superior stability and inhibitory activity, thus allowing administration at low dosages, i.e., less than 5 mg/kg body weight of the recipient per day. In one embodiment, the antisense strand of a dsRNA has a 1 to 10 nucleotide overhang at the 3' end and/or the 5' end. In one embodiment, the sense strand of a dsRNA has a 1 to 10 nucleotide overhang at the 3' end and/or the 5' end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

[00171] The terms "blunt" or "***blunt ended***" as used herein in reference to double-stranded oligonucleotide mean that there are no unpaired nucleotides or nucleotide analogs at a given terminal end of a double-stranded oligonuleotide, i.e., no nucleotide overhang. One or both ends of a double-stranded oligonucleotide can be blunt. Where both ends are blunt, the oligonucleotide is said to be double-blunt ended. To be clear, a "double-blunt ended" oligonucleotide is a double-stranded oligonucleotide that is blunt at both ends, i.e., no nucleotide overhang at either end of the molecule. Most often such a molecule will be double-stranded over its entire length. When only one end of is blunt, the oligonucleotide is said to be single-blunt ended. To be clear, a "single-blunt ended" oligonucleotide is a double-stranded oligonucleotide that is blunt at only one end, i.e., no nucleotide overhang at one end of the molecule. Generally, a single-blunt ended oligonucleotide is blunt ended at the 5'-end of sense stand.

[00172] A RNA effector molecule as described herein can contain one or more ***mismatches*** to the target sequence. For example, a RNA effector molecule as described herein

contains no more than three mismatches. If the antisense strand of the RNA effector molecule contains mismatches to a target sequence, it is preferable that the area of mismatch not be located in the center of the region of complementarity. If the antisense strand of the RNA effector molecule contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5' or 3' end of the region of complementarity. For example, for a 23-nucleotide RNA effector molecule agent RNA strand which is complementary to a region of a target gene, the RNA strand generally does not contain any mismatch within the central 13 nucleotides. The methods described herein, or methods known in the art, can be used to determine whether a RNA effector molecule containing a mismatch to a target sequence is effective in inhibiting the expression of a target gene. Consideration of the efficacy of RNA effector molecules with mismatches in inhibiting expression of a target gene is important, especially if the particular region of complementarity in a target gene is known to have polymorphic sequence variation within the population.

[00173] In some embodiments, the RNA effector molecule is a promoter-directed RNA (***pdRNA***) which is substantially complementary to at least a portion of a noncoding region of an mRNA transcript of a target gene. In one embodiment, the pdRNA is substantially complementary to at least a portion of the promoter region of a target gene mRNA at a site located upstream from the transcription start site, e.g., more than 100, more than 200, or more than 1,000 bases upstream from the transcription start site. In another embodiment, the pdRNA is substantially complementary to at least a portion of the 3'-UTR of a target gene mRNA transcript. In one embodiment, the pdRNA comprises dsRNA of 18-28 bases optionally having 3' di- or tri-nucleotide overhangs on each strand. The dsRNA is substantially complementary to at least a portion of the promoter region or the 3'-UTR region of a target gene mRNA transcript. In another embodiment, the pdRNA comprises a gapmer consisting of a single stranded polynucleotide comprising a DNA sequence which is substantially complementary to at least a portion of the promoter or the 3'-UTR of a target gene mRNA transcript, and flanking the polynucleotide sequences (e.g., comprising the 5 terminal bases at each of the 5' and 3' ends of the gapmer) comprising one or more modified nucleotides, such as 2' MOE, 2'OMe, or Locked Nucleic Acid bases (LNA), which protect the gapmer from cellular nucleases.

[00174] pdRNA can be used to selectively increase, decrease, or otherwise modulate expression of a target gene. Without being limited to theory, it is believed that pdRNAs modulate expression of target genes by binding to endogenous antisense RNA transcripts which overlap with noncoding regions of a target gene mRNA transcript, and recruiting Argonaute proteins (in the case of dsRNA) or host cell nucleases (e.g., RNase H) (in the case of gapmers)

to selectively degrade the endogenous antisense RNAs. In some embodiments, the endogenous antisense RNA negatively regulates expression of the target gene and the pdRNA effector molecule activates expression of the target gene. Thus, in some embodiments, pdRNAs can be used to selectively activate the expression of a target gene by inhibiting the negative regulation of target gene expression by endogenous antisense RNA. Methods for identifying antisense transcripts encoded by promoter sequences of target genes and for making and using promoter-directed RNAs are known, *see, e.g.*, WO 2009/046397.

[00175] In some embodiments, the RNA effector molecule comprises an ***aptamer*** which binds to a non-nucleic acid ligand, such as a small organic molecule or protein, e.g., a transcription or translation factor, and subsequently modifies (e.g., inhibits) activity. An aptamer can fold into a specific structure that directs the recognition of a targeted binding site on the non-nucleic acid ligand. Aptamers can contain any of the modifications described herein.

[00176] In some embodiments, the RNA effector molecule comprises an ***antagomir***. Antagomirs are single stranded, double stranded, partially double stranded or hairpin structures that target a microRNA. An antagomir consists essentially of or comprises at least 10 or more contiguous nucleotides substantially complementary to an endogenous miRNA and more particularly a target sequence of an miRNA or pre-miRNA nucleotide sequence. Antagomirs preferably have a nucleotide sequence sufficiently complementary to a miRNA target sequence of about 12 to 25 nucleotides, such as about 15 to 23 nucleotides, to allow the antagomir to hybridize to the target sequence. More preferably, the target sequence differs by no more than 1, 2, or 3 nucleotides from the sequence of the antagomir. In some embodiments, the antagomir includes a non-nucleotide moiety, e.g., a cholesterol moiety, which can be attached, e.g., to the 3' or 5' end of the oligonucleotide agent.

[00177] In some embodiments, antagomirs are stabilized against nucleolytic degradation by the incorporation of a modification, e.g., a nucleotide modification. For example, in some embodiments, antagomirs contain a phosphorothioate comprising at least the first, second, and/or third internucleotide linkages at the 5' or 3' end of the nucleotide sequence. In further embodiments, antagomirs include a 2'-modified nucleotide, e.g., a 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA). In some embodiments, antagomirs include at least one 2'-O-methyl-modified nucleotide.

[00178] In some embodiments, the RNA effector molecule is a promoter-directed RNA (***pdRNA***) which is substantially complementary to at least a portion of a noncoding region of an

mRNA transcript of a target gene. The pdRNA can be substantially complementary to at least a portion of the promoter region of a target gene mRNA at a site located upstream from the transcription start site, e.g., more than 100, more than 200, or more than 1,000 bases upstream from the transcription start site. Also, the pdRNA can substantially complementary to at least a portion of the 3'-UTR of a target gene mRNA transcript. For example, the pdRNA comprises dsRNA of 18 to 28 bases optionally having 3' di- or tri-nucleotide overhangs on each strand. The dsRNA is substantially complementary to at least a portion of the promoter region or the 3'-UTR region of a target gene mRNA transcript. In another embodiment, the pdRNA comprises a gapmer consisting of a single stranded polynucleotide comprising a DNA sequence which is substantially complementary to at least a portion of the promoter or the 3'-UTR of a target gene mRNA transcript, and flanking the polynucleotide sequences (e.g., comprising the 5 terminal bases at each of the 5' and 3' ends of the gapmer) comprising one or more modified nucleotides, such as 2'MOE, 2'OMe, or Locked Nucleic Acid bases (LNA), which protect the gapmer from cellular nucleases.

[00179] pdRNA can be used to selectively increase, decrease, or otherwise modulate expression of a target gene. Without being limited to theory, pdRNAs can modulate expression of target genes by binding to endogenous antisense RNA transcripts which overlap with noncoding regions of a target gene mRNA transcript, and recruiting Argonaute proteins (in the case of dsRNA) or host cell nucleases (e.g., RNase H) (in the case of gapmers) to selectively degrade the endogenous antisense RNAs. In some embodiments, the endogenous antisense RNA negatively regulates expression of the target gene and the pdRNA effector molecule activates expression of the target gene. Thus, in some embodiments, pdRNAs can be used to selectively activate the expression of a target gene by inhibiting the negative regulation of target gene expression by endogenous antisense RNA. Methods for identifying antisense transcripts encoded by promoter sequences of target genes and for making and using promoter-directed RNAs are known. *See, e.g.*, WO 2009/046397.

[00180] Expressed interfering RNA (***eiRNA***) can be used to selectively increase, decrease, or otherwise modulate expression of a target gene. Typically, eiRNA, the dsRNA is expressed in the first transfected cell from an expression vector. In such a vector, the sense strand and the antisense strand of the dsRNA can be transcribed from the same nucleic acid sequence using e.g., two convergent promoters at either end of the nucleic acid sequence or separate promoters transcribing either a sense or antisense sequence. Alternatively, two plasmids can be cotransfected, with one of the plasmids designed to transcribe one strand of the dsRNA while the other is designed to transcribe the other strand. Methods for making and using eiRNA

effector molecules are known in the art. *See, e.g.,* WO 2006/033756; U.S. Patent Pubs. No. 2005/0239728 and No. 2006/0035344.

[00181] In some embodiments, the RNA effector molecule comprises a small single-stranded Piwi-interacting RNA (***piRNA*** effector molecule) which is substantially complementary to at least a portion of a target gene, as defined herein, and which selectively binds to proteins of the Piwi or Aubergine subclasses of Argonaute proteins. Without being limited to a particular theory, it is believed that piRNA effector molecules interact with RNA transcripts of target genes and recruit Piwi and/or Aubergine proteins to form a ribonucleoprotein (RNP) complex that induces transcriptional and/or post-transcriptional gene silencing of target genes. A piRNA effector molecule can be about 10 to 50 nucleotides in length, about 25 to 39 nucleotides in length, or about 26 to 31 nucleotides in length. *See, e.g.,* U.S. Patent Pub. No. 2009/0062228.

[00182] ***MicroRNAs*** are a highly conserved class of small RNA molecules that are transcribed from DNA in the genomes of plants and animals, but are not translated into protein. Pre-microRNAs are processed into miRNAs. Processed microRNAs are single stranded ~17-25 nucleotide (nt) RNA molecules that become incorporated into the RNA-induced silencing complex (RISC) and have been identified as key regulators of development, cell proliferation, apoptosis and differentiation. They are believed to play a role in regulation of gene expression by binding to the 3'-untranslated region of specific mRNAs. MicroRNAs cause post-transcriptional silencing of specific target genes, e.g., by inhibiting translation or initiating degradation of the targeted mRNA. In some embodiments, the miRNA is completely complementary with the target nucleic acid. In other embodiments, the miRNA has a region of noncomplementarity with the target nucleic acid, resulting in a "bulge" at the region of non-complementarity. In some embodiments, the region of noncomplementarity (the bulge) is flanked by regions of sufficient complementarity, e.g., complete complementarity, to allow duplex formation. For example, the regions of complementarity are at least 8 to 10 nucleotides long (e.g., 8, 9, or 10 nucleotides long).

[00183] miRNA can inhibit gene expression by, e.g., repressing translation, such as when the miRNA is not completely complementary to the target nucleic acid, or by causing target RNA degradation, when the miRNA binds its target with perfect or a high degree of complementarity.In further embodiments, the RNA effector molecule can include an oligonucleotide agent which targets an endogenous miRNA or pre-miRNA. For example, the RNA effector can target an endogenous miRNA which negatively regulates expression of a target gene, such that the RNA effector alleviates miRNA-based inhibition of the target gene.

The oligonucleotide agent can include naturally occurring nucleobases, sugars, and covalent internucleotide (backbone) linkages and/or oligonucleotides having one or more non-naturally-occurring features that confer desirable properties, such as enhanced cellular uptake, enhanced affinity for the endogenous miRNA target, and/or increased stability in the presence of nucleases. In some embodiments, an oligonucleotide agent designed to bind to a specific endogenous miRNA has substantial complementarity, e.g., at least 70%, 80%, 90%, or 100% complementary, with at least 10, 20, or 25 or more bases of the target miRNA. Exemplary oligonucleiotde agents that target miRNAs and pre-miRNAs are described, for example, in U.S. Patent Pubs. No. 20090317907, No. 20090298174, No. 20090291907, No. 20090291906, No. 20090286969, No. 20090236225, No. 20090221685, No. 20090203893, No. 20070049547, No. 20050261218, No. 20090275729, No. 20090043082, No. 20070287179, No. 20060212950, No. 20060166910, No. 20050227934, No. 20050222067, No. 20050221490, No. 20050221293, No. 20050182005, and No. 20050059005.

[00184] A miRNA or pre-miRNA can be 10 to 200 nucleotides in length, for example from 16 to 80 nucleotides in length. Mature miRNAs can have a length of 16 to 30 nucleotides, such as 21 to 25 nucleotides, particularly 21, 22, 23, 24, or 25 nucleotides in length. miRNA precursors can have a length of 70 to 100 nucleotides and can have a hairpin conformation. In some embodiments, miRNAs are generated *in vivo* from pre-miRNAs by the enzymes cDicer and Drosha. miRNAs or pre-miRNAs can be synthesized *in vivo* by a cell-based system or can be chemically synthesized. miRNAs can comprise modifications which impart one or more desired properties, such as superior stability, hybridization thermodynamics with a target nucleic acid, targeting to a particular tissue or cell-type, and/or cell permeability, e.g., by an endocytosis-dependent or -independent mechanism. Modifications can also increase sequence specificity, and consequently decrease off-site targeting.

[00185] In further embodiments, the RNA effector molecule can comprise an oligonucleotide agent which targets an endogenous miRNA or pre-miRNA. For example, the RNA effector can target an endogenous miRNA which negatively regulates expression of a target gene, such that the RNA effector alleviates miRNA-based inhibition of the target gene.

[00186] As used herein, the phrase "***in the presence of at least one RNA effector molecule***" encompasses exposure of the cell to a RNA effector molecule experessed within the cell, e.g., shRNA, or exposure by exogenous addition of the RNA effector molecule to the cell, e.g., delivery of the RNA effector molecule to the cell, optionally using an agent that facilitates uptake into the cell. A portion of a RNA effector molecule is substantially complementary to at least a portion of the target gene RNA, such as the coding region, the promoter region, the 3'

untranslated region (3'-UTR), or a long terminal repeat (LTR) of the target gene RNA. RNA effector molecules disclosed herein include a RNA strand (the antisense strand) having a region which is 30 nucleotides or less in length, e.g., 10 to 200 nucleotides in length, or 19 to 24 nucleotides in length, which region is substantially complementary to at least a portion of a target gene which encodes a protein that affects one or more aspects of the production of a immunogenic agent, such as the yield, purity, homogeneity, biological activity, or stability of the immunogenic agent. A RNA effector molecule interacts with RNA transcripts of a target gene and mediates its selective degradation or otherwise prevents its translation. In various embodiments of the present invention, the RNA effector molecule is at least one gapmer, or siRNA, miRNA, dsRNA, saRNA, shRNA, piRNA, tkRNAi, eiRNA, pdRNA, antagomir, or ribozyme.

[00187] Double-stranded and single-stranded oligonucleotides that are effective in inducing RNA interference are also referred to as siRNA, RNAi agent, or iRNA agent, herein. These RNA interference inducing oligonucleotides associate with a cytoplasmic multi-protein complex known as RNAi-induced silencing complex (RISC). Without being bound by theory, RNA interference leads to Argonaute-mediated post-transcriptional cleavage of target gene mRNA transcripts. In many embodiments, single-stranded and double-stranded RNAi agents are sufficiently long that they can be cleaved by an endogenous molecule, e.g. by Dicer, to produce smaller oligonucleotides that can enter the RISC machinery and participate in RISC mediated cleavage of a target sequence, e.g., a target mRNA.

[00188] In some embodiments, the RNAs provided herein identify a site in a target transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features RNA effector molecules that target within one of such sequences. Such a RNA effector molecule will generally include at least 10 contiguous nucleotides from one of the sequences provided coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a target gene.

[00189] The phrase "***genome information***" as used herein and throughout the claims and specification is meant to refer to sequence information from partial or entire genome of an organism, including protein coding and non-coding regions. These sequences are present every cell originating from the same organisms. As opposed to the transcriptome sequence information, genome information comprises not only coding regions, but also, for example, intronic sequences, promoter sequences, silencer sequences and enhancer sequences. Thus, the "genome information" can refer to, for example a human genome, a mouse genome, a rat genome. One can use complete genome information or partial genome information to add an

additional dimension to the database sequences to increase the potential targets to modify with a RNA effector molecule.

[00190] The phrase "***play a role***" refers to any activity of a transcript or a protein in a molecular pathway known to a skilled artisan or identified elsewhere in this specification. Such pathways an cellular activities include, but are not limited to apoptosis, cell division, glycosylation, growth rate, a cellular productivity, a peak cell density, a sustained cell viability, a rate of ammonia production or consumption, or a rate of lactate production.

[00191] A "***bioreactor***", as used herein, refers generally to any reaction vessel suitable for growing and maintaining host cells such that the host cells produce an immunogenic agent, and for recovering such immunogenic agent. Bioreactors described herein include cell culture systems of varying sizes, such as small culture flasks, Nunc multilayer cell factories, small high yield bioreactors (e.g., MiniPerm, INTEGRA-CELLine), spinner flasks, hollow fiber-WAVE bags (Wave Biotech, Tagelswangen, Switzerland), and industrial scale bioreactors. In some embodiments, the immunogenic agent is produced in a "***large scale culture***" bioreactor having a 1 L capacity or more, suitable for pharmaceutical or industrial scale production of immunogenic agents (e.g., a volume of at least 1 L, least 2 L, at least 5 L, at least 10 L, at least 25 L, at least 50 L, at least 100 L, or more, inclusive), often including means of monitoring pH, glucose, lactate, temperature, and/or other bioprocess parameters. In one embodiment , a large scale culture is at least 1 L in volume.

[00192] In one embodiment , a large scale culture is at least 2 L in volume. In one embodiment , a large scale culture is at least 5 L in volume. In one embodiment , a large scale culture is at least 25 L in volume. In one embodiment , a large scale culture is at least 40 L in volume. In one embodiment , a large scale culture is at least 50 L in volume. In one embodiment, a large scale culture is at least 100 L in volume.

[00193] A "***host cell***", as used herein, is any cell, cell culture, cellular biomass or tissue, capable of being grown and maintained in cell culture under conditions allowing for production and recovery of useful quantities of an immunogenic agent, as defined herein. A host cell can be derived from a yeast, insect, amphibian, fish, reptile, bird, mammal or human, or can be a hybridoma cell. Host cells can be unmodified cells or cell lines, or cell lines which have been genetically modified (e.g., to facilitate production of an immunogenic agent). In some embodiments, the host cell is a cell line that has been modified to allow for growth under desired conditions, such as in serum-free media, in cell suspension culture, or in adherent cell culture. As used herein, "hamster" refers to *Cricetulus griseus* (Chinese hamster).

[00194] A mammalian host cell can be advantageous where the immunogenic agent is a mammalian recombinant polypeptide, particularly if the polypeptide is a biotherapeutic agent or is otherwise intended for administration to or consumption by humans. In some embodiments, the host cell is a CHO cell, which is a cell line used for the expression of many recombinant proteins. Additional mammalian cell lines used commonly for the expression of recombinant proteins include 293HEK cells, HeLa cells, COS cells, NIH/3T3 cells, Jurkat Cells, NSO cells. and HUVEC cells.

[00195] In one embodiment, the host cell is a Madin Darby canine kidney (MDCK) cell. MDCK cells are routinely used by those of skill in the art for virus/vaccine production.

[00196] In some embodiments, the host cell is a CHO cell derivative that has been modified genetically to facilitate production of recombinant proteins or other immunogenic agents. For example, various CHO cell strains have been developed which permit stable insertion of recombinant DNA into a specific gene or expression region of the cells, amplification of the inserted DNA, and selection of cells exhibiting high level expression of the recombinant protein. Examples of CHO cell derivatives useful in methods provided herein include, but are not limited to, CHO-Kl cells, CHO-DUKX, CHO-DUKX B1, CHO-DG44 cells, CHO-ICAM-1 cells, and CHO-hlFNγ cells. Methods for expressing recombinant proteins in CHO cells are known in the art and are described in, e.g., U.S. Patents No. 4,816,567 and No. 5,981,214.

[00197] Examples of human cell lines useful in methods provided herein include the cell lines 293T (embryonic kidney), 786-0 (renal), A498 (renal), A549 (alveolar basal epithelial), ACHN (renal), BT-549 (breast), BxPC-3 (pancreatic), CAKI-1 (renal), Capan-1 (pancreatic), CCRF-CEM (leukemia), COLO 205 (colon), DLD-1 (colon), DMS 114 (small cell lung), DU145 (prostate), EKVX (non-small cell lung), HCC-2998 (colon), HCT-15 (colon), HCT-116 (colon), HT29 (colon), HT-1080 (fibrosarcoma), HEK 293 (embryonic kidney), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), HL-60(TB) (leukemia), HOP-62 (non-small cell lung), HOP-92 (non-small cell lung), HS 578T (breast), HT-29 (colon adenocarcinoma), IGR-OV1 (ovarian), IMR32 (neuroblastoma), Jurkat (T lymphocyte), K-562 (leukemia), KM12 (colon), KM20L2 (colon), LAN5 (neuroblastoma), LNCap.FGC (Caucasian prostate adenocarcinoma), LOX IMVI (melanoma), LXFL 529 (non-small cell lung), M14 (melanoma), M19-MEL (melanoma), MALME-3M (melanoma), MCFlOA (mammary epithelial), MCF7 (mammary), MDA-MB-453 (mammary epithelial), MDA-MB-468 (breast), MDA-MB-231 (breast), MDA-N (breast), MOLT-4 (leukemia), NCI/ADR-RES (ovarian), NCI-H226 (non-small cell lung), NCI-H23 (non-small cell lung), NCI-H322M (non-small cell lung), NCI-H460

(non-small cell lung), NCI-H522 (non-small cell lung), OVCAR-3 (ovarian), OVCAR-4 (ovarian), OVCAR-5 (ovarian), OVCAR-8 (ovarian), P388 (leukemia), P388/ADR (leukemia), PC-3 (prostate), PERC6® (E1-transformed embryonal retina), RPMI-7951 (melanoma), RPMI-8226 (leukemia), RXF 393 (renal), RXF-631 (renal), Saos-2 (bone), SF-268 (CNS), SF-295 (CNS), SF-539 (CNS), SHP-77 (small cell lung), SH-SY5Y (neuroblastoma), SK-BR3 (breast), SK-MEL-2 (melanoma), SK-MEL-5 (melanoma), SK-MEL-28 (melanoma), SK-OV-3 (ovarian), SN12K1 (renal), SN12C (renal), SNB-19 (CNS), SNB-75 (CNS) SNB-78 (CNS), SR (leukemia), SW-620 (colon), T-47D (breast), THP-1 (monocyte-derived macrophages), TK-10 (renal), U87 (glioblastoma), U293 (kidney), U251 (CNS), UACC-257 (melanoma), UACC-62 (melanoma), UO-31 (renal), W138 (lung), and XF 498 (CNS).

[00198] Examples of non-human primate cell lines useful in methods provided herein include the cell lines monkey kidney (CVI-76), African green monkey kidney (VERO-76), green monkey fibroblast (COS-1), and monkey kidney (CVI) cells transformed by SV40 (COS-7). Additional mammalian cell lines are known to those of ordinary skill in the art and are catalogued at the American Type Culture Collection catalog (Manassas, VA).

[00199] Additional examples of rodent cell lines useful in methods provided herein include the cell lines baby hamster kidney (BHK) (e.g., BHK21, BHK TK), mouse Sertoli (TM4), buffalo rat liver (BRL 3A), mouse mammary tumor (MMT), rat hepatoma (HTC), mouse myeloma (NS0), murine hybridoma (Sp2/0), mouse thymoma (EL4), murine embryonic (NIH/3T3, 3T3 Ll), rat myocardial (H9c2), mouse myoblast (C2C12), and mouse kidney (miMCD-3).

[00200] In some embodiments, the host cell is a multipotent stem cell or progenitor cell. Examples of multipotent cells useful in methods provided herein include murine embryonic stem (ES-D3) cells, human umbilical vein endothelial (HuVEC) cells, human umbilical artery smooth muscle (HuASMC) cells, human differentiated stem (HKB-Il) cells, human mesenchymal stem (hMSC) cells, and induced pluripotent stem (iPS) cells.

[00201] In some embodiments, the host cell is a plant cell. Examples of plant cells that grow readily in culture include *Arabidopsis thaliana* (cress), *Allium sativum* (garlic) *Taxus chinensis*, *T. cuspidata*, *T. baccata*, *T. brevifolia* and *T. mairei* (yew), *Catharanthus roseus* (periwinkle), *Nicotiana benthamiana* (solanaceae), *N. tabacum* (tobacco) including tobacco cells lines such as NT-1 or BY-2 (NT-1 cells are available from ATCC, No. 74840, *see also* U.S. Patent No. 6,140,075), *Oryza sativa* (rice), *Lycopersicum esulentum* (tomato), *Medicago sativa* (alfalfa), *Glycine max* (soybean), *Medicago truncatula* and *M. sativa* (clovers), *Phaseolus vulgaris* (bean), *Solanum tuberosum* (potato), *Beta vulgaris* (beet), *Saccharum spp.* (sugarcane),

Tectona grandis (teak), *Musa spp.* (banana), *Phyllostachys nigra* (bamboo), *Vitis vinifera* and *V. gamay* (grape), *Popuius alba* (poplar), *Elaeis guineensis* (oil palm), *Ulmus spp.* (elm), *Thalictrum minus* (meadow rue), *Tinospora cordifolia* ( ), *Vinca rosea* (vinca), *Sorghum spp.*, *Lolium perenne* (ryegrass), *Cucumis sativus* (cucumber), *Asparagus officinalis*, *Brucea javanica* (Yadanxi), *Doritaenopsis* and *Phalaenopsis* (orchids), *Rubus chamaemorus* (cloudberry), *Coffea arabica*, *Triticum timopheevii* (wheat), *Actinidia deliciosa* (kiwi), *Typha latifolia* (cattail), *Azadirachta indica* (neem), *Uncaria tomentosa* and *U. guianensis* (cat's claw), *Platycodon grandiflorum* (balloon flower), *Calotropis gigantea* (mikweed), *Kosteletzkya virginica* (mallow), *Pyrus malus* (apple), *Papaver somniferum* (opium poppy), *Citrus ssp.*, *Choisya ternata* (mock orange), *Galium mollugo* (madder), *Digitalis lanata* and *D. purpurea* (foxglove), *Stevia rebaudiana* (sweetleaf), *Stizolobium hassjoo* (purselane), *Panicum virgatum* (switchgrass), *Rudgea jasminoides*, *Panax quinquefolius* (American ginseng), *Cupressus macrocarpa* and *C. arizonica* (cypress), *Vetiveria zizanioides* (vetiver grass), *Withania somnifera* (Indian ginseng), *Vigna unguiculata* (cowpea), *Phyllanthus niruri* (spurge), *Pueraria tuberosa* and *P. lobata* (kudzu), *Glycyrrhiza echinata* (liquorice), *Cicer arietinum* (chick pea), *Silybum marianum* (milk thistle), *Callistemon citrinus* (bottle brush tree), *Astragalus chrysochlorus* (cuckoo flower), *Coronilla vaginalis*, such as cell line 39 RAR (crown vetch), *Salvia miltiorrhiza* (red sage), *Vigna radiata* (mung bean), *Gisekia pharnaceoides*, *Datura tatula* and *D. stramonium* (devil's trumpet), and *Zea mays spp.* (maize/corn).

[00202] The plant cell cultures provided herein are not limited to any particular method for transforming plant cells. Technology for introducing DNA into plant cells is well-known to those of skill in the art. *See, e.g.*, U.S. Patent Application Pub. No. 2010/0009449. Basic methods for delivering foreign DNA into plant cells have been described, including chemical methods (Graham & van der Eb, 54 Virol. 536-39 (1973); Zatloukal et al., 660 Ann. NY Acad. Sci. 136-53 (1992)); physical methods, including microinjection (Capeechi, 22 Cell 479-88 (1980), electroporation (Wong & Neumann, 107 Biochem. Biophys. Res. Commn. 584-87 (1982); Fromm et al., 82 PNAS 5824-28 (1985); U.S. Patent No. 5,384,253), and the "gene gun" (Johnston & Tang, 43 Met. Cell. Biol. 353-65 (1994); Fynan et al., 90 PNAS 11478-82 (1993)); viral methods (Clapp, 20 Clin. Perinatol. 155-68 (1993); Lu et al., 178 J. Exp. Med. 2089-96 (1993); Eglitis & Anderson, 6 Biotechs. 608-14 (1988); Eglitis et al., 241 Avd. Exp. Med. Biol. 19-27 (1988); and receptor-mediated methods (Curiel et al., 88 PNAS 8850-54 (1991); Curiel et al., 3 Hum. Gen. Ther. 147-54 (1992); Wagner et al., 89 PNAS 6099-103 (1992). Transgenic plant is herein defined as a plant cell culture, plant cell line, plant tissue culture, lower plant, monocot plant cell culture, dicot plant cell culture, or progeny thereof derived from

a transformed plant cell or protoplast, wherein the genome of the transformed plant contains foreign DNA, introduced by laboratory techniques, not originally present in a native, non-transgenic plant cell of the same species.

[00203] In some embodiments, the host cell is fungal, such as *Sacharomyces cerevisiae*, *Pichia pastoris* or *P. methanolica*, *Rhizopus*, *Aspergillus*, *Scizosacchromyces pombe*, *Hansanuela polymorpha*, or *Kluyveromyces lactis*. *See, e.g.*, Petranovic & Vemuri, 144 J. Biotech. 204-11 (2009); Bollok et al., 3 Recent Pat. Biotech. 192-201 (2009); Takegawa et al., 53 Biotech. Appl. Biochem. 227-35 (2009); Chiba & Akeboshi, 32 Biol. Pharm. Bull. 786-95 (2009).

[00204] In some embodiments, the host cell is an insect cell, such as Sf9 cell line (derived from pupal ovarian tissue of *Spodoptera frugiperda*); Hi-5 (derived from *Trichoplusia ni* egg cell homogenates); or S2 cells (from *Drosophila melanogaster*).

[00205] In some embodiments, the host cells are suitable for growth in suspension cultures. Suspension-competent host cells are generally monodisperse or grow in loose aggregates without substantial aggregation. Suspension-competent host cells include cells that are suitable for suspension culture without adaptation or manipulation (e.g., hematopoietic cells, lymphoid cells) and cells that have been made suspension-competent by modification or adaptation of attachment-dependent cells (e.g., epithelial cells, fibroblasts).

[00206] In some embodiments, the host cell is an attachment dependent cell which is grown and maintained in adherent culture. Examples of human adherent cell lines useful in methods provided herein include the cell lines human neuroblastoma (SH-SY5Y, IMR32, and LAN5), human cervical carcinoma (HeLa), human breast epithelial (MCFlOA), human embryonic kidney (293T), and human breast carcinoma (SK-BR3).

[00207] In some embodiments, the host cell is a cell line that has been modified to allow for growth under desired conditions, such as in serum-free media, in cell suspension culture, or in adherent cell culture. The host cell can be, for example, a human Namalwa Burkitt lymphoma cell (BLcl-kar-Namalwa), baby hamster kidney fibroblast (BHK), CHO cell, Murine myeloma cell (NS0, SP2/0), hybridoma cell, human embryonic kidney cell (293 HEK), human retina-derived cell (PER.C6® cells, U.S. Patent No. 7,550,284), insect cell line (Sf9, derived from pupal ovarian tissue of *Spodoptera frugiperda*; or Hi-5, derived from *Trichoplusia ni* egg cell homogenates; *see also* U.S. Patent No. 7,041,500), Madin-Darby canine kidney cell (MDCK), primary mouse brain cells or tissue, primary calf lymph cells or tissue, primary monkey kidney cells, embryonated hens' egg, primary chicken embryo fibroblast (CEF), Rhesus fetal lung cell (FRhL-2), Human fetal lung cell (WI-38, MRC-5), African green monkey kidney epithelial cell

(Vero, CV-1), Rhesus monkey kidney cell (LLC-MK2), or yeast cell. Additional mammalian cell lines commonly used for the expression of recombinant proteins include, but are not limited to, HeLa cells, COS cells, NIH/3T3 cells, Jurkat Cells, and human umbilical vein endothelial cells (HUVEC) cells.

[00208] Host cells can be unmodified or genetically modified (e.g., a cell from a transgenic animal). For example, CEFs from transgenic chicken eggs can have one or more genes essential for the IFN pathway, e.g., interferon receptor, STAT1, etc., disrupted, i.e., a trangenic "knockout." *See, e.g.*, Sang, 12 Trends Biotech. 415 (1994); Perry et al., 2 Transgenic Res. 125 (1993); Stern, 212 Curr Top Micro. Immunol. 195-206 (1996); Shuman, 47 Experientia 897 (1991). Also, the cell can be modified to allow for growth under desired conditions, e.g., incubation at 30°C.

[00209] In some embodiments, the host cells are suitable for growth in suspension cultures. Suspension-competent host cells are generally monodisperse or grow in loose aggregates without substantial aggregation. Suspension-competent host cells include cells that are suitable for suspension culture without adaptation or manipulation (e.g., hematopoietic cells, lymphoid cells) and cells that have been made suspension-competent by modification or adaptation of attachment-dependent cells (e.g., epithelial cells, fibroblasts). In some embodiments, the host cell is an attachment dependent cell which is grown and maintained in adherent culture. In some embodiments, the host cell is contained in an egg, such as a fish, amphibian, or avian egg.

[00210] "Isolating immunogenic agent from the host cell" means at least one step in separating the immunogenic agent away from host cellular material, e.g., the host cell, host cell culture medium, host cellular biomass, or host tissue. Thus, isolating immunogenic agents that are secreted into, and ultimately harvested from, the host cell culture media are encompassed in the phrase "isolated from the host cell." A useful quantity includes an amount, including an aliquot or sample, used to screen for or monitor production, including monitoring modulation of target gene expression.

[00211] The present invention provides for the production of immunogenic agents, including an antigen, antigenic polypeptide, a metabolite, an intermediate, a viral antigen, bacterial antigen, fungal antigen, parasite antgen, virus particle, defective virus, live attenuated virus, killed virus, or vaccine. Immunogenic agents can include any immunogenic substance capable of being produced by a host cell and recovered in useful quantities, including but not limited to, polypeptides, glycoproteins and "biologics" such as a a vaccine that is synthesized from living organisms or their products, and used as a preventive, or therapeutic agent. Thus,

immunogenic agents can be used for a wide range of applications, including as biotherapeutic agents, vaccines, research or diagnostic reagents, and the like.

[00212] In some embodiments, the immunogenic agent is a polypeptide. The polypeptide can be a recombinant polypeptide or a polypeptide endogenous to the host cell. In some embodiments, the polypeptide is a glycoprotein and the host cell is a mammalian cell. Non-limiting examples of polypeptides that can be produced according to methods provided herein include receptors, membrane proteins, cytokines, chemokines, hormones, enzymes, growth factors, growth factor receptors, antibodies, antibody derivatives and other immune effectors, interleukins, interferons, erythropoietin, integrins, soluble major histocompatibility complex antigens, binding proteins, transcription factors, translation factors, oncoproteins or proto-oncoproteins, muscle proteins, myeloproteins, neuroactive proteins, tumor growth suppressors, structural proteins, and blood proteins (e.g., thrombin, serum albumin, Factor VII, Factor VIII, Factor IX, Factor X, Protein C, von Willebrand factor, etc.) to which an immune response is desired.

[00213] As used herein, a polypeptide encompasses glycoproteins or other polypeptides which have undergone post-translational modification, such as deamidation, glycosylation, and the like. In some embodiments, the immunogenic agent is an aberrantly glycosylated protein. For example, many cancer antigens are known to be aberrantly glycoylated, particularly involving fucosyl residues. Moriwaki & Miyoshi, 2 World J. Heparol., 151-61 (2010). Thus, in one embodiment, the production of a cancer antigen is enhanced by modulating expression of a target gene encoding a fucosyltransferase, such as FUT8 (for example, by contacting a host CHO cell by use of a corresponding RNA effector molecule comprising an an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:209841-210227). In a particular embodiment, methods are provided for enhancing production of a fucosylated immunogen (e.g., a recombinant cancer antigen) by contacting a cell (e.g., CHO cell) with one or more RNA effector molecules that comprise at least 16 contiguous nucleotides of a nucleotide sequence (e.g., at least 17, at least 18, at least 19 nucleotides or more) to modulate fucosylation of the biological product. For example, the cell can be contacted with one or more RNA effector molecules of SEQ ID NOs:3152714-3152753, wherein the contacting modulates expression of the CHO cell fucosyltransferase (FUT8).

[00214] In one embodiment, production of the immunogenic agent is enhanced by contacting the host cell with at least one RNA effector molecule against target genes selected from the group consisting of FUT8, TSTA3, and GMDS, e.g., to modulate fucosylation. In one

embodiment, at least two RNA effector molecules against target genes selected from the group consisting of FUT8, TSTA3, and GMDS are used. In one aspect of these embodiments, the host cell can be further contacted with with a RNA effector molecule that targets a gene that encodes a sialytransferase, e.g., CHO cell ST3 β-galactoside α-2,3-sialyltransferase 1 (SEQ ID NO:2088), ST3 β-galactoside α-2,3-sialyltransferase 4 (SEQ ID NO:2167), ST3 β-galactoside α-2,3-sialyltransferase 3 (SEQ ID NO:3411), ST3 β-galactoside α-2,3-sialyltransferase 5 (SEQ ID NO:3484), ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6 (SEQ ID NO:4186) or ST3 β-galactoside α-2,3-sialyltransferase 2 (SEQ ID NO:4319). Targeting sialyltransferases can also be advantageous in the context of altering host cell membrane-associated sialic acid viral receptors, as discussed further herein.

[00215] In one embodiment the RNA effector molecule is an siRNA having a sequence selected from the group consisting of CHO cell ST3 β-galactoside α-2,3-sialyltransferase 1 (SEQ ID NOs:681105-681454), ST3 β-galactoside α-2,3-sialyltransferase 4 (SEQ ID NOs:707535-707870), ST3 β-galactoside α-2,3-sialyltransferase 3 (SEQ ID NOs:1131123-1131445), ST3 β galactoside α-2,3-sialyltransferase 5 (SEQ ID NOs:1155324-1155711), ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6 (SEQ ID NOs:1391079-1391449), or ST3 β-galactoside α-2,3-sialyltransferase 2 (SEQ ID NOs:1435989-1436317).

[00216] In other embodiments, the immunogenic agent is an immunogenic viral, bacterial, allergen, fungal, parasite, protozoan, or recombinant protein derived from an expression vector.

[00217] Another example approach for producing viral-based vaccines involves the use of attenuated live virus vaccines, which are capable of replication but are not pathogenic, and, therefore, provide lasting immunity and afford greater protection against disease. The conventional methods for producing attenuated viruses involve the chance isolation of host range mutants, many of which are temperature sensitive, e.g., the virus is passaged through unnatural hosts, and progeny viruses which are immunogenic, yet not pathogenic, are selected. Efficient vaccine production requires the growth of large quantities of virus produced in high yields from a host system. Different types of virus require different growth conditions in order to obtain acceptable yields. The host in which the virus is grown is therefore of great significance. As a function of the virus type, a virus can be grown in embryonated eggs, primary tissue culture cells, or in established cell lines.

[00218] Thus, in some embodiments of the present invention, the immunogenic agent is a viral product, for example, naturally occurring viral strains, variants or mutants; mutagenized viruses (e.g., generated by exposure to mutagens, repeated passages and/or passage in non-

permissive hosts), reassortants (in the case of segmented viral genomes), and/or genetically engineered viruses (e.g., using the "reverse genetics" techniques) having the desired phenotype. The viruses of these embodiments can be attenuated; i.e., they are infectious and can replicate *in vivo*, but generate low titers resulting in subclinical levels of infection that are generally non-pathogenic.

[00219] Additionally, the immunogenic agent of the present invention can be derived from an intracellular parasite against which production of an immunogenic agent can be enhanced using the compositions, cells, and/or methods of the present invention, e.g., using a RNA effector molecule. For example, alternative embodiments of the present invention provide for production of a bacterial immunogen in a eukaryotic cell. These bacteria include *Shigella flexneri*, *Listeria monocytogenes*, *Rickettsiae tsutsugamushi*, *Rickettsiae rickettsiae*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Chlamydia* ssp. Additional embodiments of the present invention provide for production of a protozoan immunogen in a eukaryotic cell. These protozoa include *Plasmodium falciparum*, *Tripanosoma cruzi*, and *Leishmania donovani*.

[00220] In some embodiments, the enhancement of production of an immunogenic agent is achieved by improving viability of the cells in culture. As used herein, the term "improving cell viability" refers to an increase in cell density (e.g., as assessed by a Trypan Blue exclusion assay) or a decrease in apoptosis (e.g., as assessed using a TUNEL assay) of at least 10% in the presence of a RNA effector molecule(s) compared with the cell density or apoptosis levels in the absence of such a treatment. In some embodiments, the increase in cell density or decrease in apoptosis in response to treatment with a RNA effector molecule(s) is at least 20%, 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 even 100% compared to untreated cells. In some embodiments, the increase in cell density in response to treatment with a RNA effector molecule(s) is at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 1000-fold or higher than the cell density in the absence of the RNA effector molecule(s).

[00221] "Bioprocessing" as used herein is an exemplary process for the industrial-scale production of an immunogenic agent (e.g., a recombinant antigenic polypeptide) in cell culture (e.g., in a mammalian host cell), that typically includes the following steps: (a) inoculating mammalian host cells (e.g., that comprises either a virus, or a transgene that encodes a recombinant antigenic polypeptide) into a seed culture vessel containing cell culture medium and propagating the cells to reach a minimum threshold cross-seeding density; (b) transferring the propagated seed culture cells, or a portion thereof, to a large-scale bioreactor; (c) propagating

the large-scale culture under conditions allowing for rapid growth and cell division until the cells reach a predetermined density; (d) maintaining the culture under conditions that disfavor continued cell growth and/or host cell division and facilitate expression of the antigenic protein or virus particles.

[00222] Steps (a) to (c) of the above method generally comprise a "growth" phase, whereas step (d) generally comprises a "production" phase. In some embodiments, fed batch culture or continuous cell culture conditions are tailored to enhance growth and division of the host cells in the growth phase and to disfavor cell growth and/or division and facilitate expression of the immunogenic agent during the production phase. For example, in some embodiments, an immunogenic agent is expressed at levels of about 1 mg/L, about 2.5 mg/L, about 5 mg/L, about 1 g/L, about 5 g/L, about 15 g/L, or higher. The rate of cell growth and/or division can be modulated by varying culture conditions, such as temperature, pH, dissolved oxygen (dO_2) and the like. For example, suitable conditions for the growth phase can include a pH of between about pH 6.5 and pH 7.5, a temperature between about 30°C to 38°C, and a dO_2 between about 5% to 90% saturation. In some embodiments, the expression of a heterologous protein can be enhanced in the production phase by inducing a temperature shift to a lower culture temperature (e.g., from about 37°C to about 30°C), increasing the concentration of solutes in the cell culture medium, or adding a toxin (e.g., sodium butyrate) to the cell culture medium. In some embodiments, the expression of a heterologous protein can be enhanced in the production phase by inducing a temperature shift to about 28°C, e.g., to increase protein expression in the absence of call division (*see, e.g.*, Example 11). A variety of additional protocols and conditions for enhancing growth and/or protein expression during the production phase are known in the art.

[00223] The host cells can be cultured in a stirred tank bioreactor system in a fed batch culture process in which the host cells and culture medium are supplied to the bioreactor initially and additional culture nutrients are fed, continuously or in discrete increments, throughout the cell culture process. The fed batch culture process can be semi-continuous, wherein periodically whole culture (including cells and medium) is removed and replaced by fresh medium. Alternatively, a simple batch culture process can be used in which all components for cell culturing (including the cells and culture medium) are supplied to the culturing vessel at the start of the process. A continuous perfusion process can also be used, in which the cells are immobilized in the culture, e.g., by filtration, encapsulation, anchoring to microcarriers, or the like, and the supernatant is continuously removed from the culturing vessel and replaced with fresh medium during the process.

[00224] In one embodiment, after the production phase the immunogenic agent is recovered from the cell culture medium using various methods known in the art. For example, recovering a secreted heterologous protein typically involves removal of host cells and debris from the medium, for example, by centrifugation or filtration. In some cases, particularly if the immunogenic agent is a protein is not secreted, protein recovery can also be performed by lysing the cultured host cells, e.g., by mechanical shear, osmotic shock, or enzymatic treatment, to release the contents of the cells into the homogenate. The protein can then be separated from subcellular fragments, insoluble materials, and the like by differential centrifugation, filtration, affinity chromatography, hydrophobic interaction chromatography, ion-exchange chromatography, size exclusion chromatography, electrophoretic procedures (e.g., preparative isoelectric focusing (IEF)), ammonium sulfate precipitation, and the like. Procedures for recovering and purifying particular types of proteins are known in the art.

[00225] In some embodiments, it is desirable to adapt cells to serum free media and adapt adherent cells to cell growth in suspension. In some embodiments, cells are adapted to grow in serum-free medium. In one aspect of the invention, adaptation of cells is facilitated by increasing cell placisity by using a RNA effector molecule that targets genes involved in control of plasticity. For example, a RNA effector targeting cell cycle regulators (e.g., cyclin kinase and others described herein) (*see, e.g.*, Table 13, that identifies example CHO cyclin kinase target genes and exemplary siRNAs (antisense strand)); histone and DNA methylases (see Tables 1-2, that identify example CHO target genes and exemplary siRNAs (anti-sense stand)); p53 (*see* Table 13, that identifies example CHO target genes and exemplary siRNAs (antisense strand); and stress response proteins for example, heat shock proteins (e.g., HSP90, etc.) (see Table 15, that identifies example CHO target genes and exemplary siRNAs (antisense strand)), and the like can be used. In one embodiment, a RNA effector targets a transcript that encodes transformation related protein p53 (CHO4957.1) comprising SEQ ID NO:4957. In one embodiment, the RNA effector molecule targeting p53 comprises at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:1649857-1650157.

Table 2. Histone Deacetylase

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID Nos:
1754	2157	histone deacetylase 6	10.782	567757-568119
1979	2085	histone deacetylase 5	7.779	644628-644970
2337	1975	histone deacetylase 1	59.419	765392-765715
2781	1861	histone deacetylase 3	24.855	916015-916347
3049	1780	histone deacetylase 7	2.965	1007551-1007926
3374	1701	histone deacetylase 2	14.591	1118498-1118826

Table 2. Histone Deacetylase				
4712	1390	histone deacetylase 4	1.236	1566324-1566700
5878	1129	histone deacetylase 8	1.863	1972862-1973238

Table 3. Histone Demethylase				
SEQ ID NO:	cons L	Description	Avg Cov	siRNA SEQ ID NOs:
8124	593	jumonji C domain-containing histone demethylase 1 homolog D (*S. cerevisiae*)	0.097	2740320-2740607
3143	1759	KDM1 lysine (K)-specific demethylase 6B	0.901	1039895-1040219
3732	1616	KDM3B lysine (K)-specific demethylase 3B	1.408	1238921-1239289
1277	2344	lysine (K)-specific demethylase 1	23.583	404752-404996
46	4190	lysine (K)-specific demethylase 2A	3.834	24130-24506
804	2588	lysine (K)-specific demethylase 2B	2.962	249009-249279
2238	2001	lysine (K)-specific demethylase 3A	2.287	731689-732019
5937	1116	lysine (K)-specific demethylase 4A	0.332	1994536-1994923
4730	1387	lysine (K)-specific demethylase 4C	0.743	1572325-1572714
3157560	3436	lysine (K)-specific demethylase 5A	0.649	3201397-3201496
4012	1547	lysine (K)-specific demethylase 5B	0.291	1332770-1333138
207	3330	lysine (K)-specific demethylase 5C	4.939	74541-74774

[00226] The terms “system”, “computing device”, and “computer-based system” refer to the computer hardware, associated software, and data storage devices used to analyze the information of the present invention. In one embodiment, the computer-based systems of the present invention comprises one or more central processing units (e.g., CPU, PAL, PLA, PGA), input means (e.g., keyboard, cursor control device, touch screen), output means (e.g., computer display, printer) and data storage devices (e.g., RAM, ROM, volatile and non-volatile memory devices, hard disk drives, network attached storage, optical storage devices, magnetic storage devices, solid state storage devices). As such, any convenient computer-based system can be employed in the present invention. Further, the computing device can included an embedded system based on a combination computing hardware and associated software or firmware.

[00227] A “processor” includes any hardware and/or software combination which can perform the functions under program control. For example, any processor herein can be a programmable digital microprocessor such as available in the form of an embedded system, a programmable controller, mainframe, server or personal computer (desktop or portable). Where the processor is selectively programmable, suitable programs, software or firmware can be communicated from a remote location to the processor, or previously saved in a computer program product (such as a portable or fixed computer readable storage medium, whether magnetic, optical or solid state device based). For example, a magnetic medium or optical disk

can store the program or operating instructions and can be read and transferred to each processor at its corresponding station.

[00228] "Computer readable medium" as used herein refers to any storage or transmission medium that participates in providing instructions and/or data to a computer for execution and/or processing. Examples of storage media include floppy disks, magnetic media (tape, disk), UBS, optical media (CD-ROM, DVD, Blu-Ray), solid state media, a hard disk drive, a RAM, a ROM or integrated circuit, a magneto-optical disk, or a computer readable card such as a PCMCIA card and the like, whether or not such devices are internal or external to the computer. A file containing information can be "stored" on computer readable medium, where "storing" means recording information such that it is accessible and retrievable at a later date by a computer.

[00229] With respect to computer readable media, "permanent memory" or "non-volatile memory" refers to memory that is permanently stored on a data storage medium. Permanent memory is not erased by termination of the electrical supply to a computer or processor. A computer hard-drive, ROM, CD-ROM, floppy disk and DVD are all examples of permanent memory. Random Access Memory (RAM) is an example of non-permanent or volatile memory.

[00230] To "record" or "store" data, programming or other information on a computer readable medium refers to a process for storing information, using any convenient method. Any convenient data storage structure can be chosen, based on the means used to access the stored information.

[00231] A "memory" or "memory unit" refers to any device which can store information for subsequent retrieval by a processor, and can include magnetic or optical devices (such as a hard disk, floppy disk, CD, or DVD), or solid state memory devices (such as volatile or non-volatile RAM). A memory or memory unit can have more than one physical memory device of the same or different types (for example, a memory can have multiple memory devices such as multiple hard drives or multiple solid state memory devices or some combination of hard drives and solid state memory devices).

[00232] This application describes a variety of genes, transcripts, proteins, etc. using known names for the nucleic acid sequence. To the extent a specific sequence identifier is not cross-referenced to such a name, the artisan can readily do so by known means. For example, there are numerous searchable sites such as GeneCards.org (a collaborative searchable, integrated, database of human genes that provides concise genomic, transcriptomic, genetic, proteomic, functional and disease related information on all known and predicted human genes; database developed at the Crown Human Genome Center, Department of Molecular Genetics, the Weizmann Institute of Science), and publications that form the basis of such sites. One can

readily use the name to locate the sequence and using such sequence cross-reference the Sequence No. used herein. Similarly, by looking for complementary sequences of at least 15 nucleic acids identify the corresponding siRNAs to such genes.

[00233] Throughout the specification, in some cases we have given the gene abbreviation or alias of the target gene and corresponding siRNA SEQ ID NOs for that gene. In some cases we have given the full gene name of the target gene, the corresponding SEQ ID NO. for the target gene (e.g.,transcript sequence) as well as example siRNA SEQ ID NOs directed against the target gene. In various embodiments of the invention, the RNA effector molecule is a siRNA that comprises an antisense strand comprising at least 16 contiguous nucleotides of a siRNA nucleotide sequence of any of the siRNA sequences identified herein by SEQ ID NO., *see, e.g.*, Tables 1-16, 21-25, 27-30, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 50, 51-61, 64, 65 and 66.

[00234] It should be understood that the siRNAs identified by SEQ ID NO. are often referred to herein within a range of SEQ ID NOs, e.g., from SEQ ID NOs: 2480018-2480362. The range includes all SEQ ID NOs: within the range, e.g., SEQ ID NO: 2480018, SEQ ID NO:2480019, SEQ ID NO: 2480020, etc., all the way to SEQ ID NO: 2480362.

II. Enhancing bioprocessing

[00235] The invention provides methods for enhancing the production of immunogenic agents using the RNA effector molecules described herein. The methods generally comprise contacting a cell with a RNA effector molecule, a portion of which is complementary to a target gene, and maintaining the cell in culture (e.g., a large-scale bioreactor) for a time sufficient to modulate expression of the target gene, wherein the modulation enhances production of the immunogenic agent from the cell, and isolating the immunogenic agent from the cell. The RNA effector molecule(s) can be added to the cell culture medium used to maintain the cells under conditions that permit production of an immunogenic agent, e.g., to provide transient modulation of the target gene thereby enhancing expression of the immunogen.

[00236] As known to those of skill in the art liposome mediated delivery of siRNA using lipid polynucleotide carriers is commonly used in research applications. As described in PCT publication WO 2009/012173, however, the use of lipid polynucleotide carriers, e.g., common liposome transfection reagents, has been found to be detrimental when used in bioprocessing of protein. Polynucleotide carriers have been reported to be toxic to host cells due to toxicity such that they impair the ability of host cells to produce the desired immunogenic agent on an industrial level. In addition, polynucleotide carriers have been observed to cause adverse and unwanted changes in the phenotype of host cells, e.g., CHO cells, compromising the ability of

the host cells to produce the immunogenic agent of interest. Accordingly, the artisan would expect that the use of such polynucleotide carriers would hinder a cells ability to produce a desired protein.

[00237] Surprisingly, as described herein, RNA effector molecules (e.g., targeting BAX, BAK and/or LDH) can be delivered transiently to host cells in culture by using polynucleotide carriers (e.g., lipid formulated mediated delivery) during the bioprocessing procedure in large scale cultures (e.g., 1 L and 40 L) without detrimental effects on the cells, e.g., cell viability and density is maintained. Thus, large scale production of immunogenic agents can be done, on an industrial scale, using lipid reagents to facilitate RNA effector uptake in cells when they are in culture (e.g., suspension culture), for example, resulting in transient modulation of genes that increase protein production. It should be understood, however, that embodiments of the invention are not limited to delivery of RNA effector molecules by lipid formulation mediated delivery.

[00238] In one embodiment, the production of an immunogenic agent is enhanced by contacting cultured cells with a RNA effector molecule provided herein during the production phase to modulate expression of a target gene encoding a protein that affects protein expression, post-translational modification, folding, secretion, and/or other processes related to production and/or recovery of the immunogenic agent. In further embodiments, the production of an immunogenic agent is enhanced by contacting cultured cells with a RNA effector molecule that inhibits cell growth and/or cell division during the production phase.

[00239] In some embodiments, the production of an immunogenic agent in a cultured host cell is enhanced by contacting the cell with a RNA effector molecule which modulates expression of a protein of a contaminating virus, thus reducing the contaminant's infectivity and/or viral load in the host cell. In additional embodiments, production of an immunogenic agent in a cultured host cell is enhanced by contacting the cell with a RNA effector molecule which modulates expression of a host cell protein involved in viral infection, e.g., a cell membrane ligand, or viral reproduction, thus reducing the infectivity and/or load of contaminating viruses in the host cell.

[00240] In some embodiments, host cell target genes useful for modulation include those described in Table 1 as follows:

Table 1. Focused Immune Response Targets

SEQ ID NO:	cons L	Description	Avg Cov	siRNA SEQ ID NOs:
166	3461	xenotropic and polytropic retrovirus receptor 1	0.95	62021-62362
680	2676	polymerase (RNA) III (DNA directed) polypeptide E	5.84	211082-211316

2455	1943	host cell factor C1	2.096	805085-805458
2525	1927	myxovirus (influenza virus) resistance 2	8.118	829145-829432
2543	1922	beclin 1, autophagy related	22.681	835365-835694
3179	1750	polymerase (RNA) III (DNA directed) polypeptide D	5.685	1052412-1052729
3259	1732	polymerase (RNA) III (DNA directed) polypeptide C	15.023	1079448-1079786
3885	1577	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	11.687	1290692-1291012
4201	1500	eukaryotic translation initiation factor 2 α kinase 3	2.46	1396283-1396617
4256	1491	polymerase (RNA) III (DNA directed) polypeptide B	1.005	1414629-1414949
4266	1488	tumor susceptibility gene 101	23.4	1417992-1418306
4832	1362	mitochondrial antiviral signaling protein	1.615	1607184-1607527
5436	1229	polymerase (RNA) III (DNA directed) polypeptide F	0.45	1814931-1815240
5608	1188	caspase 12	0.856	1875252-1875646
5618	1187	myeloid differentiation primary response gene 88	1.629	1878827-1879137
5799	1146	lysosomal trafficking regulator	0.206	1944185-1944541
5948	1114	interferon regulatory factor 7	2.718	1998635-1999022
7260	823	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	0.166	2454994-2455378
7439	778	B-cell leukemia/lymphoma 2	0.149	2513854-2514170
7465	772	zinc finger CCCH type, antiviral 1	0.346	2522447-2522771
7670	721	myxovirus (influenza virus) resistance 1	0.687	2588615-2588951
7683	718	toll-like receptor 3	0.226	2593179-2593525
7716	710	polymerase (RNA) III (DNA directed) polypeptide H	2.352	2604412-2604804
7764	698	polymerase (RNA) III (DNA directed) polypeptide G	0.231	2620918-2621272
7929	651	interleukin 23, α subunit p19	0.852	2676772-2677097
8096	601	barrier to autointegration factor 1	10.185	2731441-2731749
8245	562	calcitonin gene-related peptide-receptor component protein	0.987	2778256-2778534
8318	541	T-cell specific GTPase	0.193	2802893-2803167
8531	490	interleukin 15	1.901	2874576-2874952
9014	389	polymerase (RNA) III (DNA directed) polypeptide K	0.509	3021834-3022134
9395	285	2'-5' oligoadenylate synthetase 1B	0.156	3108340-3108557
9402	282	ISG15 ubiquitin-like modifier	1.263	3109784-3109974
9724	148	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	0.096	3149990-3150001
9741	139	NLR family, pyrin domain containing 3	0.035	3150878-3150975
3157613	530	radical S-adenosyl Met domain containing 2	0.148	3252217-3252316

[00241] In some embodiments, the enhancement of production of an immunogenic agent upon modulation of a target gene is detected by monitoring one or more measurable bioprocess parameters, such as a parameter selected from the group consisting of: cell density, pH, oxygen levels, glucose levels, lactic acid levels, temperature, and protein production. Protein production

can be measured as specific productivity (SP) (the concentration of a product, such as a heterologously expressed polypeptide, in solution) and can be expressed as mg/L or g/L; in the alternative, specific productivity can be expressed as pg/cell/day. An increase in SP can refer to an absolute or relative increase in the concentration of a product produced under two defined set of conditions (e.g., when compared with controls not treated with RNA effector molecule(s)).

[00242] In some embodiments, the enhancement of production of an immunogenic agent, upon modulation of a target gene, is detected by monitoring one or more measurable bioprocess parameters, such as cell density, medium pH, oxygen levels, glucose levels, lactic acid levels, temperature, viral protein, or viral particle production. For example, protein production can be measured as specific productivity (SP) (the concentration of a product in solution) and can be expressed as mg/L or g/L; in the alternative, specific productivity can be expressed as pg/cell/day. An increase in SP can refer to an absolute or relative increase in the concentration of an immunogenic agent produced under two defined set of conditions. Alternatively, viral particle products can be titrated by well known plaque assays, measured as plaque forming units per mL (PFU/mL).

[00243] In some embodiments, RNA effector compositions include two or more RNA effector molecules, e.g., comprise two, three, four or more RNA effector molecules. In various embodiments, the two or more RNA effector molecules are capable of modulating expression of the same target gene and/or one or more additional target genes. Advantageously, certain compositions comprising multiple RNA effector molecules are more effective in enhancing production of an immunogenic agent, or one or more aspects of such production, than separate compositions comprising the individual RNA effector molecules.

[00244] In other embodiments, a plurality of different RNA effector molecules are contacted with the cell culture and permit modulation of one or more target genes. In one embodiment, at least one of the plurality of different RNA effector molecules is a RNA effector molecule that modulates expression of glutaminase, glutamine dehydrogenase, or LDH. In another embodiment, RNA effector molecules targeting Bax and Bak are co-administered to a cell culture during production of the immunogenic agent and can optionally contain at least one additional RNA effector molecule or agent. In another embodiment, a plurality of different RNA effector molecules is contacted with the cells in culture to permit modulation of Bax, Bak and LDH expression. In another embodiment, a plurality of different RNA effector molecules is contacted with the cells in culture to permit modulation of expression of Bax and Bak, as well as glutaminase and/or glutamine dehydrogenase.

[00245] When a plurality of different RNA effector molecules are used to modulate expression of one or more target genes the plurality of RNA effector molecules can be contacted with cells simultaneously or separately. In addition, each RNA effector molecule can have its own dosage regimen. For example, one can prepare a composition comprising a plurality of RNA effector molecules are contacted with a cell. Alternatively, one can administer one RNA effector molecule at a time to the cell culture. In this manner, one can easily tailor the average percent inhibition desired for each target gene by altering the frequency of administration of a particular RNA effector molecule. For example, strong inhibition (e.g., >80% inhibition) of lactate dehydrogenase (LDH) may not always be necessary to significantly improve production of an immunogenic agent and under some conditions it may be preferable to have some residual LDH activity. Thus, one may desire to contact a cell with a RNA effector molecule targeting LDH at a lower frequency (e.g., less often) or at a lower dosage (e.g., lower multiples over the IC_{50}) than the dosage for other RNA effector molecules. Contacting a cell with each RNA effector molecule separately can also prevent interactions between RNA effector molecules that can reduce efficiency of target gene modulation. For ease of use and to prevent potential contamination it may be preferred to administer a cocktail of different RNA effector molecules, thereby reducing the number of doses required and minimizing the chance of introducing a contaminant to the cell or cell culture.

[00246] In some embodiments, the production of an immunogenic agent is enhanced by contacting cultured cells with a RNA effector molecule provided herein during the growth phase to modulate expression of a target gene encoding a protein that affects cell growth, cell division, cell viability, apoptosis, nutrient handling, and/or other properties related to cell growth and/or division. In further embodiments, the production of a heterologous protein is enhanced by contacting cultured cells with a RNA effector molecule which transiently inhibits expression of the heterologous protein during the growth phase.

[00247] In yet further embodiments, the modulation of expression (e.g., inhibition) of a target gene by a RNA effector molecule can be alleviated by contacting the cell with second RNA effector molecule, wherein at least a portion of the second RNA effector molecule is complementary to a target gene encoding a protein that mediates RNAi in the host cell. For example, the modulation of expression of a target gene can be alleviated by contacting the cell with a RNA effector molecule that inhibits expression of an argonaute protein (e.g., Argonaute-2) or other component of the RNAi pathway of the cell. In one embodiment, the immunogenic agent is a recombinant protein and expression of the product is transiently inhibited by contacting the cell with a first RNA effector molecule targeted to the transgene

encoding the immunogenic agent. The inhibition of expression of the immunogenic agent is then alleviated by contacting the host cell with a second RNA effector molecule targeted against a gene encoding a protein of the RNAi pathway of the cell.

Host cell immune response

[00248] In additional embodiments, production of an immunogenic agent in a host cell is further enhanced by introducing a RNA effector molecule that modulates expression of a host cell protein involved in microbial infection or replication such that the infectivity, load, and/or production of the immunogenic agent is increased. Modulating a host cell immune response can also be beneficial in the production of certain immunogenic agents that are themselves involved in modulating the immune response (e.g., influenza and the like).

[00249] For example, several human, mammalian and avian viruses are introduced into and/or cultivated in cells for either virus production or heterologous protein expression (e.g., ultimately for vaccine production). Infection or transfection results in the accumulation of an immunogenic agent, such as live virus particles, which can be collected from either cells or cell media after a suitable incubation period. For example, the standard method of vaccine production consists of culturing cells, infecting with a live virus (e.g., rotavirus, influenza, yellow fever), incubation, harvesting of cells or cell media, downstream processing, and filling and finishing. For the classic inactived influenza vaccine, purification, inactivation, and stabilization of this harvested immunogenic agent yields vaccine product, which techniques are well known in the art.

[00250] Recombinant DNA technology and genetic engineering techniques, in theory, can afford a superior approach to producing an attenuated virus because specific mutations are deliberately engineered into the viral genome. The genetic alterations required for attenuation of viruses are not always predictable, however. In general, the attempts to use recombinant DNA technology to engineer viral vaccines have been directed to the production of subunit vaccines which contain only the protein subunits of the pathogen involved in the immune response, expressed in recombinant viral vectors such as vaccinia virus or baculovirus. More recently, recombinant DNA techniques have been utilized to produce herpes virus deletion mutants or polioviruses that mimic attenuated viruses found in nature or known host range mutants.

[00251] The yield of an immunogenic agent, such as an attenuated live influenza virus or an immunomodulatory polypeptide, made in a host cell can be adversely affected by the immune response of the host cell, e.g., the interferon response of the host cell in which the virus or viral vector is replicated. Additionally, the infected host cell(s) can become apoptotic before viral

yield is maximized. Thus, although these attenuated viruses are immunogenic and non-pathogenic, they are often difficult to propagate in conventional cell substrates for the purposes of making vaccines. Hence, some embodiments of the present invention provide for compositions and methods using a RNA effector molecules to modulate the expression of adverse host cell responses and therefore increase yield. For example, some embodiments of the present invention relate to contacting a cell with a RNAi-based product siRNA prior to, during or after the viral or vector administration, to inhibit cellular and anti-viral processes that compromise the yield and quality of the product harvest.

[00252] The use of cell-based bioprocesses for the manufacture of immunogenic agents is enhanced, in some embodiments, by modulating expression of a target gene affecting the host cell's reaction to viral infection. This approach is useful where the immunogenic agent is viral or otherwise immunomodulatory, or where viral vectors are used to introduce heterologous proteins into the host cell.

[00253] For example, in some embodiments the target gene is a cell interferon protein or a protein associated with interferon signaling. In particular, the gene can be an interferon gene such as IFN-α (e.g., *Gallus* IFN-α, GeneID: 396398); IFN-β (e.g., *Gallus* IFN-β, GeneID: 554219); or IFN-γ (e.g., *Gallus* IFN-γ, GeneID: 396054). Thus, for example, IFN-β expression can be modulated by use of corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3156155-3156180 (*Gallus*, sense), SEQ ID NOs:3156181-3156206 (*Gallus*, antisense), SEQ ID NOs:3155493-3155540 (*Canis*, sense), SEQ ID NOs:3155445-3155492 (*Canis*, antisense), depending on the cultured cell.

[00254] Alternatively, the target gene can be an interferon receptor such as IFNAR1 (interferon α, β and ω receptor 1) (e.g., *Gallus* IFNAR1, GeneID: 395665), IFNAR2 (interferon α, β and ω receptor 2) (e.g., *Gallus* IFNAR2, GeneID: 395664), IFNGR1 (interferon γ receptor 1) (e.g., *Gallus* IFNGR1, GeneID: 421685) or IFNGR2 (interferon γ receptor 2 (interferon γ transducer 1)) (e.g., *Gallus* IFNGR2, GeneID: 418502). Thus, for example, IFNAR1 expression can be modulated by use of corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:2436536-2436863 (CHO cell, antisense), SEQ ID NOs:3154605-3154633 (*Gallus*, sense), SEQ ID NOs:3154634-3154662 (*Gallus*, antisense), SEQ ID

NOs:3155397-3155444 (*Canis*, sense), SEQ ID NOs:3155445-3155492 (*Canis*, antisense), depending on the cultured cell.

[00255] In some embodiments, the gene can be associated with interferon signaling such as STAT-1 (signal transducer and activator of transcription 1) (e.g., *Gallus* Stat1, GeneID: 424044), STAT-2, STAT-3 (e.g., *Gallus* Stat3, GeneID:420027), STAT-4 (e.g., *Gallus* Stat4, GeneID: 768406), STAT-5 (e.g., *Gallus* Stat5, GeneID: 395556; JAK-1 (Janus kinase 1) (e.g., *Gallus* Jak1, GeneID: 395681; JAK-2 (e.g., *Gallus* Jak2, GeneID: 374199), JAK-3 (e.g., *Gallus* Jak3, GeneID: 395845), IRF1 (interferon regulatory factor 1) (e.g., *Gallus* IRF1, GeneID: 396384), IRF2 (e.g., *Gallus* GeneID: 396115), IRF3, IRF4 (e.g., *Gallus* GeneID: 374179), IRF5 (e.g., *Gallus* GeneID: 430409), IRF6 (e.g., *Gallus* GeneID: 419863), IRF7 (e.g., *Gallus* GeneID: 396330), IRF8 (e.g., *Gallus* GeneID:396385), IRF 9, or IRF10 (e.g., *Gallus* GeneID: 395243).

[00256] Thus, for example, IRF3 expression can be modulated by use of corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:1430473-1430786 (CHO cell, antisense), SEQ ID NOs:3288948-3289249 (*Gallus*, sense), SEQ ID NOs:3289250-3289551 (*Gallus*, antisense), SEQ ID NOs:3290142-3290445 (*Canis*, sense), SEQ ID NOs:320446-320749 (*Canis*, antisense), depending on the cultured cell.

[00257] Similarly, the target gene can encode an interferon-induced protein such as 2′,5′ oligoadenylate synthetases (2-5 OAS); an interferon-induced antiviral protein; RNaseL (ribonuclease L (2',5'-oligoisoadenylate synthetase-dependent) (e.g., *Gallus* GeneID: 424410 (Silverman et al., 14 J. Interferon Res. 101-04 (1994)); dsRNA-dependent protein kinase (PKR) aka: eukaryotic translation initiation factor 2-α kinase 2 (EIF2AK2) (Li et al., 106 PNAS 16410-05 (2009)); Mx (MX1 myxovirus (influenza virus) resistance 1, interferon-inducible protein p78) (e.g., *Gallus* MX, GeneID: 395313; Haller et al., 9 Microbes Infect. 1636-43 (2007)); IFITM1, IFITM2, IFITM3 (Brass et al., 139 Cell 1243-54 (2009)); Proinflammatory cytokines; MYD88 (myeloid differentiation primary response gene) up-regulated upon viral challenge (e.g., *Gallus* Myd88, GeneID: 420420); or TRIF (toll-like receptor adaptor molecule 1) (e.g., *Gallus* TRIF, GeneID: 100008585), Hghighi et al., Clin. Vacc. Immunol. (Jan. 13, 2010).

[00258] Thus, for example, MX1 expression can be modulated by use of corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a

sequence selected from the group consisting of SEQ ID NOs:2588615-2588951 (CHO cell, antisense), SEQ ID NOs:326682-3286975 (*Gallus*, sense), SEQ ID NOs:3286976-3287269 (*Gallus*, antisense), SEQ ID NOs:3286132-3286406 (*Canis*, sense), SEQ ID NOs:3286407-3286681 (*Canis*, antisense), depending on the cultured cell.

[00259] Also, for example IFTM1 expression can be modulated by use of corresponding RNA effector molecule having an oligonucleotide strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3155115-3155161 (*Canis*, sense), SEQ ID NOs:3155162-3155208 (*Canis*, antisense).

[00260] Addtionally, IFITM2 expression can be modulated by use of corresponding RNA effector molecule having an oligonucleotide strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3156587-3156633 (CHO cell, sense), SEQ ID NOs:3156634-3156680 (CHO cell, antisense), SEQ ID NOs:2685171-2685550 (CHO cell, antisense), SEQ ID NOs:3155209-3155255 (*Canis*, sense), SEQ ID NOs:3155256-3155302 (*Canis*, antisense), depending on the cultured cell.

[00261] Likewise, IFITM3 expression can be modulated by use of corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide having a sequence selected from the group consisting of SEQ ID NOs:3156681-3156727 (CHO cell, sense), SEQ ID NOs:3156728-3156774 (CHO cell, antisense), SEQ ID NOs:2696169-2696546 (CHO cell, antisense), SEQ ID NOs:3155303-3155349 (*Canis*, sense), SEQ ID NOs:3155350-3155350 (*Canis*, antisense), depending on the cultured cell.

[00262] Further regarding example interferon-induced expression, PKR (EIF2AK2) expression can be modulated by use of corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from Tables 67 and 68, as follows:

Table 67. Example target PKR (EIF2AK2) oligonucletides

Gallus PKR Sense	*Gallus* PKR Antisense
CCACUGAGUGAUUCAGCCU	AGGCUGAAUCACUCAGUGG
GGUACAGGCGUUGGUAAGA	UCUUACCAACGCCUGUACC
CAGGCGUUGGUAAGAGUAA	UUACUCUUACCAACGCCUG
GAAUGUGCAUACUUCGGAU	AUCCGAAGUAUGCACAUUC
CAUACUUCGGAUGUAGUGA	UCACUACAUCCGAAGUAUG
GACAUUGCAGCUAGUUGAU	AUCAACUAGCUGCAAUGUC
CAUUGCAGCUAGUUGAUUA	UAAUCAACUAGCUGCAAUG

Table 67. Example target PKR (EIF2AK2) oligonucletides	
Gallus PKR Sense	*Gallus* PKR Antisense
CCACGCUCCAAUGUAUUCU	AGAAUACAUUGGAGCGUGG
GUAAUUAGUGGUCAUGUAU	AUACAUGACCACUAAUUAC
CAUGAACUCAGUAAUUCCU	AGGAAUUACUGAGUUCAUG
GAGUCAUGGGGUAUUACCU	AGGUAAUACCCCAUGACUC
GGUAUUACCUUUAAAGACU	AGUCUUUAAAGGUAAUACC
GAAAGACAUGUCCCUAUCU	AGAUAGGGACAUGUCUUUC
GAGCCUUCAAAUUGUCGGA	UCCGACAAUUUGAAGGCUC
GAGUAUUGGCACCUAAUUU	AAAUUAGGUGCCAAUACUC
GGUUUCGUCAGCAGUAUAA	UUAUACUGCUGACGAAACC
CUAUGCAAUCAAACGAGUU	AACUCGUUUGAUUGCAUAG
GUUAAUAAAUAGGAACGUA	UACGUUCCUAUUUAUUAAC
GCUCGCGAAUCUUGAACAU	AUGUUCAAGAUUCGCGAGC
CGCGAAUCUUGAACAUGAA	UUCAUGUUCAAGAUUCGCG
GAAUUCUAUCGUAGCUGUU	AACAGCUACGAUAGAAUUC
GAAUAUAUUCCUAUCAUAU	AUAUGAUAGGAAUAUAUUC
CUUUGGUCUCGUGACUUCU	AGAAGUCACGAGACCAAAG
CCCUCUGACUAAGAACCGA	UCGGUUCUUAGUCAGAGGG
GAGGAACACAGUCAUAUAU	AUAUAUGACUGUGUUCCUC
GAUAUGGAAAGGAAGUAGA	UCUACUUCCUUUCCAUAUC
GGUAUGGCAGGAUGUUAGA	UCUAACAUCCUGCCAUACC
CCAGGUACCCAUAAUCAAA	UUUGAUUAUGGGUACCUGG
GACAACUCGCAUAAAGCUU	AAGCUUUAUGCGAGUUGUC
CACUUCUUUUAGGUGAACU	AGUUCACCUAAAAGAAGUG
CCUUAAGUAUUUAGCUUUU	AAAAGCUAAAUACUUAAGG
GUUCUUCCUUAUAGGAACA	UGUUCCUAUAAGGAAGAAC
CAGGUAGGGUCCUCUUAAU	AUUAAGAGGACCCUACCUG
GUAGGGUCCUCUUAAUACA	UGUAUUAAGAGGACCCUAC
CUCCUAUACAGUACGGUUU	AAACCGUACUGUAUAGGAG
CUAUACAGUACGGUUUUAA	UUAAAACCGUACUGUAUAG
GUACGGUUUUAAUCGCCUA	UAGGCGAUUAAAACCGUAC
GGUUUUAAUCGCCUAUUAU	AUAAUAGGCGAUUAAAACC
GAUUAUAGGUGUACCUGAA	UUCAGGUACACCUAUAAUC
GUCAGCUCAACAUAAGGUA	UACCUUAUGUUGAGCUGAC
CUGAUUGACCGUUACUCUU	AAGAGUAACGGUCAAUCAG
GACCGUUACUCUUUGGUUA	UAACCAAAGAGUAACGGUC
CGUUACUCUUUGGUUAUAU	AUAUAACCAAAGAGUAACG
GGUUAUAUACUUAAGAGAU	AUCUCUUAAGUAUAUAACC
CUUAAGAGAUUUCUCGUUU	AAACGAGAAAUCUCUUAAG
GAUUUCUCGUUUGACUAAA	UUUAGUCAAACGAGAAAUC
CUCGUUUGACUAAAUAAGA	UCUUAUUUAGUCAAACGAG

Table 68. Example target PKR (EIF2AK2) oligonucletides	
Canis PKR Sense	*Canis* PKR Antisense
CAGAAAGGUACUUAAGUAU	AUACUUAAGUACCUUUCUG
AGAAAGGUACUUAAGUAUA	UAUACUUAAGUACCUUUCU
AAAGGUACUUAAGUAUAAU	AUUAUACUUAAGUACCUUU
UACUUAAGUAUAAUGAACU	AGUUCAUUAUACUUAAGUA
AAGUAUAAUGAACUGUCUA	UAGACAGUUCAUUAUACUU
GGACCUGCACAUAACUUAA	UUAAGUUAUGUGCAGGUCC
ACUUAAGAUUUACAUUCCA	UGGAAUGUAAAUCUUAAGU
AGCCAAAUUAGCUCUUGAA	UUCAAGAGCUAAUUUGGCU
AAACAAGGCGGUUAGUUCU	AGAACUAACCGCCUUGUUU

Table 68. Example target PKR (EIF2AK2) oligonucletides	
Canis PKR Sense	*Canis* PKR Antisense
UUAGAAGGCGUUGGGAAUU	AAUUCCCAACGCCUUCUAA
UAGAAGGCGUUGGGAAUUA	UAAUUCCCAACGCCUUCUA
AUUACAUAGGCCGUAUGAA	UUCAUACGGCCUAUGUAAU
UUACAUAGGCCGUAUGAAU	AUUCAUACGGCCUAUGUAA
UACAUAGGCCGUAUGAAUA	UAUUCAUACGGCCUAUGUA
GAAGGAACAACUAUCUGUA	UACAGAUAGUUGUUCCUUC
AGAAAGAUUUCAUUGCAGA	UCUGCAAUGAAAUCUUUCU
ACAUUUGGCUGCUAAAUUU	AAAUUUAGCAGCCAAAUGU
UUGCAUAUGAACAGAUACA	UGUAUCUGUUCAUAUGCAA
AUUGUAACAGGGACAAUGU	ACAUUGUCCCUGUUACAAU
CUCUGAGCAAUGCCAGAUA	UAUCUGGCAUUGCUCAGAG
ACACAGUGGAACUCAGGUU	AACCUGAGUUCCACUGUGU
GAAAUAGAACCAAUUGGCU	AGCCAAUUGGUUCUAUUUC
AAUAGAACCAAUUGGCUCA	UGAGCCAAUUGGUUCUAUU
GCUCAGGUGGAUAUGGUCA	UGACCAUAUCCACCUGAGC
GAUUUAUGUUAUUAAACGU	ACGUUUAAUAACAUAAAUC
UUUAUGUUAUUAAACGUGU	ACACGUUUAAUAACAUAAA
UAUGUUAUUAAACGUGUUA	UAACACGUUUAAUAACAUA
AUGUUAUUAAACGUGUUAA	UUAACACGUUUAAUAACAU
UGUUAUUAAACGUGUUAAA	UUUAACACGUUUAAUAACA
AAGGUAGAACGGGAAGUAA	UUACUUCCCGUUCUACCUU
AGCGCUUGAUCACGUAAAU	AUUUACGUGAUCAAGCGCU
GCGCUUGAUCACGUAAAUA	UAUUUACGUGAUCAAGCGC
CGCUUGAUCACGUAAAUAU	AUAUUUACGUGAUCAAGCG
AUCACGUAAAUAUCGUGCA	UGCACGAUAUUUACGUGAU
UAUCGUGCACUACCGUAGU	ACUACGGUAGUGCACGAUA
CCUUCAAGAACAACUAAGU	ACUUAGUUGUUCUUGAAGG
UCUGUGAUAAAGGAACAUU	AAUGUUCCUUUAUCACAGA
CAUUGGAGCAAUGGAUUGA	UCAAUCCAUUGCUCCAAUG
GGCUAAUUCUUGCAGAACU	AGUUCUGCAAGAAUUAGCC
UACAUAUGUCCCACUGUUU	AAACAGUGGGACAUAUGUA
CUAAGGGCUGGCAAGUUCU	AGAACUUGCCAGCCCUUAG
ACUUGAGCCCAUGAAACGA	UCGUUUCAUGGGCUCAAGU
GCCCAUGAAACGACCUAAU	AUUAGGUCGUUUCAUGGGC
CAUGAAACGACCUAAUGCA	UGCAUUAGGUCGUUUCAUG
GAAACGACCUAAUGCAUCU	AGAUGCAUUAGGUCGUUUC
AUAUUAGAGCCCUUCUAAA	UUUAGAAGGGCUCUAAUAU
UCUUCUAGGGUAUUUACCU	AGGUAAAUACCCUAGAAGA

[00263] In another embodiment, the immunogenic agent is produced by a cell transfected with one or more retroviral vectors. Upon transfection with a first retroviral vector, expression of the retroviral vector Env and/or Gag molecule is transiently inhibited by contacting the cell with a first RNA effector molecule (i.e., targeting the *env* gene or *gag* gene), allowing more efficient transfection with a second retroviral vector. For example, a first retroviral vector can encode a first peptide and a second retroviral vector can encode a second peptide (such that the recombinant immunogenic agent contains both peptides). Additionally, the inhibition of

expression can be alleviated by introducing into the cell an additionally RNA effector molecule targeted against a gene encoding a protein of the RNAi pathway.

[00264] In some embodiments, the target gene is a regulatory element or gene of an endogenous retrovirus (ERV) of the cell. For example, in particular embodiments the target gene can encode an ERV LTR, env protein, or gag protein. In some embodiments, the target gene is a gene of a latent virus such as a herpesvirus, adenovirus, vesivirus, or circovirus. In particular embodiments, for example, the target gene can encode a polypeptide or protein, such as a latent HSV glycoprotein D or PCV-1 Rep protein (described elsewhere herein). Provided herein in Table 64 are exemplary RNA effector molecules for targeting PCV-1:

Table 64. Duplexes targeting PCV-1 with modified nucleotides			
Duplex No	Sense	Antisense	
1	uAGAAAuAAGuGGuGGGAudTsdT	AAcACCcACCUCUuAUGGGdTsdT	
2	AAuAAGuGGuGGGAuGGAudTsdT	uAAGGGUGAAcACCcACCUdTsdT	
3	AuAAGuGGuGGGAuGGAuAdTsdT	UuAAGGGUGAAcACCcACCdTsdT	
4	uAAGuGGuGGGAuGGAuAudTsdT	AUuAAGGGUGAAcACCcACdTsdT	
5	GuGGuGGGAuGGAuAucAudTsdT	uAUuAAGGGUGAAcACCcAdTsdT	
6	GGAuGGAuAucAuGGAGAAdTsdT	UuAUuAAGGGUGAAcACCCdTsdT	
7	uGGAuAucAuGGAGAAGAAdTsdT	AAGCUCCCGuAUUUUGUUUdTsdT	
8	AuAucAuGGAGAAGAAGuudTsdT	AAGGGAGAUUGGAAGCUCCdTsdT	
9	ucAuGGAGAAGAAGuuGuudTsdT	UUCCUCUCCGcAAAcAAAAdTsdT	
10	uGGAGAAGAAGuuGuuGuudTsdT	AAACCUUCCUCUCCGcAAAdTsdT	
11	GGAGAAGAAGuuGuuGuuudTsdT	UUCcAAACCUUCCUCUCCGdTsdT	
12	GAGAAGAAGuuGuuGuuuudTsdT	uACCCUCUUCcAAACCUUCdTsdT	
13	AGAAGuuGuuGuuuuGGAudTsdT	UUCuACCCUCUUCcAAACCdTsdT	
14	AGuuGuuGuuuuGGAuGAudTsdT	AAUUCGcAAACCCCUGGAGdTsdT	
15	GuuGuuGuuuuGGAuGAuudTsdT	AAAUUCGcAAACCCCUGGAdTsdT	
16	uuuuAuGGcuGGuuAccuudTsdT	uAGcAAAAUUCGcAAACCCdTsdT	
17	uGGcuGGuuAccuuGGGAudTsdT	UUCUuAGcAAAAUUCGcAAdTsdT	
18	cuGGuuAccuuGGGAuGAudTsdT	AAGUCUGCUUCUuAGcAAAdTsdT	
19	GAGAcuGuGuGAccGGuAudTsdT	AAAGUCUGCUUCUuAGcAAdTsdT	
20	cuGuGuGAccGGuAuccAudTsdT	AAAAGUCUGCUUCUuAGcAdTsdT	
21	uGuGuGAccGGuAuccAuudTsdT	uAAAAGUCUGCUUCUuAGCdTsdT	
22	ccGGuAuccAuuGAcuGuAdTsdT	UuAAAAGUCUGCUUCUuAGdTsdT	
23	ccAuuGAcuGuAGAGAcuAdTsdT	UUcACCUUGUuAAAAGUCUdTsdT	
24	GuAuuuuGAuuAccAGcAAdTsdT	uACcACUUcACCUUGUuAAdTsdT	
25	uAuuuuGAuuAccAGcAAudTsdT	AuACcACUUcACCUUGUuAdTsdT	
26	cAGGAAuGGuAcuccucAAdTsdT	AAuACcACUUcACCUUGUUdTsdT	
27	cAGcuGuAGAAGcucucuAdTsdT	AAAuACcACUUcACCUUGUdTsdT	
28	AGcuGuAGAAGcucucuAudTsdT	UUCGCUUUCUCGAUGUGGCdTsdT	
29	uAucGGAGGAuuAcuAcuudTsdT	UUCCUUUCGCUUUCUCGAUdTsdT	
30	AucGGAGGAuuAcuAcuuudTsdT	UuAUUCUGCUGGUCGGUUCdTsdT	
31	GAGGAuuAcuAcuuuGcAAdTsdT	UUCUUuAUUCUGCUGGUCGdTsdT	
32	AGGAuuAcuAcuuuGcAAudTsdT	uACUGcAGuAUUCUUuAUUdTsdT	
33	cuAcuuuGcAAuuuuGGAAdTsdT	UuACUGcAGuAUUCUUuAUdTsdT	

34	uuGGAAGAcuGcuGGAGAAdTsdT	UUuACUGcAGuAUUCUUuAdTsdT	
35	AAGAcuGcuGGAGAAcAAudTsdT	AUGUGGCCUUCUUuACUGCdTsdT	
36	AGAAcAAuccAcGGAGGuAdTsdT	uAUGUGGCCUUCUUuACUGdTsdT	
37	AcccGAAGGccGAuuuGAAdTsdT	AAGuAUGUGGCCUUCUUuAdTsdT	
38	uGcccuuuucccAuAuAAAdTsdT	uAAGuAUGUGGCCUUCUUUdTsdT	

[00265] In some embodiments, the target gene is an endogenous non-ERV gene. For example, the target gene can encode the immunogenic agent, or a portion thereof, when the immunogenic agent is a polypeptide.

[00266] Production of an immunogenic agent can also be enhanced by reducing the expression of a protein that binds to the immunogenic agent or its vector. For example, in producing a recombinant protein it can be advantageous to reduce or inhibit expression of a receptor/ligand produced by an ERV, so that its expression in the host cell does not inhibit super-infection by the recombinant vector. It is known to a skilled artisan that a receptor can be a cell surface receptor or an internal (e.g., nuclear) receptor. The expression of the binding partner can be modulated by contacting the host cell with a RNA effector molecule directed at the receptor gene according to methods described herein.

[00267] In additional embodiments, the target gene is a cell protein that mediates viral infectivity, such as TLR3 that detects dsRNA (e.g., *Gallus* TLR3, GeneID: 422720), TLR7 that detects ssRNA (e.g., *Gallus* TLR7, GeneID: 418638), TLR21, that recognizes unmethylated DNA with CpG motifs (e.g., *Gallus* Tlr3, GeneID: 415623), RIG-1 involved with viral sensing (Myong et al., 323 Science 1070-74 (2009)); LPGP2 and other RIG-1-like receptors, which are positive regulators of viral sensing (Satoh et al., 107 PNAS 1261-62 (2010); Nakhaei et al., 2009); TRIM25 (e.g., *Gallus* Trim25, GeneID: 417401; Gack et al., 5 Cell Host Microb. 439-49 (2009)); or MAVS/VISA/IPS-1/Gardif (MAVS), which interacts with RIG-1 to initiate an antiviral signaling cascade (*see* Cui et al., 29 Mol. Cell. 169-79 (2008); Kawai et al., 6 Nat. Immunol. 981-88 (2005)).

[00268] Thus, for example, TLR3 expression can be modulated by use of corresponding RNA effector molecule(s) having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3156491-3156538 (CHO cell, sense), SEQ ID NOs:3156539-3156586 (CHO cell, antisense), SEQ ID NOs:2593179-2593525 (CHO cell, antisense), SEQ ID NOs:3155965-3156011 (*Gallus*, sense), SEQ ID NOs:3156012-3156058 (*Gallus*, antisense), SEQ ID NOs:315777-3155823 (*Canis*, sense) and SEQ ID NOs:3155824-3155870 (*Canis*, antisense), depending on the cultured cell.

[00269] Additionally, for example, MAVS expression can be modulated by use of corresponding RNA effector molecule(s) having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3156397-3156443 (CHO cell, sense), SEQ ID NOs:3156444-3156490 (CHO cell, antisense), SEQ ID NOs:1607184-1607527 (CHO cell, antisense), SEQ ID NOs:3286682-3286975 (*Gallus*, sense), SEQ ID NOs:3286976-3287269 (*Gallus*, antisense), SEQ ID NOs:3286132-3286406 (*Canis*, sense) and SEQ ID NOs:3286407-3286681 (*Canis*, antisense), depending on the cultured cell.

[00270] There are host cell proteins that impact viral replication in a specific fashion, yet the exact mechanisms for this activity is unresolved. For example, the suppression of the cellular protein casein kinase 2β (CSKN2B) increases influenza replication, protein production and viral titer. Marjuki et al., 3 J. Mol. Signal. 13 (2008). CSKN2B expression can be modulated by use of corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:2634978-2635358 (CHO cell, antisense), SEQ ID NOs:3289552-3289846 (*Gallus*, sense), SEQ ID NOs:3289847-3290141 (*Gallus*, antisense), SEQ ID NOs:3288368-3288657 (*Canis*, sense), SEQ ID NOs:3288658-3288947 (*Canis*, antisense), depending on the cultured cell.

[00271] A composition, in alternative embodiments, can comprise one or more RNA effector molecules capable of modulating expression of one or multiple genes relating to a common biological process or property of the cell, for example the interferon signaling pathway including IFN, STAT proteins or other proteins in the JAK-STAT signaling pathway, IFNRA1 and/or IFNRA2. For example, viral infection results in swift innate response in infected cells against potential lytic infection, transformation and/or apoptosis, which is characterized by the production of IFNα and IFNβ. This signaling results in activation of IFN-stimulated genes (ISGs) that mediate the effects of IFN. IFN regulatory factor (IRFs) are family of nine cellular factors that bind to consensus IFN-stimulated response elements (ISREs) and induce other ISGs. *See* Kirshner et al., 79 J. Virol. 9320-24 (2005). The IFNs increase the expression of intrinsic proteins including TRIM5α, Fv, Mx1, eIF2α and 2'-5' OAS, and induce apoptosis of virus-infected cells and cellular resistance to viral infection. Koyam et al., 43 Cytokine 336-41 (2008). Hence, a particular embodiment provides for a RNA effector molecule that targets a IFNRA1 gene. Other embodiments target one or more genes in the IFN signaling pathway.

[00272] Inhibition of IFN signaling responses can be determined by measuring the phosphorylated state of components of the IFN pathway following viral infection, e.g., IRF-3,

which is phosphorylated in response to viral dsRNA. In response to type I IFN, Jak1 kinase and TyK2 kinase, subunits of the IFN receptor, STAT1, and STAT2 are rapidly tyrosine phosphorylated. Thus, in order to determine whether the RNA effector molecule inhibits IFN responses, cells can be contacted with the RNA effector molecule, and following viral infection, the cells are lysed. IFN pathway components, such as Jak1 kinase or TyK2 kinase, are immunoprecipitated from the infected cell lysates, using specific polyclonal sera or antibodies, and the tyrosine phosphorylated state of the kinase determined by immunoblot assays with an anti-phosphotyrosine antibody. *See, e.g.*, Krishnan et al., 247 Eur. J. Biochem. 298-305 (1997). A decreased phosphorylated state of any of the components of the IFN pathway following infection with the virus indicates decreased IFN responses by the virus in response to the RNA effector molecule(s).

[00273] Efficacy of IFN signaling inhibition can also be determined by measuring the ability to bind specific DNA sequences or the translocation of transcription factors induced in response to viral infection, and RNA effector molecule treatment, e.g., targeting IRF3, STAT1, STAT2, etc. In particular, STAT1 and STAT2 are phosphorylated and translocated from the cytoplasm to the nucleus in response to type I IFN. The ability to bind specific DNA sequences or the translocation of transcription factors can be measured by techniques known to skilled artisan, e.g., electromobility gel shift assays, cell staining, etc. Another approach to measuring inhibition of IFN induction determines whether an extract from the cell culture producing the desired viral product and contacted with a RNA effector molecule is capable of conferring protective activity against viral infection. More specifically, for example, cells are infected with the desired virus and contacted with a RNA effector. Approximately 15 to 20 hours post-infection, the cells or cell media are harvested and assayed for viral titer, or by quantitative product-enhanced reverse transcriptase (PERT) assay, immune assays, or *in vivo* challenge.

Host cell receptors

[00274] In some embodiments, the target gene is a host cell gene (endogenous) that encodes or is involved in the synthesis or regulation of a membrane receptor or other moiety. Modulating expression of the cell membrane can increase or decrease viral infection (e.g., by increasing or decreasing receptor expression), or can increase recovery of product that would otherwise adsorb to host cell membrane (by decreasing receptor expression).

[00275] For example, many viruses adhere to host cell-surface heparin, including PCV (Misinzo et al., 80 J. Virol. 3487-94 (2006); CMV (Compton et al., 193 Virology 834–41 (1993)); pseudorabies virus (Mettenleiter et al., 64 J. Virol. 278–86 (1990)); BHV-1 (Okazaki et

al., 181 Virology 666–70 (1991)); swine vesicular disease virus (Escribano-Romero et al., 85 Gen. Virol. 653-63 (2004)); and HSV (WuDunn & Spear, 63 J. Virol. 52–58 (1989)). Additionally, enveloped viruses having infectivity associated with surface heparin binding include HIV-1 (Mondor et al., 72 J. Virol. 3623-34 (1998)); AAV-2 (Summerford & Samulski, 72 J. Virol. 1438–45 (1998)); equine arteritis virus (Asagoe et al., 59 J. Vet. Med. Sci. 727–28 (1997)); Venezuelan equine encephalitis virus (Bernard et al., 276 Virology 93–103 (2000)); Sindbis virus (Byrnes & Griffin, 72 J. Virol. 7349–56 (1998); Chung et al., 72 J. Virol. 1577–85 (1998)); swine fever virus (Hulst et al., 75 J. Virol. 9585–95 (2001)); porcine reproductive and respiratory syndrome virus (Jusa et al., 62 Res. Vet. Sci. 261–64 (1997)); and RSV (Krusat & Streckert, 142 Arch. Virol. 1247–54 (1997)). A number of non-enveloped virus associate with cell surface heparin as well. Some picornaviridae family members associate with cell-surface heparin, including, foot-and-mouth disease virus (FMDV) (binds in *in vitro* culture) (Fry et al., 18 EMBO J. 543–54 (1999); Jackson et al., 70 J. Virol. 5282–87 (1996)); coxsackie virus B3 (CVB3) (Zautner et al., 77 J. Virol. 10071–77 (2003)); Theiler's murine encephalomyelitis virus (Reddi & Lipton, 76 J. Virol. 8400–07 (2002)); and certain echovirus serotypes (Goodfellow et al., 75 J. Virol. 4918–21 (2001)).

[00276] Hence, in particular embodiments of the present invention, cellular expression of heparin can be modulated in order to decrease or increase viral adsorption to the host cell. For example, one or more RNA effector molecule(s) can target one or more genes associated with heparin synthesis or structure, such as epimerases, xylosyltransferases, galactosyltransferases, N-acetylglucosaminyl transferases, glucuronosyltransferases, or 2-O-sulfotransferases. *See, e.g.*, Rostand & Esko, 65 Infect. Immun. 1-8 (1997).

[00277] In the instance where the expression of cell-surface heparin is increased, a RNA effector molecule can target genes associated with heparin degradation, such as genes encoding heparanase (hep) (e.g., mouse hep GeneID: 15442, mouse hep 2 GeneID: 545291, rat hep GeneID: 64537, rat hep 2 GeneID: 368128, human HEP GeneID: 10855, human HEP 2 GeneID: 60495, *Xenopus* hep GeneID: 100145320, wild pig *Sus scrofa* hep GeneID: 100271932, *Gallus* hep GeneID: 373981, *Gallus* hep 2 GeneID: 423834, dog hep GeneID: 608707, bovine hep GeneID: 8284471, *Callithrix* monkey hep GeneID: 100402671, *Callithrix* hep 2 GeneID: 100407598, *P. troglodytes* hep GeneID: 461206, rabbit hep GeneID: 100101601, Rhesus Macaque hep GeneID: 707583, or zebrafish hep GeneID: 563020). *See* Gingis-Velitski et al., 279 J. Biol. Chem. 44084-92 (2004).

[00278] Similarly, the infectivity of influenza virus is dependent on the presence of sialic acid on the cell surface (Pedroso et al., 1236 Biochim. Biophys. Acta 323-30 (1995), as is the

infectivity of rotaviruses (Isa et al., 23 Glycoconjugate J. 27-37 (2006); Fukudome et al., 172 Virol. 196-205 (1989)), other reoviruses (Paul et al., 172 Virol. 382-85 (1989)), and bovine coronaviruses (Schulze & Herrler, 73 J. Gen. Virol. 901-06 (1992)). Additional host cell-surface receptors include VCAM1 for encephalomyocarditis virus (Huberm 68 J. Virol. 3453-58 (1994); integrin VLA-2 for Echovirus (Bergelson et al., 1718-20 (1992); and members of the immunoglobulin super-family for poliovirus (Mendelson et al., 56 Cell 855-65 (1989). As such, a RNA effector targeting a host sialidase gene can be used to modulate host cell infectivity.

[00279] Thus, in some embodiments the gene target includes a host cell gene involved in sialidase (*see* Wang et al., 10 BMC Genomics 512 (2009)). For example, because influenza binds to cell surface sialic acid residues, decreased sialidase can increase the rate of purification. Target genes include, for example, NEU2 sialidase 2 (cytosolic sialidase) (*Gallus* Neu2, GeneID: 430542); NEU3 sialidase 3 (membrane sialidase) (*Gallus* Neu3, GeneID: 68823); solute carrier family 35 (CMP-sialic acid transporter) member A1 (Slc35A1). Example RNA effector molecules targeting SCL35A1 can have the sequences provided in SEQ ID NOs:3154345-3154368 (*Gallus*, sense) and SEQ ID NOs:3154369-3154392 (*Gallus*, antisense); and for SCL35A2, SEQ ID NOs:464674-465055 (CHO cell, antisense). For UDP-N-acetylglucosamine 2-epimerase/ N-acetylmannosamine kinase (Gne), example siRNAs include SEQ ID NOs:2073971-2074368 (CHO cell, antisense), SEQ ID NOs:3154297-3154320 (*Gallus*, sense) and SEQ ID NOs:3154321-3154344 (*Gallus*, antisense)); cytidine monophospho-N-acetylneuraminic acid synthetase (Cmas), example siRNAs showh in SEQ ID NOs:1633101-1633406 (CHO cell, antisense), SEQ ID NOs:3154249-3154272 (*Gallus*, sense) and SEQ ID NOs:3154273-3154296 (*Gallus*, antisense)); UDP-Gal:βGlcNAc β1,4-galactosyltransferase (B4GalT1), example siRNAs having sequences chosen from SEQ ID NOs:2528454-2528763 (CHO cell, antisense), SEQ ID NOs:3154153-3154176 (*Gallus*, sense) and SEQ ID NOs:3154177-3154200 (*Gallus*, antisense)); and UDP-Gal:βGlcNAc β1,4-galactosyltransferase, polypeptide 6 (B4GalT6), example siRNAs in SEQ ID NOs:1635173-1635561 (CHO cell, antisense), SEQ ID NOs:3154201-3154224 (*Gallus*, sense) and SEQ ID NOs:3154225-3154248 (*Gallus*, antisense).

Host cell viability

[00280] In some embodiments, the production of an immunogenic agent in a host cell is enhanced by introducing into the cell an additional RNA effector molecule that affects cell growth, cell division, cell viability, apoptosis, nutrient handling, and/or other properties related to cell growth and/or division within the cell. The target gene can also encode a host cell protein

that directly or indirectly affects one or more aspects of the production of the immunogenic agent. Examples of target genes that affect the production of polypeptides include genes encoding proteins involved in the secretion, folding or post-translational modification of polypeptides (e.g., glycosylation, deamidation, disulfide bond formation, methionine oxidation, or pyroglutamation); genes encoding proteins that influence a property or phenotype of the host cell (e.g., growth, viability, cellular pH, cell cycle progression, apoptosis, carbon metabolism or transport, lactate formation, susceptibility to viral infection or RNAi uptake, activity or efficacy); and genes encoding proteins that impair the production of an immunogenic agent by the host cell (e.g., a protein that binds or co-purifies with the immunogenic agent).

[00281] In some embodiments of the invention, the target gene encodes a host cell protein that indirectly affects the production of an immunogenic agent such that inhibiting expression of the target gene enhances production of the immunogenic agent. For example, the target gene can encode an abundantly expressed host cell protein that does not influence directly production of the immunogenic agent, but indirectly decreases its production, for example by utilizing cellular resources that could otherwise enhance production of the immunogenic agent.

[00282] In some embodiments, Ago1 (Eukaryotic translation initiation factor 2C, 1); BLK (B lymphoid tyrosine kinase); CCNB3 (Cyclin B3); HILI (piwi-like 2 (Drosophila); HIWI1 (piwi-like 2 (Drosophila); HIWI2 (piwi-like 2 (Drosophila); HIWI3(piwi-like 2 (Drosophila); is targeted using the methods and compositions described herein.

[00283] For optimal production of an immunogenic agent in cell-based bioprocesses described herein, it is desirable to maximize cell viability. Accordingly, in one embodiment, production of an immunogenic agent is enhanced by modulating expression of a cell protein that affects apoptosis or cell viability, such as Bax (BCL2-associated X protein), for example; Bak (BCL2-antagonist/killer 1) (e.g., *Gallus* Bak, GeneID: 419912), LDHA (lactate dehydrogenase A) (e.g., *Gallus* LdhA, GeneID: 396221), LDHB (e.g., *Gallus* LdhB, GeneID: 373997), BIK; BAD (SEQ ID NOs:3049436-3049721), BID (SEQ ID NOs:2582517-2582823), BIM, HRK, BCLG, HR, NOXA, PUMA (SEQ ID NOs:1712045-1712425), BOK (BCL2-related ovarian killer) (e.g., *Mus musculus* Bok, GeneID: 395445, *Gallus* Bok, GeneID: 995445, human BOK, GeneID: 666), BOO, BCLB, CASP2 (apoptosis-related cysteine peptidase 2) (e.g., *Gallus* Casp2, GeneID: 395857) (SEQ ID NOs:2718675-2719039), CASP3 (apoptosis-related cysteine peptidase) (e.g., *Gallus* Casp3, GeneID: 395476) (SEQ ID NOs:1924836-1925195), CASP6 (e.g., *Gallus* Casp6, GeneID: 395477 (SEQ ID NOs:2408466-2408843); CASP7 (e.g., *Gallus*, GeneID: 423901 (SEQ ID NOs:2301618-2301960); CASP8 (e.g., *Gallus* Casp8, GeneID: 395284, human CASP8 GeneD:841, *M. musculus* Casp8, GeneID: 12370, *Canis*

Casp8, GeneID:488473) (SEQ ID NOs:2995593-2995870); CASP9 (e.g., *Gallus* Casp9, GeneID: 426970) (SEQ ID NOs:1412589-1412860), CASP10 (e.g., *Gallus* Casp10, GeneID: 424081), BCL2 (B-cell CLL/lymphoma 2) (e.g., *Gallus* Bcl2, GeneID: 396282), p53 (e.g., *Gallus* p53, GeneID: 396200) (SEQ ID NOs:1283506-1283867), APAF1, HSP70 (e.g., *Gallus* Hsp70, GeneID: 423504) (SEQ ID NOs:3147029-3147080); TRAIL (TRAIL-LIKE TNF-related apoptosis inducing ligand-like) (e.g., *Gallus* Trail, GeneID: 395283), BCL2L1 (BCL2-like 1) (e.g., *Gallus* Bcl2L1, GeneID: 373954) BCL2L13 (BCL2-like 13 [apoptosis facilitator]) (e.g., *Gallus* Bcl2l13, GeneID: 418163, human BCL2L13, GeneID: 23786), BCL2L14 (BCL2-like 14 [apoptosis facilitator]) (e.g., *allus* Bcl2l14, GeneID: 419096), FASLG (Fas ligand [TNF superfamily, member 6]) (e.g., *Gallus* Faslg, GeneID: 429064), DPF2 (D4, zinc and double PHD fingers family 2) (e.g., *Gallus* Dpf2, GeneID: 429064), AIFM2 (apoptosis-inducing factor mitochondrion-associated 2) (e.g., human AIFM2, GeneID: 84883, *Gallus* Aifm2, GeneID: 423720), AIFM3 (e.g., *Gallus* Aifm3, GeneID: 416999), STK17A (serine/threonine kinase 17a [apoptosis-inducing]) (e.g., *Gallus* Stk17A, GeneID: 420775), APITD1 (apoptosis-inducing, TAF9-like domain 1) (e.g., *Gallus* Apitd1, GeneID: 771417), SIVA1 (apoptosis-inducing factor) (e.g., *Gallus* Siva1, GeneID: 423493), FAS (TNF receptor superfamily member 6) (e.g., *Gallus* Fas, GeneID: 395274), TGFβ2 (transforming growth factor β 2) (e.g., *Gallus* TgfB2, GeneID: 421352), TGFBR1 (transforming growth factor, β receptor I) (e.g., *Gallus* TgfR1, GeneID: 374094), LOC378902 (death domain-containing tumor necrosis factor receptor superfamily member 23) (*Gallus* GeneID: 378902), or BCL2A1 (BCL2-related protein A1) (e.g., *Gallus* Bcl2A1, GeneID: 395673). For example, the BAK protein is known to down-regulate cell apoptosis pathways. Suyama et al., S1 Nucl. Acids. Res. 207-08 (2001).

[00284] For example, LDHA expression can be modulated by use of a corresponding RNA effector molecule comprising an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the nucleotides in SEQ ID NOs:3154553-3154578 (*Gallus*, sense), SEQ ID NOs:3154579-3154604 (*Gallus*, antisense), SEQ ID NOs:3152540-3152603 (CHO cell), SEQ ID NOs:3152843-3152823 (CHO cell), SEQ ID NOs:1297283-1297604 (CHO cell, antisense), SEQ ID NOs:3155589-3155635 (*Canis*, sense), SEQ ID NOs:3154971-3155018 (*Canis*, antisense).

[00285] Further, for example, the Bak protein is known to down-regulate cell apoptosis pathways. Thus, RNA effector molecules that target Bak can be used to suppress apoptosis and increase product yield, and can comprise an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the nucleotides in SEQ ID NOs:3152412-3152475 (CHO cell), SEQ ID NOs:3152804-3152813), SEQ ID NOs:2259855-

220161 (CHO cell, antisense), SEQ ID NOs:3154393-3154413 (*Gallus*, sense), SEQ ID NOs:3154414-3154434 (*Gallus*, antisense), SEQ ID NOs:3154827-3154874 (*Canis*, sense), SEQ ID NOs:3154875-3154922 (*Canis*, antisense). *See also* Suyama et al., S1 Nucl. Acids. Res. 207-08 (2001). A particular embodiment thus provides for a RNA effector molecule that targets the Bak gene. A particular embodiment thus provides for a RNA effector molecule that targets the BAK1 gene.

[00286] Similarly, Bax protein is known to down -regulate cell apoptosis pathways. Thus, RNA effector molecules that target chicken Bax can be used to suppress apoptosis and increase immunogen product yield, and can comprise an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the nucleotides inSEQ ID NOs:3154393-3154413 (*Gallus*, sense), SEQ ID NOs:315414-3154434 (*Gallus*, antisense), SEQ ID NOs:3152412-3152539 (CHO cell), SEQ ID NOs:3152794-3152803 (CHO cell), SEQ ID NOs:3023234-3023515 (CHO cell, antisense), SEQ ID NOs:3154923-3154970 (*Canis*, sense), and SEQ ID NOs:3154971-3155018 (*Canis*, antisense).

[00287] In some embodiments, administration of RNA effector molecule/s targeting at least one gene involved in apoptosis (e.g., Bak, Bax, caspases etc.) is followed by a administration of glucose to the cell culture medium in order to increase cell density and switch cells to a lactate utilization mode. In some embodiments the concentration of glucose is increased at least 2-fold, at least 3-fold, at least 4 fold, or at least 5-fold.

[00288] Another embodiment provides for a plurality of different RNA effector molecules is contacted with the cells in culture to permit modulation of Bax, Bak and LDH expression. In another embodiment, RNA effector molecules targeting Bax and Bak are co-administered to a cell culture during production of the immunogenic agent and can optionally contain at least one additional RNA effector molecule or agent.

[00289] Alternatively, one can administer one RNA effector molecule at a time to the cell culture. In this manner, one can easily tailor the average percent inhibition desired for each target gene by altering the frequency of administration of a particular RNA effector molecule. For example, > 80% inhibition of lactate dehydrogenase (LDH) may not always be necessary to significantly improve production of an immunogenic agent and under some conditions may even be detrimental to cell viability. Thus, one may desire to contact a cell with a RNA effector molecule targeting LDH at a lower frequency (e.g., less often) than the frequency of contacting with the other RNA effector molecules (e.g., Bax/Bak). Alternatively, the cell can be contacted with a RNA effector molecule targeting LDH at a lower dosage (e.g., lower multiples over the IC_{50}) than the dosage for other RNA effector molecules (e.g., Bax/Bak). For ease of use and to

prevent potential contamination it may be preferred to administer a cocktail of different RNA effector molecules, thereby reducing the number of doses required and minimizing the chance of introducing a contaminant to the cell culture.

[00290] The production of an immunogenic agent in cell-based bioprocesses described herein can also be optimized by targeting genes that have been identified through screens. These include, for example, PUSL1 (pseudouridylate synthase-like 1) (CHO-Pusl1: SEQ ID NO:3157237; siRNA SEQ ID NOs:3249217–3249316); TPST1 (tyrosylprotein sulfotransferase 1) (e.g., *Gallus* Tpst1, GeneID: 417546) (CHO TPST1: SEQ ID NO:2613, corresponding siRNAs: SEQ ID NOs:858808-859104), and WDR33 (WD repeat domain 33) (e.g., *Gallus* Wdr33, GeneID: 424753) (CHO: SEQ ID NO:3433, corresponding siRNAs: SEQ ID NOs:1138341-1138649) (Brass et al., 139 Cell 1243-54 (2009)); Nod2 (nucleotide-binding oligomerization domain containing 2) (CHO: SEQ ID NO:6858; siRNA SEQ ID NOs:2322123-2322429) (Sabbah et al., 10 Nat. Immunol. 1973-80 (2009)); MCT4 (solute carrier family 16, member 4 [monocarboxylic acid transporter 4]) (e.g., *Gallus* Mct4, GeneID: 395383), ACRC (acidic repeat containing) (e.g., *Gallus* AcrC, GeneID :422202), AMELY, ATCAY (cerebellar, Cayman type [caytaxin]) (e.g., *Gallus* Atcay, GeneID: 420094), ANP32B (acidic [leucine-rich] nuclear phosphoprotein 32 family member) (e.g., *Gallus* Anp32B, GeneID: 420087), DEFA3, DHRS10, DOCK4 (dedicator of cytokinesis 4) (e.g., *Gallus* Dock4, GeneID: 417779), FAM106A, FKBP1B (FK506 binding protein 1B) (e.g., human FKCB1B, GeneID: 2281, *M. musculus* Fkbp1b, GeneID: 14226, *Gallus* Fkbp1B, GeneID: 395254), IRF3, KBTBD8 (kelch repeat and BTB [POZ] domain containing 8) (e.g., *Gallus* Kbtbd8, GeneID: 416085), KIAA0753 (e.g., *Gallus* Kiaa0753, GeneID: 417681), LPGAT1 (lysophosphatidyl-glycerol acyltransferase 1) (e.g., *Gallus* Lpgat1, GeneID: 421375), MSMB (microseminoprotein β) (e.g., *Gallus* Msmb, GeneID: 423773), NFS1 (nitrogen fixation 1 homolog) (e.g., *Gallus* Nfs1, GeneID: 419133), NPIP, NPM3 (nucleophosmin/nucleoplasmin 3) (e.g., *Gallus* Npm3, GeneID: 770430), SCGB2A1, SERPINB7, SLC16A4 (solute carrier family 16, member 4 [monocarboxylic acid transporter 5]) (e.g., *Gallus* Slc16a4, GeneID: 419809), SPTBN4 (spectrin, β, non-erythrocytic 4) (e.g., *Gallus* SptBn4, GeneID: 430775), or TMEM146 (Krishnan et al., 2008).

[00291] Other target genes that can be affected to optimize immunogen production include genes associated with cell cycle and/or cell proliferation, such as CDKN1B (cyclin-dependent kinase inhibitor 1B, p27, kip1) (e.g., *Gallus* Cdkn1b, GeneID: 374106), a target for which a siRNA against p27kip1 induces proliferation (Kikuchi et al., 47 Invest. Opthalmol. 4803-09 (2006)); or FOX01, a target for which a siRNA induces aortic endothelial cell

proliferation (Fosbrink et al., J. Biol. Chem. 19009-18 (2006). Thus, for example, in CEF or other chicken cells, the expression of CDKN2A, associated with cell division, can be modulated using a corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3154663-3154696 (*Gallus*, sense) and SEQ ID NOs:3154697-3154730 (*Gallus*, antisense).

[00292] Reactive oxygen species (ROS) are toxic to host cells and can mediate non-specific oxidation, degradation and/or cleavage and other structural modifications of the immunogenic agent that lead to increased heterogeneity, decreased biological activity, lower recoveries, and/or other impairments to of biologics produced by methods provided herein. Accordingly, production of an immunogenic agent is enhanced by modulating expression of a pro-oxidant enzyme, such as a CHO cell protein selected from the group consisting of: NAD(p)H oxidase, peroxidase such as a glutathione peroxidase (e.g., glutathione peroxidase 1, glutathione peroxidase 4, glutathione peroxidase 8 (putative), glutathione peroxidase 3, encoded by the oligonucleotides of SEQ ID NO:7213, NO:7582, NO:8011, and NO:9756, respectively (RNA effector molecules: SEQ ID NOs:2439217-2439612, NOs:2560559-2560895, NOs:2703865-2704225, NOs:3151589-3151685, respectively), myeloperoxidase, constitutive neuronal nitric oxide synthase (cnNOS), xanthine oxidase (XO) (SEQ ID NOs:374846-375216) and myeloperoxidase (MPO), 15-lipoxygenase-1 (SEQ ID NOs:2480018-2480362), NADPH cytochrome c reductase, NAPH cytochrome c reductase, NADH cytochrome b5 reductase (SEQ ID NOs:569460-569777, NOs:1261910-1262218, NOs:2195311-2195681, NOs:3146048-3146071, NOs:259827-260060, respectively), and cytochrome P4502E1.

[00293] Additionally, protein production can be enhanced by modulating expression of a protein that affects the cell cycle of host cells (e.g., CHO cells) such as a cyclin (e.g., cyclin M4, cyclin J, cyclin T2, cyclin-dependent kinase inhibitor 1A (P21), cyclin-dependent kinase inhibitor 1B, cyclin M3, cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4), cyclin E2, S100 calcium-binding protein A6 (calcyclin), cyclin-dependent kinase 5, regulatory subunit 1 (p35), cyclin T1, inhibitor of CDK, cyclin A1 interacting protein 1, by use of corresponding a RNA effector molecule comprising an an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:2447340-2447632, NOs:2463782-2464073, NOs:2466004-2466274, NOs:2659502-2659871, NOs:2731076-2731440, NOs:2748583-2748914, NOs:2895015 2895359, NOs:2904183-2904530, NOs:2966362-2966657, NOs:3088848-3089061, NOs:3107706-3107919, and NOs:3122589-

3122734, respectively), or a cyclin dependent kinase (CDK). In some embodiments, the cyclin-dependent kinase is a CHO cell cyclin-dependent kinase selected from the group consisting of: CDK2 (SEQ ID NOs:1193336-1193684), CDK4 (SEQ ID NOs:1609522-1609852), P10 (SEQ ID NOs:3013998-3014274), P21 (SEQ ID NOs:2659502-2659871), P27 (SEQ ID NOs:2731076-2731440), p53, P57, p16INK4a, P14ARF, and CDK4 (SEQ ID NOs:1609522-1609852). For example, in various embodiments, the expression of one or more proteins that affect cell cycle progression can be transiently modulated during the growth and/or production phases of heterologous protein production in order to enhance expression and recovery of heterologous proteins.

[00294] In addition, production of excess ammonia in bioprocessing is a common problem in large scale cell culture. High ammonia concentrations result in reduced cell and product yields, depending on cell line and process conditions. Liberation of ammonia is thought to occur through the breakdown of glutamine to glutamate by glutaminase, and/or through the conversion of glutamate to a-ketoglutarate by glutamate dehydrogenase. Therefore, in one embodiment, biologics production can be enhaced by modulating expression of a protein that affects ammonia production, such as glutaminase or glutamate dehydrogenase. A particular embodiment provides for a RNA effector that targets CHO cell glutaminase having the transcript of SEQ ID NO:311 (CHO311.1). In one embodiment the RNA effector is a siRNA selected from SEQ ID NOs:105170-105438, which target glutaminase. In another embodiment, the RNA effector targets CHO cell glutamate dehydrogenase having SEQ ID NO:569 (CHO569.1). In one embodiment the RNA effector is a siRNA selected from SEQ ID NOs:177779-178010, which target CHO cell glutamate dehydrogenase 1.

[00295] It is known that production of lactic acid in cell cultures inhibits cell growth and influences metabolic pathways involved in glycolysis and glutaminolysis (Lao & Toth, 13 Biotech. Prog., 688-91 (1997)). The accumulation of lactate in cells is caused mainly by the incomplete oxidation of glucose to CO_2 and H_2O, in which most of the glucose is oxidized to pyruvate and finally converted to lactate by lactate dehydrogenase (LDH). The accumulation of lactic acid in cells is detrimental to achieving high cell density and viability. Accordingly, in one embodiment, immunogenic protein production is enhanced by modulating expression of a protein that affects lactate formation, such as lactate dehydrogenase A (LDHA). Hence, a particular embodiment provides for a RNA effector molecule that targets the LDHA1 gene.

[00296] In some embodiments, glucose utilization of cells is manipulated by modulation espression of e.g., target genes Myc and AKT. In one embodiment the target gene is CHO myelocytomatosis oncogene comprising the sequence of SEQ ID NO:2185 (CHO2185.1). In one

embodiment the RNA effector molecule is a siRNA having a sequence selected from SEQ ID NOs:713438-713745. In one embodiment the RNA effector molecule is a siRNA having a sequence selected from SEQ ID NOs:713438-713473. In one embodiment the target gene is CHO thymoma viral proto-oncogene-1 comprising the nucleotides of SEQ ID NO:1793 (CHO1793.1). In one embodiment the RNA effector molecule is a siRNA having a sequence selected from SEQ ID NOs:581286-581643. In one embodiment the RNA effector molecule is a siRNA having a sequence selected from SEQ ID NOs:581286-581334.

[00297] In one embodiment, a cell culture is treated as described herein with RNA effector molecules that permit modulation of Bax, Bak and LDH expression. In another embodiment, the RNA effector molecules targeting Bax, Bak and LDH can be administered in combination with one or more additional RNA effector molecules and/or agents. Provided herein is a cocktail of RNA effector molecules targeting Bax, Bak and LDH expression, which can optionally be combined with additional RNA effector molecules or other bioactive agents as described herein.

[00298] In some embodiments, production of an immunogenic agent is enhanced by modulating expression of a protein that affects cellular pH, such as LDH or lysosomal V-type ATPase.

[00299] In some embodiments, production of an immunogenic agent is enhanced by modulating expression of a protein that affects carbon metabolism or transport, such as GLUT1, for example, by contacting the cell with a RNA effector molecule wherein the RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide having the nucleotide sequence selected from the group consisting of SEQ ID NOs:438155-438490, GLUT2, GLUT3, GLUT4, PTEN (SEQ ID. NOs:6091-6940) (with a RNA effector molecule wherein the RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the nucleotide sequence selected from the group consisting of SEQ ID NOs:69091-69404 (CHO cell, antisense), or LDH (with a RNA effector molecule wherein the RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs:1297283-1297604) – see also Table 10 with LDHs).

Table 4. GLUTS and PTEN

SEQ ID NO:	Transcript No.	consL	Description	Avg Coverage	siRNA SEQ ID NOs:

Table 4. GLUTS and PTEN					
SEQ ID NO:	Transcript No.	consL	Description	Avg Coverage	siRNA SEQ ID NOs:
1375	CHO1375.1	2298	solute carrier family 2 (facilitated glucose transporter), member 1	14.092	438155-438490
6869	CHO6869.1	910	solute carrier family 2, (facilitated glucose transporter), member 8	0.818	2325698-2325997
7909	CHO7909.1	656	solute carrier family 2 (facilitated glucose transporter), member 13	0.689	2669929-2670303
189	CHO189.1	3384	PTEN (phosphatase and tensin homolog)	0.633	69091-69404

[00300] In some embodiments, production of an immunogenic agent is enhanced by modulating expression of cofilin (for example a muscle cofilin 2, or non-muscle cofilin-1). In one embodiment, a cell with a RNA effector molecule wherein the RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs:435213-435610, targeting the CHO muscle cofilin 2 (SEQ ID NO:1366). In another embodiment, a cell with a RNA effector molecule wherein the RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs:1914036-1914356, targeting the CHO non-muscle cofilin 1 (SEQ ID NO:5716).

[00301] In some embodiments, production of an immunogenic agent is enhanced by modulating expression of a protein that affects uptake or efficacy of a RNA effector molecule in host cells, such as ApoE, Mannose/GalNAc-receptor, and Eri1. In various embodiments, the expression of one or more proteins that affects RNAi uptake or efficacy in cells is modulated according to a method provided herein concurrently with modulation of one or more additional target genes, such as a target gene described herein, in order to enhance the degree and/or extent of modulation of the one or more additional target genes.

[00302] In some embodiments, the production of an immunogenic agent is enhanced by inducing a stress response in the host cells which causes growth arrest and increased productivity. A stress response can be induced, e.g., by limiting nutrient availability, increasing solute concentrations, or low temperature or pH shift, and oxidative stress. Along with increased productivity, stress responses can also have adverse effects on protein folding and secretion. In some embodiments, such adverse effects are ameliorated by modulating the expression of a target gene encoding a stress response protein, such as a protein that affects protein folding and/or secretion described herein.

[00303] In some embodiments, production of an immunogenic agent is enhanced by modulating expression of a protein that affects cytoskeletal structure, e.g. altering the equilibrium between monomeric and filamentous actin. In one embodiment the target gene encodes cofilin and a RNA effector molecule inhibits expression of cofilin. In one embodiment, at least one RNA effector molecule increases expression of a target gene selected from the group consisting of: cytoplasmic actin capping protein (CapZ), Ezrin (VIL2), and Laminin A. *See, e.g.*, Table 5, as follows:

Table 5. Example hamster genes and siRNAs (antisense strand) targeting Laminin and CapZ

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
763	2614	capping protein (actin filament) muscle Z-line, α 1	5.404	235917-236159
3104	1768	capping protein (actin filament) muscle Z-line, α 2	15.011	1026343-1026702
3590	1647	capping protein (actin filament) muscle Z-line, β	60.716	1190654-1190998
5752	1156	capping protein (actin filament), gelsolin-like	62.723	1927144-1927507
1081	2436	ezrin	31.498	339220-339540
122	3653	laminin, α 5	10.318	48814-49139
8777	444	laminin, α 2	0.046	2954307-2954650
3157936	2200	laminin, α 3	0.41	3160721-3160820

[00304] The modulation of expression (e.g., inhibition) of a target gene by a RNA effector molecule can be further alleviated by introducing a second RNA effector molecule, wherein at least a portion of the second RNA effector molecule is complementary to a target gene encoding a protein that mediates RNAi in the host cell. For example, the modulation of expression of a target gene can be alleviated by introducing into the cell a RNA effector molecule that inhibits expression of an Argonaute protein (e.g., argonaute-2) or other component of the RNAi pathway of the cell. In one embodiment, the immunogenic agent is transiently inhibited by contacting the cell with a first RNA effector molecule targeted to the immunogenic agent. The inhibition of expression of the immunogenic agent is then alleviated by introducing into the cell a second RNA effector molecule targeted against a gene encoding a protein of the RNAi pathway.

[00305] Additionally, the production of a desired immunogenic agent can be enhanced by introducing into the cell a RNA effector molecule during the production phase to modulate expression of a target gene encoding a protein that affects protein expression, post-translational modification, folding, secretion, and/or other processes related to production and/or recovery of the desired immunogenic agent. Alternatively, the production of an immunogenic agent is enhanced by introducing into the cell a RNA effector molecule which inhibits cell growth and/or cell division during the production phase.

Post-translational processing

[00306] Post-translational modifications can require additional bioprocess steps to separate modified and unmodified polypeptides, increasing costs and reducing efficiency of biologics production. Accordingly, in some embodiments, in production of a polypeptide agent in a cell is enhanced by modulating the expression of a target gene encoding a protein that affects post-translational modification. In additional embodiments, biologics production is enhanced by modulating the expression of a first target gene encoding a protein that affects a first post-translational modification, and modulating the expression of a second target gene encoding a protein that affects a second post-translational modification.

[00307] More specifically, proteins expressed in eukaryotic cells can undergo several post-translational modifications that can impair production and/or the structure, biological activity, stability, homogeneity, and/or other properties of the immunogenic agent. Many of these modifications occur spontaneously during cell growth and polypeptide expression and can occur at several sites, including the peptide backbone, the amino acid side-chains, and the amino and/or carboxyl termini of a given polypeptide. In addition, a given polypeptide can comprise several different types of modifications. For example, proteins expressed in avian and mammalian cells can be subject to acetylation, acylation, ADP-ribosylation, amidation, ubiquitination, methionine oxidation, disulfide bond formation, methylation, demethylation, sulfation, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, hydroxylation, iodination, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, gluconoylation, sequence mutations, N-terminal glutamine cyclization and deamidation, and asparagine deamidation. N-terminal asparagine deamidation can be reduced by contacting the cell with a RNA effector molecule targeting the N-terminal Asn amidase (encoded, for example, by SEQ ID NO:5950), wherein the RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs:1999410-1999756.

[00308] In some embodiments, immunogen production is enhanced by modulating expression of a target gene which encodes a protein involved in protein deamidation. Proteins can be deamidated via several pathways, including the cyclization and deamidation of N-terminal glutamine and deamidation of asparagine. Thus, in one embodiment, the protein involved in protein deamidation is N-terminal asparagine amidohydrolase. Protein deamidation can lead to altered structural properties, reduced potency, reduced biological activity, reduced efficacy, increased immunogenicity, and/or other undesirable properties and can be measured by

several methods, including but not limited to, separations of proteins based on charge by, e.g., ion exchange chromatography, HPLC, isoelectric focusing, capillary electrophoresis, native gel electrophoresis, reversed-phase chromatography, hydrophobic interaction chromatography, affinity chromatography, mass spectrometry, or the use of L-isoaspartyl methyltransferase.

[00309] When the immunogenic agent comprises a glycoprotein, such as a viral product having viral surface membrane proteins or monoclonal antibody having glycosylated amino acid residues, biologics production can be enhanced by modulating expression of a target gene that encodes a protein involved in protein glycosylation. ***Glycosylation*** patterns are often important determinants of the structure and function of mammalian glycoproteins, and can influence the solubility, thermal stability, protease resistance, antigenicity, immunogenicity, serum half-life, stability, and biological activity of glycoproteins.

[00310] In various embodiments, the protein that affects glycosylation is selected from the group consisting of: dolichyl-diphosphooligosaccharide-protein glycosyltransferase (SEQ ID NOs:2742894-2743239), UDP glycosyltransferase, UDP-Gal:βGlcNAc beta 1,4-galactosyltransferase (SEQ ID NOs:851115-851489, NOs:1552461-1552728, NOs:1562813-1563108, and NOs:1635173-1635561), UDP-galactose-ceramide galactosyltransferase, fucosyltransferase (SEQ ID NOs:209841-210227), protein O-fucosyltransferase (SEQ ID NOs:916726-917035), N-acetylgalactosaminytransferase (SEQ ID NOs:57147-57422, NOs:65737-65999, NOs:1013002-1013376, NOs:1363583-1363970, NOs:1546609-1546999, NOs:1965217-1965613, NOs:2876241-2876595), particularly T4 (SEQ ID NOs:2876241-2876595), O-GlcNAc transferase (SEQ ID NOs:607012-607348), oligosaccharyl transferase (SEQ ID NOs:89738-90024, NOs:262368-262621), O-linked N-acetylglucosamine transferase, and α-galactosidase (SEQ ID NOs:1600968-1601288) and β-galactosidase (SEQ ID NOs:690601-690989).

[00311] In other embodiments. The protein that affects glycosylation is selected, for example, from Table 6, as follows:

Table 6. O-linked glycosylation

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
150	3549	UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1	11.757	57147-57422
178	3411	UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2	22.835	65737-65999
1720	2167	protein-O-mannosyltransferase 2	1.099	555946-556293
1869	2123	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)	0.839	607012-607348

Table 6. O-linked glycosylation				
SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
3065	1776	UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10	1.546	1013002-1013376
4007	1548	protein-O-mannosyltransferase 1	1.418	1331135-1331436
4654	1402	UDP-N-acetyl-α-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7	0.782	1546609-1546999
5740	1158	protein O-linked mannose β1,2-N-acetylglucosaminyltransferase	2.323	1922712-1923111
6857	913	protein O-fucosyltransferase 1	0.441	2321807-2322122
258	3197	STT3, subunit of the oligosaccharyltransferase complex, homolog B (*S. cerevisiae*)	25.073	89738-90024
1114	2420	ribophorin II	272.65	350422-350752
2417	1954	mannoside acetylglucosaminyltransferase 2	5.098	792371-792746
2614	1903	dolichyl-di-phosphooligosaccharide-protein glycotransferase	179.1	859105-859389
4441	1452	dolichyl pyrophosphate phosphatase 1	2.663	1476398-1476763
4945	1339	mannoside acetylglucosaminyltransferase 5	0.5	1645857-1646201
5594	1191	mannoside acetylglucosaminyltransferase 1	3.072	1870192-1870557
5740	1158	protein O-linked mannose β1,2-N-acetylglucosaminyltransferase	2.323	1922712-1923111
8007	632	asparagine-linked glycosylation 6 homolog (yeast, α-1,3,-glucosyltransferase)	1.15	2702432-2702775
8404	518	keratinocyte associated protein 2	6.913	2832647-2833030

[00312] In further embodiments, production of an immunogenic glycoprotein is enhanced by modulating expression of a sialidase or a sialytransferase enzyme. Terminal sialic acid residues of glycoproteins are particularly important determinants of glycoprotein solubility, thermal stability, resistance to protease attack, antigenicity, and specific activity. For example, when terminal sialic acid is removed from serum glycoproteins, the desialylated proteins have significantly decreased biological activity and lower circulatory half-lives relative to sialylated counterparts. The amount of sialic acid in a glycoprotein is the result of two opposing processes, i.e., the intracellular addition of sialic acid by sialytransferases and the removal of sialic acid by sialidases. Thus, in some embodiments, production of a glycoprotein is enhanced by inhibiting expression of a sialidase and/or activating expression of a sialytransferase. Example sialyltransferase targets and exemplary siRNAs are found in Table 7, as follows:

Table 7. Example sialyltransferase targets				
SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
2088	2048	ST3 β-galactoside α-2,3-sialyltransferase 1	5.651	681105-681454
2167	2021	ST3 β-galactoside α-2,3-sialyltransferase 4	13.01	707535-707870
3411	1689	ST3 β-galactoside α-2,3-sialyltransferase 3	3.964	1131123-1131445

3484	1672	ST3 β-galactoside α-2,3-sialyltransferase 5	21.148	1155324-1155711
4186	1504	ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6	5.237	1391079-1391449
4319	1476	ST3 β-galactoside α-2,3-sialyltransferase 2	1.043	1435989-1436317
3157960	2282	ST8 α-N-acetyl-neuraminide α-2,8-sialyltransferase 4	1.629	3246817-3246916
3158211	343	ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 4	0.282	3260605-3260704

[00313] In some embodiments, immunogenic agent production is enhanced by modulating expression of a glutaminyl cyclase which catalyzes the intramolecular cyclization of N-terminal glutamine residues into pyroglutamic acid, liberating ammonia (pyroglutamation). Glutaminyl cyclase modulation can be accomplished by contacting the cell with a RNA effector molecule targeting the glutaminyl cyclase gene (for example, hamster glutaminyl cyclase encoded by SEQ ID NO:5486), wherein the RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs:1832626-1832993.

[00314] In some embodiments, production of immunogenic agents containing disulfide bonds is enhanced by modulating expression of a protein that affects disulfide bond oxidation, reduction, and/or isomerization, such as protein disulfide isomerase or sulfhydryl oxidase. Disulfide bond formation can be particularly problematic for the production of multi-subunit proteins or peptides in eukaryotic cell culture. Examples of multi-subunit proteins or peptides include receptors, extracellular matrix proteins, immunomodulators, such as MHC proteins, full chain antibodies and antibody fragments, enzymes and membrane proteins.

[00315] In some embodiments, protein production is enhanced by modulating expression of a protein that affects methionine oxidation. Reactive oxygen species (ROS) can oxidize methionine (Met) to methionine sulfoxide (MetO), resulting in increased degradation and product heterogeneity, and reduced biological activity and stability. In some embodiments, the target gene encodes a methionine sulfoxide reductase, which catalyzes the reduction of MetO residues back to methionine. For example, wherein the CHO cell RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs:2044387-2044676, SEQ ID NOs:2557492-2557809, and SEQ ID NOs:3076104-3076309.

[00316] Immunogenic agents (including some live attenuated viruses) produced in cell culture on an industrial-scale are typically secreted by cultured cells and recovered and purified from the surrounding cell culture media. In general, the rate of protein production and the yield of recovered protein is directly related to the rate of protein folding and secretion by the host cells. For example, an accumulation of misfolded proteins in the endoplasmic reticulum (ER) of host cells can slow or stop secretion via the unfolded protein response (UPR) pathway. The UPR is triggered by stress-sensing proteins in the ER membrane which detect excess unfolded proteins. UPR activation leads to the upregulation of chaperone proteins (e.g., Bip) which bind to misfolded proteins and facilitate proper folding. UPR activation also upregulates the transcription factors XBP-1 (e.g., CHO cell SEQ ID NOs:187955-188152) and CHOP (e.g., CHO cell SEQ ID NOs:2813622-2813956). CHOP generally functions as a negative regulator of cell growth, differentiation and survival, and its upregulation via the UPR causes cell cycle arrest and increases the rate of protein folding and secretion to clear excess unfolded proteins from the cell. Hence, cell cycle can be promoted initially, then repressed during virus production phase to increase viral product yield. An increase the rate of immunogenic protein secretion by the host cells can be measured by, e.g., monitoring the amount of protein present in the culture media over time.

[00317] The present invention provides methods for enhancing the production of a secreted polypeptide in cultured eukaryotic host cells by modulating expression of a target gene which encodes a protein that affects protein secretion by the host cells. In some embodiments, the target gene encodes a protein of the UPR pathway, such as IRE1, PERK, ATF4 (CHO cell, SEQ ID NOs:1552067-1552460), ATF6 (CHO cell, SEQ ID NOs:570138-570498), eIF2α (CHO cell, SEQ ID NOs:1828122-1828492), GRP78 (CHO cell, SEQ ID NOs:292590-292837), GRP94 (CHO cell, SEQ ID NOs:180574-180954), calreticulin (CHO cell, SEQ ID NOs:895691-896051) or a variant thereof, or a protein that regulates the UPR pathway, such as a transcriptional control element (e.g., the cis-acting UPR element (UPRE)).

[00318] Other target genes involved in protein secretion are listed in Table 8, which identifies example hamster transcript target genes and exemplary siRNAs (antisense strand):

Table 8. Example Chinese hamster secretory pathway targets

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
8	4838	myosin VA	2.412	12025-12278
584	2751	transmembrane emp24-like trafficking protein 10 (yeast)	22.212	182087-182337
1448	2267	glycyl-tRNA synthetase	58.453	462911-463286
2119	2036	ADP-ribosylation factor interacting protein 1	1.425	691369-691690

2236	2001	MON1 homolog A (yeast)	8.293	730977-731347
2859	1843	retinoid X receptor α	3.715	942750-943051
3432	1685	lipase maturation factor 1	6.857	1138015-1138340
4066	1533	WD repeat domain 77	15.26	1350827-1351146
4826	1363	N-acetylglucosamine-1-phosphate transferase, α and β subunits	0.701	1605188-1605495
5380	1240	K intermediate/small conductance Ca-activated channel, subfamily N, member 4	8.029	1795510-1795838
5799	1146	lysosomal trafficking regulator	0.206	1944185-1944541
7480	768	endoplasmic reticulum protein 29	24.355	2526951-2527343
8119	595	serglycin	9.946	2738723-2739031
3157722	251	forkhead box A1	0.147	3261005-3261104

[00319] In some embodiments, the protein that affects protein secretion is a ***molecular chaperone*** selected from the group consisting of: Hsp40 (e.g., CHO cell SEQ ID NOs:677203-677558), HSP47 (e.g., CHO cell SEQ ID NOs:777036-777317), HSP60 (e.g., CHO cell SEQ ID NOs: 494743-495086), Hsp70 (e.g., CHO cell SEQ ID NOs:3147029-3147080), HSP90, HSP100, protein disulfide isomerase (e.g., CHO cell SEQ ID NOs:72748-72996), peptidyl prolyl isomerase (e.g., CHO cell SEQ ID NOs:38781-39067, NOs:1074139-1074475, NOs:1127061-1127426, NOs:1649170-1649515, NOs:2197146-2197532, NOs:2253978-2254373, NOs:2261765-2262058, NOs:2275330-2275633, NOs:2579547-2579908, and NOs:3115010-3115199), calnexin (e.g., CHO cell SEQ ID NOs:61559-61785), Erp57 (e.g., CHO cell SEQ ID NOs:774355-774677), and BAG-1.

[00320] In some embodiments, the protein that affects protein secretion is selected from the group consisting of: gamma-secretase, p115, a signal recognition particle (SRP) protein, secretin, and a kinase (e.g., MEK).

[00321] The production of immunogenic agents in cell culture can be negatively affected by proteins which have an affinity for the immunogenic agent or a molecule or factor that binds specifically to the immunogenic agent. For example, a number of heterologous proteins have been shown to bind the glycoproteins heparin and heparan sulfate at host cell surfaces. This can lead to the co-purification of heparin, heparan sulfate, and/or heparin/heparan sulfate-binding proteins with recombinant protein products, decreasing yield and reducing homogeneity, stability, biological activity, and/or other properties of the recovered proteins. Examples of heterologous proteins which have been shown to bind heparin and/or heparan sulfate include BMP3 (bone morphogenetic protein 3 or osteogenin), TNF-α, GDNF, TGF-β family members, and HGF. Therefore, in one embodiment, the production of a heterologous protein, such as BMP3, TNF-α, GDNF, TGF-β family members, or HGF, or another immunogenic agent in cultured host cells is enhanced by contacting the cells with a RNA effector molecule which

modulates (e.g., inhibits) expression and/or production of heparin and/or heparan sulfate. In one embodiment, the level of heparin and/or heparan sulfate is reduced by modulating expression of a host cell enzyme involved in the production of heparin and/or heparan sulfate, such as a host cell xylotransferase (SEQ ID NOs:1554774-1555054).

[00322] In some embodiments, for example when in immunogenic agent is a viral particle, such as an influenza virus, target genes can include those involved in reducing sialic acid from the host cell surface, which reduces virus binding, and therefore increases recovery of the virus in cell culture media (i.e., less virus remains stuck on host cell membranes). These targets include: solute carrier family 35 (CMP-sialic acid transporter) member A1 (SLC35A1) (e.g., CHO gene inferred from *M. muscuslus* Slac35a1, GeneID:24060) (*Gallus* target gene sequences selected from SEQ ID NOs:3154345-3154368 and NOs:3154369-3154392) (CHO cell target gene sequences selected from SEQ ID NOs:464674-465055), solute carrier family 35 (UDP-galactose transporter), member A2 (SLC35A2) (e.g., CHO gene inferred from *M. muscuslus* Slc35a2, GeneID: 22232) UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) (e.g., CHO gene inferred from *M. muscuslus* Gne, GeneID: 10090) (*Gallus* target gene sequences selected from SEQ ID NOs:3154297-3154320 and NOs:3154321-3154344) (CHO cell target gene sequences selected from SEQ ID NOs:2073971-2074368), cytidine monophospho-N-acetylneuraminic acid synthetase (Cmas) (e.g., CHO gene inferred from *M. muscuslus* Cmas, GeneID: 12764) (*Gallus* target gene sequences selected from SEQ ID NOs:3154249-3154272 and NOs:3154273-3154296) (CHO cell target gene sequences selected from SEQ ID NOs:1633101-1633406), UDP-Gal:βGlcNAc β1,4-galactosyltransferase (B4GalT1) (e.g., CHO gene inferred from *M. muscuslus* B4galT1, GeneID: 14595) (*Gallus* target gene sequences selected from SEQ ID NOs:3154153-3154176 and NOs:3154177-3154200) (CHO cell target gene sequences selected from SEQ ID NOs:2528454-2528763), and UDP-Gal:βGlcNAc β1,4-galactosyltransferase, polypeptide 6 (B4GalT6) (e.g., CHO gene inferred from *M. muscuslus* B4GalT6, GeneID: 56386) (*Gallus* target gene sequences selected from SEQ ID NOs:3154201-3154224 and NOs:3154225-3154248) (CHO cell target gene sequences selected from SEQ ID NOs:1635173-1635561).

[00323] Additional targets can include those involved in avian host sialidase (*see* Wang et al., 10 BMC Genomics 512 (2009)), because influenzae binds to cell surface sialic acid residues, thus decreased sialidase can increase the rate of infection or purification: NEU2 sialidase 2 (cytosolic sialidase) (e.g., *Gallus* Neu2, GeneID: 430542) and NEU3 sialidase 3 (membrane sialidase) (e.g., *Gallus* Neu3, GeneID: 68823). Additional target genes include miRNA antagonists that can be used to determine if this is the basis of some viruses not growing well in

cells, for example Dicer (dicer 1, ribonuclease type III) because knock-down of Dicer leads to a modest increase in the rate of infection (Matskevich et al., 88 J. Gen. Virol. 2627-35 (2007)); or ISRE (interferon-stimulated response element), as a decoy titrate TFs away from ISRE-containing promoters. Example genes and targets associated with sialidases (neuraminidases) are shown in Table 9, as follows:

Table 9. Example sialidases (neuraminidase)

SEQ ID NO:	consL	Description	Avg Coverage	siRNA SEQ ID NOs:
4150	1513	neuraminidase 1	11.083	1378888-1379212
4816	1365	neuraminidase 2	6.612	1601657-1601952
7787	692	neuraminidase 3	0.275	2628786-2629181

[00324] The use of bioprocesses for the manufacture of immunogenic agents at an industrial scale is often confounded by the presence of pathogens, such as active viral particles, and other adventitious agents (e.g., prions), often necessitating the use of expensive and time consuming steps for their detection, removal (e.g., viral filtration) and/or inactivation (e.g., heat treatment) to conform to regulatory procedures. Such problems can be exacerbated due to the difficulty in detecting and monitoring the presence of such viruses. Accordingly, in some embodiments, methods are provided for enhancing production of an immunogenic agent by modulating expression of a target gene affecting the susceptibility of a host cell to pathogenic infection. For example, in some embodiments, the target gene is a host cell protein that mediates viral infectivity, such as the transmembrane proteins XPR1 (e.g., CHO cell SEQ ID NOs:62021-62362), RDR, Flvcr, CCR5, CXCR4, CD4, Pit1, and Pit2 (e.g., CHO cell SEQ ID NOs:3068222-3068455).

[00325] Although a target sequence is generally 10 to 30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a “window” or “mask” of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, e.g., *in silico*) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence “window” progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform

optimally can identify those RNA sequences that, when targeted with a RNA effector molecule agent, mediate the best inhibition of target gene expression. Thus, although the sequences identified herein represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

[00326] Further, it is contemplated that for any sequence identified herein, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those and sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Coupling this approach to generating new candidate targets with testing for effectiveness of RNA effector molecules based on those target sequences in an inhibition assay as known in the art or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, e.g., the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art and/or discussed herein to further optimize the molecule (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes, etc.) as an expression inhibitor.

III. Biocontamination

[00327] Cell lines used commonly in biotechnology manufacturing processes, such as CHO cells, have been demonstrated to produce retrovirus-like particles. Moreover, MMV (murine minute virus) contamination in a large-scale biologics manufacturing process has occurred, and was attributed to adventitious contamination of raw materials used in production. Consequently, international regulatory agencies require biologics manufacturers to employ a comprehensive viral clearance strategy, including characterization of cell lines and raw materials, employing robust viral inactivation and removal steps, and testing of process intermediates and final products. Multiple orthogonal steps, including chromatographic methods, physiochemical inactivation (e.g., low pH, solvent detergent), and size exclusion-based filtration, together yield cumulative inactivation and removal of viruses. *See, e.g.*, Marques et al., 25 Biotech. Prog. 483-91 (2009); Khan et al., 52 Biotech. Appl. Biochem. 293-301 (2009). Viral clearance and clearance validation are some of the most time-consuming and revenue-eating activities in bioprocessing: Downstream processing accounts for about 70% of the total

biomanufacturing cost. Chochois et al., 36 Bioprocess Intl. (June, 2009). Downstream bioprocessing filter products, alone, cost biotechnology and vaccine makers more than $1 billion annually.

[00328] Thus, in further embodiments, production is enhanced by introducing into the cell a RNA effector molecule that inhibits expression of viral proteins in host cells. More specifically, for example, latent DNA viruses (such as herpesviruses) and endogenous retroviruses (ERVs), or retroviral elements are likely present in all vertebrates. Endogenous retroviral sequences are an integral part of eukaryotic genomes, and although the majority of these sequences are defective, some can produce infectious virus, either spontaneously or upon long-term culture. ERV virus production can also be induced upon treatment with various chemical or other agents that can be part of the normal production system. Additionally, although many endogenous retroviruses do not readily re-infect their own cells, they can infect other species *in vitro* and *in vivo*. For example, two of three subgroups of pig ERVs (PERVs), can infect human cells *in vitro*.

[00329] There are at least twenty-six distinct groups of human endogenous retroviruses (HERVs); and bird, mouse, cat, and pig harbor replication-competent ERVs that are capable of interacting with related exogenous virus. Retrovirus-induced tumorigenesis can involve the generation of a novel pathogenic virus by recombination between replication-competent and -defective sequences and/or activation of a cellular oncogene by a long terminal repeat (LTR) due to upstream or downstream insertion of retrovirus sequences. Thus, the activation of an endogenous, infectious retrovirus in a cell substrate that is used for the production of biologics is an important safety concern, especially in the case of live, viral vaccines, where minimal purification and inactivation steps are used in order to preserve high vaccine potency.

[00330] Adventitious viruses represent a major risk associated with the use of cell-substrate derived biologicals, including vaccines for human use. The possibility for viral contamination exists in primary cultures and established cultures, as well as Master Cell Banks, end-of-production cells, and bulk harvest fluids. For example, this is a major obstacle to the use of neoplastic-immortalized cells for which the mechanism of transformation is unknown, because these could have a higher risk of containing oncogenic viruses. Extensive testing for the presence of potential extraneous agents is therefore required to ensure the safety of the vaccines. The most common scenarios for adventitious viral contamination of biologics include bovine viral diarrhoea virus in foetal bovine serum; porcine parvovirus in porcine substrates; and murine minute virus, reovirus, vesivirus and Cache Valley virus in CHO cell-derived bulk

harvests. The three last-named viral entities are believed to be introduced via bovine serum used during the manufacturing process (during scale-up or during the entire process).

[00331] During the production of live attenuated viral vaccines, removal of contaminating viral particles, nucleic acid, or proteins is problematic because any antiviral approach must leave the viral product intact and immunogenic. Indeed, endogenous avian viral particles have been found in commercially released human measles and mumps vaccines derived from chicken embryo fibroblasts. Moreover, endogenous viral proteins, particularly envelop proteins, often inhibit the efficiency of recombinant viral vectors used in creating transformed cell lines. Further, endogenous virus can aggravate the immune response of the host cell, often triggered during viral production or retroviral transduction. Hence, there remains a need for techniques that inhibit adventitious, latent and endogenous viral activity, and thus increase purity and yield of immunogenic agents produced in cells.

[00332] The present invention provides for enhancing production of an immunogenic agent by introducing into the cell a RNA effector molecule to modulate expression of a target gene, optionally encoding a protein, that is involved with the expression of an adventitious, latent or endogenous virus. Thus, in some embodiments, the production of an immunogenic agent in a host cell is enhanced by introducing into the cell a RNA effector molecule that inhibits expression of a latent or endogenous viral protein such that the infectivity and/or load of the desired immunogenic agent in the cell is increased.

[00333] For example, a particular advantage of cell-culture based inactivated influenza virus or influenza viral antigens is the absence of egg-specific proteins that might trigger an allergic reaction against egg proteins. Therefore, the use according to the invention is especially suitable for the prophylaxis of influenza virus infections, particularly in populations that constitute higher-risk groups, such as asthmatics, those with allergies, and also people with suppressed immune systems and the elderly.

[00334] The cultivation conditions under which a virus strain is grown in cell culture also are of great significance with respect to achieving an acceptably high yield of the strain. In order to maximize the yield of a desired virus strain, both the host system and the cultivation conditions must be adapted specifically to provide an environment that is advantageous for the production of a desired virus strain. Many viruses are restricted to very specific host systems, some of which are very inefficient with regard to virus yields. Some of the mammalian cells which are used as viral host systems produce virus at high yields, but the tumorigenic nature of such cells invokes regulatory constraints against their use for vaccine production.

[00335] The problems arising from the use of serum in cell culture and/or protein additives derived from an animal or human source added to the culture medium, e.g., the varying quality and composition of different batches and the risk of contamination with mycoplasma, viruses or BSE-agent, are well-known. In general, serum or serum-derived substances like albumin, transferrin or insulin can contain unwanted agents that can contaminate the culture and the immunogenic agents produced therefrom. Furthermore, human serum derived additives have to be tested for all known viruses, like hepatitis or HIV, which can be transmitted by serum. Bovine serum and products derived therefrom, for example trypsin, bear the risk of bovine spongiform encephalitis-contamination. In addition, all serum-derived products can be contaminated by still unknown agents. Therefore, cells and culture conditions that do not require serum or other serum derived compounds are being pursued.

[00336] For example, the production of smallpox vaccine, modified vaccinia virus Ankara (MVA) is amplified in cell cultures of primary or secondary chicken embryo fibroblasts (CEF). The CEF are obtained from embryos of chicken eggs that have been incubated for 10 to 12 days, from which the cells are then dissociated and purified. These primary CEF cells can either be used directly or after one further cell passage as secondary CEF cells. Subsequently, the primary or secondary CEF cells are infected with the MVA. For the amplification of MVA the infected cells are incubated for 2 to 3 days at 37°C. *See, e.g.*, Meyer et al., 72 J. Gen. Virol. 1031-38 (1991); Sutter et al., 12 Vaccine 1032-40 (1994). Many pox viruses replicate efficiently in CEF incubated at temperatures below 37°C, such as 30°C. *See* U.S. Patent No. 6,924,137.

[00337] The use of established mammalian cell lines, such as Madin-Darby canine kidney (MDCK) line, has been successful in replicating some viral strains. Nevertheless, a number of virus strains will not replicate in the MDCK line. In addition, fears over possible adverse effects associated with employing cells with a tumorigenic potential for human vaccine production have precluded the use of MDCK, a highly transformed cell line, in this context.

[00338] Other attempts at developing alternative vaccine production methods have been undertaken. U.S. Patent No. 4,783,411 discusses a method for preparing influenza vaccines in goldfish cell cultures. The virus particles for infecting the goldfish cell cultures, after their establishment, were obtained from chicken embryo cultures or from infected CD-I strain mice. The virus is passaged at least twice in the goldfish cell cultures, resulting in an attenuated influenza virus which can be used as a live vaccine. Additionally, African green monkey kidney epithelial cells (Vero) and chicken embryo cells (CEC) have been adapted to grow and produce influenzae virus and recombinant influenzae proteins in serum-free, protein-free media. *See* WO 96/015231.

[00339] Although the use of protein and serum free media limits the risk from adventitious virus contamination, it does not address the continued risk posed by latent viruses or endogenous retroviruses that exist in cell banks. The activation of an endogenous, infectious retrovirus in a cell substrate that is used for the production of biologics is an important safety concern, especially in the case of live, viral vaccines, where there are minimal purification and inactivation steps in order to preserve high vaccine potency.

[00340] In some embodiments, an RNA effector molecule targeting a vesivirus can be used with the methods and compositions described herein. Exemplary RNA effector molecules that target vesivirus are include, but are not limited to, those in Table 63 below:

Table 63: Duplexes targeting vesivirus with modified nucleotides

Duplex No	Sense/Antisense	Sequence
1	S	cuGuGGcAAGAcuAcucuudTsdT
	AS	AAGAGuAGUCUUGCcAcAGdTsdT
2	S	ccuAcAcAGGcAAcGAGGudTsdT
	AS	ACCUCGUUGCCUGUGuAGGdTsdT
3	S	GAAucAAAuuucAcAGAAudTsdT
	AS	AUUCUGUGAAAUUUGAUUCdTsdT
4	S	GAGuuGcGAccuGuGGAuAdTsdT
	AS	uAUCcAcAGGUCGcAACUCdTsdT
5	S	cAAGuGGGAuucAAcucAAdTsdT
	AS	UUGAGUUGAAUCCcACUUGdTsdT
6	S	GGAAcAucuAcGAuuAcAudTsdT
	AS	AUGuAAUCGuAGAUGUUCCdTsdT
7	S	GGcAAGAcuAcucuuGcuudTsdT
	AS	AAGcAAGAGuAGUCUUGCCdTsdT
8	S	cAGGcAAcGAGGuGuGcAudTsdT
	AS	AUGcAcACCUCGUUGCCUGdTsdT
9	S	GuuGAGAuGGuAAAuAcAAdTsdT
	AS	UUGuAUUuACcAUCUcAACdTsdT
10	S	GcuAAGAGAAGAcucAuuudTsdT
	AS	AAAUGAGUCUUCUCUuAGCdTsdT
11	S	cAAccAccAAAcGuAAcAAdTsdT
	AS	UUGUuACGUUUGGUGGUUGdTsdT
12	S	cAuGuucAccuAuGGuGAudTsdT
	AS	AUcACcAuAGGUGAAcAUGdTsdT
13	S	cAAGAcuAcucuuGcuuAudTsdT
	AS	AuAAGcAAGAGuAGUCUUGdTsdT
14	S	GcAucAuuGAuGAAuucGAdTsdT
	AS	UCGAAUUcAUcAAUGAUGCdTsdT
15	S	GGAAAGGuGuucuccuccAdTsdT
	AS	UGGAGGAGAAcACCUUUCCdTsdT
16	S	GAuGuuucuGAuGccAuuAdTsdT
	AS	uAAUGGcAUcAGAAAcAUCdTsdT
17	S	GcuGuuGcuAcGcuuucuudTsdT
	AS	AAGAAAGCGuAGcAAcAGCdTsdT

Table 63: Duplexes targeting vesivirus with modified nucleotides		
18	S	GuGAuGAuGGcGuGuAcAudTsdT
	AS	AUGuAcACGCcAUcAUcACdTsdT
19	S	cuAcucuuGcuuAuGccAudTsdT
	AS	AUGGcAuAAGcAAGAGuAGdTsdT
20	S	cGAcucuAAuccGGAAucAdTsdT
	AS	UGAUUCCGGAUuAGAGUCGdTsdT
21	S	ccuccAAAuAcGuGAuuAudTsdT
	AS	AuAAUcACGuAUUUGGAGGdTsdT
22	S	cuGAuGccAuuAuGucuAudTsdT
	AS	AuAGAcAuAAUGGcAUcAGdTsdT
23	S	GGuAuGccAcuAAccucuAdTsdT
	AS	uAGAGGUuAGUGGcAuACCdTsdT
24	S	GcGuGuAcAucGuAccAAAdTsdT
	AS	UUUGGuACGAUGuAcACGCdTsdT
25	S	cuucuGuucucAAucucAAdTsdT
	AS	UUGAGAUUGAGAAcAGAAGdTsdT
26	S	GAcucuAAuccGGAAucAAdTsdT
	AS	UUGAUUCCGGAUuAGAGUCdTsdT
27	S	cAAAuAcGuGAuuAuGAcAdTsdT
	AS	UGUcAuAAUcACGuAUUUGdTsdT
28	S	GcAuGAAuucGGcuucAuudTsdT
	AS	AAUGAAGCCGAAUUcAUGCdTsdT
29	S	cGuGuAcAucGuAccAAAudTsdT
	AS	AUUUGGuACGAUGuAcACGdTsdT
30	S	cuGuucucAAucucAAuAudTsdT
	AS	AuAUUGAGAUUGAGAAcAGdTsdT
31	S	cucuAAuccGGAAucAAAudTsdT
	AS	AUUUGAUUCCGGAUuAGAGdTsdT
32	S	cGuGAuuAuGAcAucAAAudTsdT
	AS	AUUUGAUGUcAuAAUcACGdTsdT
33	S	GuAccGcAAGGGAAuGcAudTsdT
	AS	AUGcAUUCCCUUGCGGuACdTsdT
34	S	cAAccAcuGccucuuAGuudTsdT
	AS	AACuAAGAGGcAGUGGUUGdTsdT
35	S	cuGuuAuGccuAAuGucuudTsdT
	AS	AAGAcAUuAGGcAuAAcAGdTsdT
36	S	cAAuAuuGAccAccAcGAudTsdT
	AS	AUCGUGGUGGUcAAuAUUGdTsdT
37	S	cGGAAucAAAuuucAcAGAdTsdT
	AS	UCUGUGAAAUUUGAUUCCGdTsdT
38	S	GuGAuuAuGAcAucAAAuAdTsdT
	AS	uAUUUGAUGUcAuAAUcACdTsdT
39	S	cAAGGGAAuGcAucGGuAudTsdT
	AS	AuACCGAUGcAUUCCCUUGdTsdT
40	S	GGGuGuGcAcucAuccAAudTsdT
	AS	AUUGGAUGAGUGcAcACCCdTsdT
41	S	cuuucuuccuAuGGAcuAAdTsdT
	AS	UuAGUCcAuAGGAAGAAAGdTsdT
42	S	cAcGAuGccuAcAcAGGcAdTsdT

Table 63: Duplexes targeting vesivirus with modified nucleotides		
	AS	UGCCUGUGuAGGcAUCGUGdTsdT
43	S	GGAAucAAAuuucAcAGAAdTsdT
	AS	UUCUGUGAAAUUUGAUUCCdTsdT
44	S	GAuuAuGAcAucAAAuAAudTsdT
	AS	AUuAUUUGAUGUcAuAAUCdTsdT
45	S	GcAucGGuAuuGcGuuGAudTsdT
	AS	AUcAACGcAAuACCGAUGCdTsdT
46	S	GGAGAAGGGuGuuGAuGuudTsdT
	AS	AAcAUcAAcACCCUUCUCCdTsdT
47	S	GcGcuucuuGAcAGAAAuudTsdT
	AS	AAUUUCUGUcAAGAAGCGCdTsdT

Endogenous retrovirus

[00341] Retroviruses replicate by reverse transcription, mediated by a RNA-dependent DNA polymerase (reverse transcriptase), encoded by the viral *pol* gene. Retroviruses also carry at least two additional genes: the *gag* gene encodes the proteins of the viral skeleton, matrix, nucleocapsid, and capsid; the *env* gene encodes the envelope glycoproteins. Additionally, retroviral transcription is regulated by promoter regions or “enhancers” situated in highly repeated regions (LTRs) which are present at both ends of the retroviral genome.

[00342] During the infection of a cell, reverse transcriptase makes a DNA copy of the RNA genome; this copy can then integrate into the host cell genome. Retroviruses can infect germ cells or embryos at an early stage and be transmitted by vertical Mendelian transmission. These endogenous retroviruses (ERVs) can degenerate during generations of the host organism and lose their initial properties. Some ERVs conserve all or part of their properties or of the properties of their constituent motifs, or acquire novel functional properties having an advantage for the host organism. These retroviral sequences can also undergo, over the generations, discrete modifications which will be able to trigger some of their potential and generate or promote pathological processes.

[00343] Human endogenous retroviral sequences (HERVs) represent a substantial part of the human genome. These retroviral regions exist in several forms: complete endogenous retroviral structures combining *gag*, *pol* and *env* motifs, flanked by repeat nucleic sequences which exhibit a significant analogy with the *LTR-gag-pol-env-LTR* structure of infectious retroviruses; truncated retroviral sequences, for example the retrotransposons lack their *env* domain; and the retroposons that lack the *env* and LTR regions. ERVs capable of shedding virus particles are often called type C ERVs.

[00344] Important ERVs include human teratocarcinoma retrovirus (HTDV), or HERV-K, an endogenous retrovirus known to produce viral particles from endogenous provirus. Löwer

et al., 68 J. Gen. Virol. 2807-15 (1987); Mold et al., 4 J. Biomed. Sci. 78082 (2005). HERV-R is another important ERV, because it has been found to be expressed in many tissues, including the adrenal cortex and various adrenal tumors such as cortical adenomas and pheochromocytomas. Katsumata et al., 66 Pathobiology 209-15 (1998). Murine leukemia virus (MLV) is another important ERV, that produces infective virus particles in rodent-derived cell culture upon induction. Khan & Sears, 106 Devel. Biol. 387-92 (2001). Indeed, cell culture changes that significantly alter the metabolic state of the cells and/or rates of protein expression (e.g., pH, temperature shifts, sodium butyrate addition) measurably increased the rate of endogenous retroviral synthesis in CHO cells. Brorson et al., 80 Biotech. Bioengin. 257-67 (2002).

[00345] An on-line database, called HERVd - Human Endogenous Retrovirus Database (NAR Molecular Biology Database Collection entry number 0495), has been compiled from the human genome nucleotide sequences, obtained mostly in the various ongoing Human Genome Projects. This provides a relatively simple and fast environment for screening HERVs, and makes it possible to continuously improve classification and characterization of retroviral families. The HERVd database now contains retroviruses from more than 90% of the human genome. Additionally, ERV sequences can be obtained readily through the National Institutes of Health's on-line "Entrez Gene" site.

[00346] Further regarding ERVs, embodiments of the present invention target at least one gene or LTR of primate/human Class I Gamma ERVs pt01-Chr10r-17119458, pt01-Chr5-53871501, BaEV, GaLV, HERV-T, HERV-R (HERV-3, ERV3 *env* gene, GeneID: 2086), HERV-E (ERVE1, GeneID: 85314), HERV-ADP, HERV-I, MER4like, HERV-FRD (ERVFRD1, Env protein, GeneID: 405754; *P. troglodytes* Env protein, GeneID: 471856; *Rattus norvegicus* Herv-frd Env polyprotein, GeneID: 290348), HERV-W (ERVWE2, ERV-W, env(C7), member 2, *P. troglodytes*, GeneID: 100190905; HERVWE1, ERV-W, env(C7), member 1, GeneID: 30816), HERV-H (HHLA1, HERV-H LTR-associating protein 1, GeneID:10086, *P. troglodytes* GeneID: 736282; Hhla1, mouse GeneID: 654498; HHLA2, HERV-H LTR-associating protein 2, GeneID: 11148; HHLA3, HERV-H LTR-associating protein 3, GeneID: 11147; *Xenopus* hhla2, GeneID:734131), HERVH-RTVLH2, HERVH-RGH2, HERV-Hconsensus, HERV-Fc1; primate/human Epsilon endogenous retrovirus hg15-chr3-152465283; primate/human Intermediate (epsilon-like) HERVL66; primate/human Class III Spuma-like ERVs HSRV, HFV, HERV-S, HERV-L, HERVL40, HERVL74; primate/human Delta ERV HTLV-1, HTLV-2; primate/human Lenti ERV (lentivirus) HIV-1, HIV-2; primate/human Class II, Beta ERVs MPMV, MMTV, HML1, HML2, HML3, HML4, HML7, HML8, HML5, HML10, HML6, HML9, human

teratocarcinoma-derived retrovirus (HTDV/HERV-K), or HERV-V (HERV-V1 Env1, GeneID: 147664; HERV-V2, HSV2, GeneID: 100271846).

[00347] Additional primate ERV genes that can be targeted by the methods of the present invention include LOC471586 (similar to ERV-BabFcenv provirus ancestral Env polyprotein, *P. troglodytes* GeneID: 471586), LOC470639 (similar to ERV-BabFcenv provirus ancestral Env polyprotein, *P. troglodytes* GeneID: 470639); LOC100138322 (similar to HERV-K_7p22.1 provirus ancestral Pol protein, *Bos taurus* GeneID: 10013822; LOC110138431 (similar to HERV-K_1q22 provirus ancestral Pol protein, *B. taurus* GeneID: 100138431; LOC100137757 (similar to HERV-K_6q14.1 provirus ancestral Gag-Pol polyprotein, *B. taurus* GeneID: 100137757); LOC100141085 (similar to HERV-K_8p23.1 provirus ancestral Pol protein, *B. taurus* GeneID: 100141085); LOC100138106 (similar to HERV-F(c)1_Xq21.33 provirus ancestral Gag polyprotein, *B. taurus* GeneID: LOC100138106); LOC100140731 (similar to HERV-W_3q26.32 provirus ancestral Gag polyprotein *B. taurus*, GeneID: 100140731); LOC100139657 (similar to HERV-W_3q26.32 provirus ancestral Gag polyprotein *B. taurus* GeneID: 100139657).

[00348] In other embodiments of the present invention, the ERV is rodent Class II, Beta ERV mouse mammary tumor (MMTV, GeneID: 2828729; MMTVgp7, GeneID: 1491863; MMTV *env* GeneID: 1491862; MMTVgp1, GeneID: 1724724; MMTVgp2, GeneID: 1724723; MMTV *pol* GeneID: 1491865; MMTV *pro*, GeneID: 1491865; MMTV *gag*, GeneID: 1491864); rodent Class I Gamma ERV MLV (Mlv1, mouse GeneID: 108317); feline Class I Gamma ERV FLV; ungulate Class I Gamma ERV PERV; ungulate Delta ERV BLV; ungulate lentivirus Visna, EIAV; ungulate Class II, Beta ERV JSRV; avian Class III, Spuma-like ERVs gg01-chr7-7163462; gg01-chrU-52190725, gg01-Chr4-48130894; avian Alpha ERVs ALV (ALV *pol* GeneID: 1491910; ALV *p2*, GeneID: 1491909; ALV *p10*, GeneID: 1491908; ALV *env*, GeneID: 1491907; ALV transmembrane protein, *tm*, GeneID: 1491906; ALV trans-acting factor, GeneID: 1491911), gg01-chr1-15168845; avian Intermediate Beta-like ERVs gg01-chr4-77338201; gg01-ChrU-163504869, gg01-chr7-5733782; Reptilian Intermediate Beta-like ERV Python-molurus; Fish Epsilon ERV WDSV; fish Intermediate (epsilon-like) ERV SnRV; Amphibian Epsilon ERV Xen1; Insect Errantivirus ERV Gypsy; or *Ty1* in *Saccharomyces cerevisiae*, yeast ORF161 (ERV-1-like protein, *Ectocarpus siliculosus* virus 1, GeneID: 920716).

[00349] Further regarding ERVs, as noted herein the HERV-K ERVs are particularly relevant because they can be activated by a variety of stimuli. Hence, aspects of the present invention target genes of the HERV-K family, including HERV-K3, GeneID: 2088; HERV-K2,

GeneID: 2087; HERV-K_11q22.1 provirus ancestral Pol protein, GeneID: 100133495; HERV-K7, GeneID: 449619; HERV-K6, GeneID: 64006; HERV-K(1), ERVK4, GeneID: 60359; and HERV-K(II), ERVK5, GeneID: 60358; LOC100133495 (HERV-K_11q22.1 provirus ancestral Pol protein, GeneID: 100133495).

[00350] As described herein, in particular aspects of the present invention the target gene is an ERV *env* gene, for example eERV family W, env(C7), member 1 (ERVWE1), GeneID: 30816; LOC147664 (HERV-V1 or EnvV1), GeneID: 147664; HERV-FRD provirus Env polyprotein (ERVFRDE1), GeneID: 405754 and GeneID: 471856; ERV sequence K, 6 (ERVK6 or HERV-K108), GeneID: 64006; ERV sequence 3 envelope protein (ERV3), GeneID: 2086 and GeneID: 100190893; ALV Env protein, GeneID: 1491907, or the Env protein of HERV-K18.

[00351] In a particular embodiment, the expression of HERV-K Env1 can be modulated by use of a corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3287270-3287569 (sense) and SEQ ID NOs:3287570-3287869 (antisense).

[00352] In addition to targeting ERV genes and regulatory sequences, some embodiments of the present invention target ERV receptors. For example, human solute carrier family 1 (neutral amino acid transporter), member 5 (SLC1A5, GeneID: 6510) is a receptor for Simian type D retrovirus and feline endogenous RD-114 virus. Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4 (Slc1a4, GeneID: 55963) and member 5 (Slc1a5, GeneID: 20514) are mouse versions of related proteins. Human solute carrier family 1 (glutamate/neutral amino acid transporter), member 4 (SLC1A4, GeneID: 6509), is used as receptor by HERV-W Env glycoprotein. Thus, inhibition of cellular viral receptors can decrease receptor interference, latent, endogenous or adventitious viral infection, and thus increase the production of immunogenic agent in the cell.

Latent virus

[00353] Bornaviruses are genus of non-segmented, negative-sense, non-retroviral RNA viruses that establish persistent infection in the cell nucleus. Elements homologous to the bornavirus nucleoprotein (N) gene exist in the genomes of several mammalian species, and produce mRNA that encodes endogenous Borna-like N (EBLN) elements. Horie et al., 463 Nature 84-87 (2010). Hence, in some embodiments of the invention, the target gene is a bornaviral gene.

[00354] Latent DNA viruses that can be targeted by the methods of the present invention include adenoviruses. For example, species of C serotype adenovirus can establish latent infection in human tissues. *See* Garnett et al., 83 J. Virol. 2417-28 (2000). Avian adenovirus and adenovirus-associated virus (AAV) proteins have been produced by specific-pathogen-free chicks, indicating that avian AAV can exist as a latent infection in the germ line of chickens. Sadasiv et al., 33 Avian Dis. 125-33 (1989); *see also* Katano et al., 36 Biotechniq. 676-80 (2004). In some embodiments of the invention, the target gene is a latent DNA virus. For example, the target gene can be the latent membrane protein (LMP)-2A from HHV-4 (EBV), GeneID: 3783751, which protein also transactivates the Env protein of HERV-K18.

[00355] Circoviridae are DNA viruses that exhibit a latent phase. Porcine circoviridae type 1 (PCV1) was found to have contaminated Vero cell banks from which rotavirus vaccine was made, causing a temporary FDA hold on administration of the vaccine. Assoc. Press, March 23 (2010). The genomes of PCV1 virus are provided herein are PCV1 AY193712.1 (SEQ ID NO:3154148), PCV1 EF533941.1 (SEQ ID NO:3154149), PCV1 FJ475129.2 (SEQ ID NO:3154150), PCV1 GU371908.1 (SEQ ID NO:3154151), and PCV1 GU722334.1 (SEQ ID NO:3154152).

[00356] An embodiment of the present invention provides for a RNA effector molecule that inhibits a PCV1 *rep* or *cap* gene. The *rep* gene of PCV1 is indispensable for replication of viral DNA. Mankertz & Hillenbrand, 279 Virol. 429-38 (2001). In a particular embodiment, the expression of PCV1 Rep protein can be modulated by use of a corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3152824-3153485 (sense), SEQ ID NOs:3153486-3154147 (antisense), and the tables provided herein.

[00357] In another particular embodiment, the expression of PCV1 Cap protein can be modulated by use of a corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3154731-3154778 (sense), SEQ ID NOs:3154778-3154826 (antisense), and the tables provided herein.

Adventitious virus

[00358] As used herein an "adventitious virus" or "adventitious viral agent" refers to a virus contaminant present within a immunogenic agent, including, for example, vaccines, cell lines and other cell-derived products. Regarding vaccine products, for example, exogenous,

adventitious ALV was found in commercial Marek's Disease vaccines propagated in CEF or DEF cell cultures by different manufacturers. Moreover, some of these vaccines were also contaminated with endogenous ALV. Fadly et al., 50 Avian Diseases 380-85 (2006); Zavala & Cheng, 50 Avian Diseases 209-15 (2006).

[00359] Other embodiments of the present invention target the genes of adventitious animal viruses, including vesivirus, porcine circovirus, lymphocytic choriomeningitis virus, porcine parvovirus, adenoassociated viruses, reoviruses, rabies virus, papillomavirus, herpesviruses, leporipoxviruses, and leukosis virus (ALV), hantaan virus, Marburg virus, SV40, SV20, Semliki Forest virus (SFV), simian virus 5 (sv5), feline sarcoma virus, porcine parvovirus, adenoassociated viruses (AAV), mouse hepatitis virus (MHV), Moloney murine leukemia virus (MoMLV or MMLV, gag protein GeneID: 1491870), murine leukemia virus (MuLV), pneumonia virus of mice (PVM), Theiler's encephalomyelitis virus (THEMV), murine minute virus (MMV or MVM, GeneID: 2828495, *vp1*, GeneID: 148592; *vp*, GeneID: 1489591; *ns1*, GeneID: 1489590), mouse adenovirus (MAV), mouse cytomegalovirus (MCMV), mouse rotavirus (EDIM), Kilham rat virus (KRV), Toolan's H-1 virus, Sendai virus (SeV, also known as murine parainfluenza virus type 1 or hemagglutinating virus of Japan (HVJ)), rat coronavirus (RCV or sialodacryoadenitis virus (SDA)), pseudorabies virus (PRV), Cache Valley virus, bovine diarrhea virus, bovine parainfluenza virus type 3, bovine respiratory syncytial virus, bovine adenoviruses, bovine parvoviruses, bovine herpesvirus 1 (infectious bovine rhinotracheitis virus), other bovine herpesviruses, bovine reovirus, other bovine herpesviruses, bovine reovirus, bluetongue viruses, bovine polyoma virus, bovine circovirus, and orthopoxviruses other than vaccinia, pseudocowpox virus (a widespread parapoxvirus that can infect humans), papillomavirus, herpesviruses, leporipoxviruses, or exogenous retroviruses.

[00360] In a particular embodiment, the expression of MMLV Gag protein can be modulated by use of a corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3287870-3288118: (sense) and SEQ ID NOs:3288119-3288367 (antisense).

[00361] In a particular embodiment, the expression of vesivirus can be modulated by use of a corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs: 3152604-3152713 and the tables provided herein.

[00362] Other embodiments target human-origin adventitious agents including HIV-1 and HIV-2; human T cell lymphotropic virus type I (HTLV-I) and HTLV-II; human hepatitis A, B, and C viruses; human cytomegalovirus (CMV); EBV; HHV 6, 7, and 8; human parvovirus B19; reoviruses; polyoma (JC/BK) viruses; SV40 virus; human coronaviruses; human papillomaviruses; influenza A, B, and C viruses; various human enteroviruses; human parainfluenza viruses; and human respiratory syncytial virus.

[00363] Parvoviridae are single-stranded DNA viruses with genomes of about 4 to 5 kilobases. This family includes: *Dependovirus* such as human helper-dependent adeno-associated virus (AAV) serotypes 1 to 8, autonomous avian parvoviruse, and adeno associated viruses (AAV 1-8); *Erythrovirus* such as bovine, chipmunk, and autonomous primate parvoviruses, including human parvoviruses B19 (the cause of Fifth disease) and V9; and *Parvovirus* that includes parvoviruses of other animals and rodents, carnivores, and pigs, including MVM. These parvoviruses can infect several cell types and have been described in clinical samples. AAVs, in particular, have been implicated in decreased replication, propagation, and growth of other virus.

[00364] MVM gains cell entry by deploying a lipolytic enzyme, phospholipase A2 (PLA2), that is expressed at the N-terminus of virion protein 1 (VP1, also called MMVgp3), the MVM minor coat protein, GeneID: 1489592. Farr et al., 102 PNAS 17148-53 (2005). Other MVM targets can be chosen from MVM VP (also called MMVgp2), GeneID: 1489591; and MVM non-structural, initiator protein (NS1, also called MMVgp1), GeneID: 1489590. In a particular embodiment, the expression of MVM NS2 protein can be modulated by use of a corresponding RNA effector molecule having an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of SEQ ID NOs:3285524-3285827 (sense) and SEQ ID NOs:3285828-3286131 (antisense).

[00365] Polyomaviruses are double-stranded DNA viruses that can infect, for example, humans, primates, rodents, rabbits, and birds. Polyomaviruses (PyV) include SV40, JC and BK viruses, Murine pneumonotropic virus, hamster PyV, murine PyV virus, and Lymphotropic papovavirus (LPV, the African green monkey papovavirus). The sequences for these viruses are available via GenBank. *See also* U.S. Patent Pub. No. 2009/0220937. Because of their tumorigenic and oncogenic potential, it is important to eliminate these viruses in cell substrates used for vaccine production.

[00366] Papillomaviridae contains more that 150 known species representing varying host-specificity and sequence homology. They have been identified in mammals (humans,

simians, bovines, canines, ovines) and in birds. Majority of the human Papillomaviruses (HPVs), including all HPV types traditionally called genital and mucosal HPVs belong to supergroup A. Within supergroup A, there are 11 groups; the most medically important of these are the human Papillomaviruses HPV 16, HPV 18, HPV 31, HPV 45, HPV 11, HPV 6 and HPV 2. Each of these has been reported as "high risk" viruses in the medical literature.

[00367] Exogenous retroviruses are known to cause various malignant and non-malignant diseases in animals over a wide range of species. These viruses infect most known animals and rodents. Examples include Deltaretroidvirus (HTLV-1, -2, -3, and-4, STLV-1, -2, and -3), Gammaretrovirus (MLV, PERV), Alpharetrovirus (Avian leucosis virus and Avian endogenous virus), and HIV 1 and 2.

[00368] Other viral families which are potential adventitious contaminants for which embodiments of the present invention are directed include: Bunyaviridae (LCMV, hantavirus), Herpesviridae (Human herpesviruses 1 through 8, Bovine herpesvirus, Canine herpesvirus and Simian cytomegalovirus), Hepadnaviridae (Hepatitis B virus), Hepeviridae (Hepatitis E virus), Deltavirus (Hepatitis delta virus), Adenoviridae (Human adenoviruses A-F and murine adenovirus), Coronaviridae, Flaviviridae (Bovine viral diarrhea virus, TBE, Yellow fever virus, Dengue viruses 1-4, WNV and hepatitis C virus), Orthomyxoviridae (influenza), Paramyxoviridae (parainfluenza, mumps, measles, RSV, Pneumonia virus of mice, Sendai virus, and Simian parainfluenza virus 5), Togaviridae (Western equine encephalomyelitis virus, rubella), Picornaviridae (Poliovirus types 1-13, coxsackie B, echovirus, rhinovirus, Human hepatitis A, Human coxsackievirus, Human cardiovirus, Human rhinovirus and Bovine rhinovirus), Reoviridae (Mouse rotavirus, reovirus type 3 and Colorado tick fever virus), and Rhabdoviridae (vesicular stomatitis virus).

[00369] For example, mouse and hamster cell banks used to make immunogenic agents can be infected with viruses known to be pathogenic to human. Mouse cell banks can carry lymphocytic choriomeningitis virus (LCM), sendai virus, hantaan virus, and/or lactic dehydrogenase virus; hampster cell banks can carry LCM, sendai virus, and/or reovirus type 3. Indeed, commercially available monoclonal antibodies produced from transgenic mouse-derived cells are tested for virus including LCM, Ectromelia (MEV), mouse encephalomyelitis virus (GDVII), Hantaan, MVM, mouse adenovirus (MAV), mouse hepatitis (MHV), pneumonia virus of mice (PVM), Polyoma, Reovirus type 3 (REO-3), Sendai (SeV), virus of epizootic diarrhea of infant mice (EDIM), mouse cytomegalovirus (MCMV), papovavirus K, and LDVH viruses; Thymic Agent virus; bovine virus diarrhea (BVD), infectious bovine rhinotracheitis (IBR), respitratory parainfluenz-3 (PI-3), papillomavirus (BPV) and adenovirus-3 (BAV-3) viruses; and

caprine (goat) adenovirus (CAV), herpesvirus (CHV), and arthritis encephalitis virus (CAEV) viruses. *See* Geigert, CHALLENGE OF CMC REGULATORY COMPLIANCE FOR BIOPHARMACEUTICALS, 109-11 (Springer, New York, NY, 2004); BLA reference No. 98-9912, Centocor, Infliximab Detailed Product Review (1997); BioProcessing J. (Fall, 2009).

[00370] In some embodiments, the production of an immunogenic agent in a host cell is enhanced by introducing into the cell an additional RNA effector molecule that affects cell growth, cell division, cell viability, apoptosis, the immune response of the cells, nutrient handling, and/or other properties related to cell growth and/or division within the cell. In further embodiments, production is enhanced by introducing into the cell a RNA effector molecule that transiently inhibits expression of immunogenic agents during the growth phase.

IV. Transcriptome

[00371] Embodiments of the present invention also provide for a set of transcripts that are expressed inCHO cells, also called "the CHO cell transcriptome", and further provides siRNA molecules designed to target any one of the transcripts of the CHO cell transcriptome. Uses of the transcriptome in a form of an organized CHO cell transcript sequence database for selecting and designing CHO cell modulating effector RNAs are also provided in the form or methods and systems. Other embodiments further provide a selection of siRNAs targeted against each of the transcripts in the CHO transcriptome, and uses thereof for engineering or modifying CHO cells, for example, for improved production of biomolecules. Accordingly, particular embodiments provide modified CHO cells.

[00372] A set of transcripts that were discovered in CHO cells pooled under different conditions, including early-, mid- and late-log phase cells, that were grown in standard conditions under chemically defined media at 37°C. The transcripts are set forth in the tables herein, and in the corresponding sequences (SEQ ID files).

[00373] The discovery of the CHO transcriptome is useful for specifically modifying one or more cellular processes in the CHO cell, for example, for the production of biomolecules in such cells. For example, based on the known expressed transcripts, one can modulate apoptosis regulating genes, cell cycle genes, DNA amplification (DHFR) regulating genes, virus gene production regulating genes, e.g., in the case of viral promoters that are used to drive biomolecule production in the cells, glycosylation-associated genes, carbon metabolism regulating genes, prooxidant enzyme encoding genes. By modulating the known expressed genes or transcripts one can further modulate protein folding, methionine oxidation, protein

pyroglutamation, disulfide bond formation, protein secretion, cell viability, specific productivity of cell, nutrient requirements, internal cell pH.

[00374] Methods for modulating production of an immunogenic agent in a host cell, particularly in a CHO cell, are provided, the methods comprising the steps of contacting the cell with a RNA effector molecule, a portion of which is complementary to at least a portion of a target gene, maintaining the cell in a bioreactor for a time sufficient to modulate expression of the target gene, wherein the modulation enhances production of the immunogenic agent and recovering the immunogenic agent from the cell.

[00375] The present disclosure includes the nucleic acid sequences of the transcripts of the CHO transcriptome, the proteins the transcripts are translated into, and some of the pathways in which the transcribed proteins play a role. The description also sets forth a compilation of siRNA molecules as RNA effector molecules designed to target the sequences of the transcriptome. Systems, including computer assisted systems, and methods, including computer assisted methods, for selecting appropriate RNA effector molecules to modulate gene expression in a cell, particularly in a CHO cell, based on the known transcriptome transcript sequences are also described.

CHO cell transcriptome:

[00376] We have discovered a defined set of transcripts expressed in a CHO cell. The defined set of transcripts in referred to herein as a "transcriptome". The transcript name, at least one pathway in which the transcript plays a role, an associated SEQ ID NO(s), and corresponding exemplary siRNA molecule SEQ ID NOs are set forth in any of the tables described herein including, for example, Tables 1-16, 21, 23, 24, 27-30, 52-61, 65 or 66. The sequences of the transcripts in the CHO cell transcriptome are set forth in the associated SEQ ID NOs:1-9771 and SEQ ID NOs:3157149-3158420.

[00377] Thus, in one embodiment, the invention provides a Chinese hamster ovary (CHO) cell transcriptome comprising a selection or a compilation of transcripts having SEQ ID NOs:1-9771. In some embodiments, the CHO transcriptome consists essentially of a selection or a compilation of transcripts having SEQ ID NOs:1-9771. In some embodiments, the CHO cell transcriptome consists of a selection or a compilation of transcripts having SEQ ID NOs:1-9771.

[00378] In some embodiments, the invention provides at least one siRNA directed to any one of the CHO cell transcriptome transcript set forth in any of the tables presented herein, see e.g., Tables 1-16, 21-25, 27-30, 52-61, 65 or 66. In some embodiments, the siRNA is selected from the group of siRNAs set forth in Tables 1-16, 21-31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 50, 51-61, 63-65 or 66. In some embodiments, not all transcript SEQ ID NOs are present in the

tables described herein. In some embodiments, the RNA effector molecule comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the nucleotide sequence selected from the group consisting of SEQ ID NOs:9772-3152399 and SEQ ID NOs:3161121-3176783. Additional targets that can be modulated for improved quality/quantity of expression are set forth herein. Provided herein are CHO transcripts, i.e. SEQ ID NO's 1-9771 and SEQ ID NOs:3157149-3158420. These transcripts can be assigned to an encoded protein name and categorized into functional groups. One can readily determine functional groups to classify a transcript to by homology to sequences known to have a particular function. In one embodiment one uses a known functional domain and looks for homology of at least 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%. *See* for example Tables 10-16**,** which correlate the SEQ ID NO transcript with a description of encoded protein and function, e.g., cell cycle/cell division transcripts of Table 13**.** Exemplary categories that transcripts can be grouped are described throughout the application and include, e.g., transcripts (i.e., target genes) that encode for proteins involved in apoptosis, cell cycle genes, DNA amplification (DHFR), glycosylation, carbon metabolism, prooxidant enzymes, protein folding, methionine oxidation, protein pyroglutamation, disulfide bond formation, protein secretion, immune response, cell nutrient requirements, and shutting down RNA Interference. For the transcripts disclosed herein whose function is not specifically recited herein, one of skill in the art can easily compare (using known algorithms and programs) the transcript sequences of SEQ ID NOs:1-9771 and SEQ ID NOs:3157149-3158420 to sequence information of transcripts found in any of various organisms and assign function and/or protein encoded name as described above. For example, one of skill in the art can use the sequence information described herein to predict protein function using any prediction methods, algorithms, and/or resources and applications found on the world wide web, as reviewed in any of Freitas et al., 7 IEEE/ACM Transactions on Computational Biology and Bioinformatics (TCBB) 172-82 (2010); Rentzscha & Orengoa, 27 Trends in Biotech. 210-19 (2009); Lowenstein et al., 10 Genome Biol. 207 (2009) or Friedberg, 7 Briefings in Bioinformatics 225-42 (2006). Alternatively, the transcript sequences can be compared to a partial or entire genome of an organism (genome information), including protein coding and non-coding regions.

[00379] One can silence target transcripts using siRNA, such as set forth in SEQ ID NOs:9772-3152399 and SEQ ID NOs:3161121-3176783. The particular siRNA can readily be matched to its corresponding target by looking for a transcript containing a complimentary sequence that is at 90% complementary. Well known algorithms can be used to determine appropriate RNA effector molecules for targeting the transcripts identified herein. For example,

one of skill in the art can use the sequence information described herein to determine appropriate RNA sequences for targeting the transcripts described herein, and for preventing/promoting an immune response to those RNA sequences, using any prediction methods, algorithms, and/or resources and applications found on the world wide web, as reviewed in, or as described in, Pappas et al., 12 Exp. Op. Therapeutic Targets 115-27 (2008); Kurreck et al., 2009, 48 Angewandte Chemie 1378-98 (2009); Gredell et al., 16 Engin. Cell Funct. by RNA Interference in Cell Engin. 175-94 (2009); PCT/US2005/044662 (June 15, 2006); PCT/US2009/039937 (October 15, 2009); or PCT/US2009/051648 (January 28, 2010).

[00380] Thus, the system described herein (i.e., to select for a sequence of at least one RNA effector molecule that is suitable for modulating protein expression in a cell) can be used to identify both the CHO transcript sequence and the RNA effector molecules (e.g., siRNAs) that can be used to modulate any particular function in the host cell. A CHO transcript is assigned function and/or encoded protein name when the transcript sequence has at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to a transcript of an organism whose function and protein name is known

Systems and methods for selecting RNA effector molecules:

[00381] Based on the known CHO transcriptome, we have developed methods and systems for selecting RNA effector molecules to affect the cells through manipulating cellular processes, for example, to improve production of biomolecules in the cells.

[00382] Accordingly, the present embodiments provide databases and system comprising and using the CHO transcriptome sequences and optionally also an organized compilation of the CHO transcriptome outlining at least one functional aspect of each of the transcript, such as the transcripts role in a particular cellular process or pathway, and the corresponding siRNAs to allow design and selection of targets and effector RNA molecules for optimization of biological processes, particularly in the CHO cells.

[00383] Functional aspects of transcripts relate to their role in, for example apoptosis, cell cycle, DNA amplification (DHFR), virus gene production, e.g., in the case of viral promoters that are used to drive biomolecule production in the cells, glycosylation, carbon metabolism, prooxidant enzymes, protein folding, methionine oxidation, protein pyroglutamation, disulfide bond formation, protein secretion, cell viability, specific productivity of cell, nutrient requirements, internal cell pH. Other cellular processes are known to a skilled artisan, and can be found, for example, at the Gene Ontology database available through the world wide web.

[00384] Accordingly as shown in Figure 16, the invention provides a system 100 for selecting a sequence of at least one RNA effector molecule suitable for modulating protein expression in a cell, the system comprising: a computing device 110, having a processor 112 and associated memory 114, and a database 120 comprising at least one cell transcriptome information, the information comprising, a sequence for each transcript of the transcriptome, and optionally, a name of the transcript, and a pathway the transcript plays a role; and at least one RNA effector molecule information, the information comprising at least the sequence of the RNA effector molecule and optionally target specificity of the RNA effector molecule, wherein each RNA effector molecule is designed to match at least one or more sequences in the at least one cell transcriptome; a computer program, stored in memory 114, executed by the computing device 110 and configured to receive from a user via a user input device 118, parameters comprising a cell type selection, a target organism selection, a cellular pathway selection, a cross-reactivity selection, a target gene name and/or sequence selection, and optionally a method of delivery selection comprising either in vivo or in vitro delivery options; and further optionally user address information; a first module configured to check the parameters against the sequences in the database for a matching combination of the parameters and transcriptome transcript sequences; and a second module to display a selected sequence of at least one RNA effector molecule suitable for modulating protein expression in the cell.

[00385] The computing device 110 and associated programs stored in memory 114 can be adapted and configured to provide a user interface, such as a graphical user interface which allows the user to input search target parameters, for example, using one or more drop down menus or structured or free form text input, and selects the appropriate parameters for finding an appropriate target in the desired cell. For example, if a user wishes to find a target for modulating carbon metabolism in a CHO cell, the user identifies the target cell as "CHO", and pathway as "carbon metabolism", and the server performs a search through the database that would identify, e.g., transcripts for Gluts, PTEN and LDH genes and matches them with the appropriate siRNA molecules from the siRNA database part. This output information can be presented to the user on a computer display 116 or other output device, such as a printer.

[00386] The system can be a stand-alone system or an internet-based system, wherein the computations and selection of effector RNA molecules is performed in same or different locations. As shown in Figure 16, the transcriptome information can be stored in database 120 and accessed by computing device 110. As used herein, the term database includes any organization of data regardless of whether it is structured or unstructured that allows retrieval of the information requested. The database can be a flat file or set of flat files stored in memory,

one or more tables stored in memory, a set of discrete data elements stored in memory. The database can also include any well known database program that allows a user to directly or indirectly (through another program) access the data. Examples of these include MICROSOFT® ACCESS®, and ORACLE® database and MYSQL® open source database.

[00387] In an alternative embodiment of the invention shown in Figure 17, the system 200 can be a network based system. The system 200 can include a server system 210 and one or more client systems 240 and 250 connected to a network 230, such as a private user network or Ethernet, or the Internet. The server system 210 and client systems 240 and 250 can be computing devices as described herein. Server system 210 can include one or more processors 212 and associated memory 214 and one or more computer programs or software adapted and configured to control the operations and functions of the server system 210. The Server system 210 can include one or more network interfaces for connecting via wire or wirelessly to the network 230. Examples of server systems include computer servers based on INTEL® and AMD microprocessor architectures available from Hewlett-Packard Development Co., LP; DELL; and APPLE® Inc.

[00388] Client systems 240 and 250 can include one or more processors 242 and 252 and associated memory 244 and 254 and one or more computer programs or software adapted and configured to control the operations and functions of the client systems 240 and 250. The client systems 240 and 250 can include one or more network interfaces for connecting via wire or wirelessly to the network 230. Examples of client systems include desktop and portable computers based on INTEL® and AMD microprocessor architectures available from Hewlett-Packard Development Co., LP; DELL; and Apple Inc., and smaller network enabled, handheld devices such as a personal digital assistant (PDA) (e.g., DROID®, HTC Corp.) smartphone (e.g., BLACKBERRY® smartphone, Research In Motion, Ltd.), iPod®, iPad™ and iPhone® devices (APPLE® Inc.).

[00389] In accordance with one embodiment, the server system 210 is a web server, for example based in Internet Information Services (IIS) for Windows® or .NET FRAMEWORK products (MICROSOFT® Corp.), or Apache open-source HTTP server (Apache Software Foundation), and uses a web-based application accessed by a remote client system via the Internet to search the database of transcriptome information to identify RNA effector molecules that can be suitable for modulating protein expression in a cell. The system can include or be connected to a fulfillment system that allows a user to select and purchase desired quantities of the identified RNA effector molecules to be delivered to the user.

[00390] One can also provide a system by selling a software to be run by a computer, wherein the databases and algorithms matching the parameters with sequence information and other information are provided to the user. The user can then either synthesize the effector RNA molecules or separately order them from a third party provider.

[00391] In some embodiments, the system further comprises a storage module for storing the at least one RNA effector molecule in a container, wherein if there are two or more RNA effector molecules, each RNA effector molecule is stored in a separate container, and a robotic handling module, which upon selection of the matching combination, selects a matching container, and optionally adds to the container additives based on a user selection for *in vivo* or *in vitro* delivery, and optionally further packages the container comprising the matching RNA effector molecule to be sent to the user address. Exemplary additives that can be added to the siRNA or a mixture of siRNAs are set forth herein.

[00392] The storage module can be a refrigerated module linked to the system components.

[00393] The system can also be linked to a nucleic acid or other biomolecule synthesizer.

[00394] The robotic handling module can be any system that can retrieve, and optionally mix components from the storage module, and or the biomolecule synthesizer, and optionally package the container(s). The robotic handling module can comprise one or more parts functioning based upon the commands from the system. The robotic handling module can be in the same or different location as where the computations are performed.

[00395] In some embodiments, the system further comprises genome information of the cell, wherein by a user selection, the RNA effector molecules can be matched to target genomic sequences, comprising promoters, enhancers, introns and exons present in the genome.

[00396] In some embodiments of the invention, the system can include hardware components or systems of hardware components and software components that carry out specific tasks (such as managing input and output of information, processing information, etc.) of the system and can be carried out by the execution of software applications on and across the one or more computing devices that make up the system. The present inventions can include any convenient type of computing device, e.g., such as a server, main-frame computer, a work station, etc. Where more than one computing device is present, each device can be connected via any convenient type of communications interconnect, herein referred to as a network, using well know interconnection technologies including, for example, Ethernet (wired or wireless - "WiFi"), BLUETOOTH® technology, ZIGBEE® wireless technology, AT&T™ 3G network, or SPRINT™ 3G or 3G/4G networks. Where more than one computing device is used, the devices

can be co-located or they can be physically separated. Various operating systems can be employed on any of the computing devices, where representative operating systems include MICROSOFT® WINDOWS® operating system, MACOS™ operating system software (APPLE® Inc.), SOLARIS® operating system (Oracle Corp.), Linux (Linux Online, Inc.), UNIX® server systems and OS/400 software (IBM Corp.), ANDROID™ (Sprint), Chrome OS (Google Inc.), and others. The functional elements of system can also be implemented in accordance with a variety of software facilitators, platforms, or other convenient method.

[00397] Items of data can be “linked” to one another in a memory when the same data input (for example, filename or directory name or search term) retrieves the linked items (in a same file or not) or an input of one or more of the linked items retrieves one or more of the others.

[00398] Figure 18 shows a diagrammatic view of the data structure according to one embodiment of the invention. In this embodiment, input field terms can be linked to Target RNA, such as by their associated sequence ID in the database and in accordance with the invention, executing a software module to search for one or more of the input field terms returns one or more sequence IDs of the Target. In addition, each Target RNA can be linked to one or more RNA effector molecules, such as by their associated sequence ID and in accordance with the invention, the for each Target identified, a software module can be executed to perform a subsequent search for some or all of Targets identified can return one or more sequence IDs for desired RNA effector molecules and return a listing of the RNA effector molecules and their sequence IDs.

[00399] Alternatively, for each target identified, a software module can be executed that implements one or more well known algorithms for determining the desired RNA effector molecules and return a listing of the RNA effector molecules and their sequence IDs.

[00400] Figure 19 shows a flow chart of the method for identifying RNA effector molecules according to one embodiment of the invention. The method 400 includes presenting the user with an input screen 402 that allows the user to input the desired parameters for finding the Target in the desired cell. The input can be free form text or one or more drop-down boxes allowing the user to select predefined terms. At step 404, the user selects the appropriate user interface element, for example a “search” button and the system searches the database for Targets associated with the input parameters. At step 406, the user can be presented with a list of Targets, each associated with a check box and the user can select or unselect the check box associated with each target to further refine their search. At step 408, the user selects the appropriate user interface element, for example a “search” button and the system can search the

database for RNA effector molecules associated with the input targets and/or use well know algorithms to determine RNA effector molecules associated with the input targets. The system can, for example, search for RNA effector molecules and if, none are found, use the well know algorithms to determine appropriate RNA effector molecules. Subsequently, the determined molecules can be added to the database and appear in subsequent searches. Alternatively, even where RNA effector molecules are found, the system can, in addition, use the well know algorithms to determine additional appropriate RNA effector molecules. At step 410, the user can be provided with optional functions such as ordering the reported RNA effector molecule from information found in the database. For example, online procurement can be provided as described in U.S. Patent Application Pub. No. 2005/0240352.

[00401] In one example of the system and the method of using the system, a person, such as a customer, is experiencing problems in protein production using a cell line. The problem can be, e.g., in post translational modification of the protein, such as in glycosylation, e.g., too much fucosylation, and /or another process, such as too much lactic acid buildup or too low yield.

[00402] The system of the invention allows the user to input parameters, such as the problem or multiple problems they are experiencing (too low cell growth rate or too much fucosylation) and/or a target gene, or transcript or multiple target genes or transcripts that they wish to modulate, such as FUT8, GMDS, and/or TSTA3, into the user interface.

[00403] The system takes the parameters and matches them with sequence data and RNA effector molecule data and delivers suggested RNA effector molecule(s) to the customer. For example, the system can match the problem to a cellular pathway, such as glycosylation, with transcripts known to play a role in glycosylation, and then matches the RNA effector molecules targeting these sequences and delivers, e.g. a list of siRNA sequences with which the customer can experiment.

[00404] If the customer wishes to receive one or more of the sequences, the customer can order or instruct the system to synthesize and/or send the appropriate nucleic acids to the customer-defined location. The system can also send instructions to a nucleotide synthesis system to make the sequences. The synthesizer can be in the same or in a remote location from the other system parts. The system can also select ready-made sequences from a storage location and provide packaging information so that the appropriate molecules can be sent to the customer-defined location. If the customer wishes to obtain different mixtures of the RNA effector molecules, such can be defined prior to submitting the final order and then the system will instruct the robotic component to mix the appropriate RNA effector molecules, such as

siRNA duplexes, e.g, comprising an antisense and sense strand, in one vial or tube or other container.

[00405] We have further discovered a set of siRNA molecules that target at least one of the transcripts in the CHO cell transcriptome. Table 1 also sets forth a set of siRNA molecules that target the transcrips in the CHO cell transcriptome.

[00406] Thus, for example, methods are provided herein for enhancing production of a recombinant antibody or a portion or derivative thereof by contacting a cell, such as a CHO cell, with one or more RNA effector molecules that permit modulation of fucosylation of the recombinant antibody or portion or derivative thereof. For example, SEQ ID NOs:3152714-3152753, can be contacted with a cell to modulate expression of the fucosyltransferase (FUT8). In another embodiment, a cell is contacted with one or more RNA effector molecules wherein the contacting modulates expression of a GDP0mannose 4,6-dehydratase (GMDS) (encoded, for example, by SEQ ID NO:5069). A RNA effector molecule targeting GMDS can comprise an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs:1688202-1688519.

[00407] In another embodiment, a cell is contacted with one or more RNA effector molecules wherein the contacting modulates expression of a gene encoding GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (encoded by TSTA3), (encoded, for example, by SEQ ID NO:5505). A RNA effector molecule targeting TSTA3 can comprise an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide molecule selected from the group consisting of SEQ ID NOs:1839578-1839937. In still another embodiment, a cell is contacted with a plurality of RNA effector molecules targeting the expression of more than one of FUT8, GMDS, and TSTA3.

[00408] Reduced sialic content of antibodies is believed to further increase ADCC. Therefore, in still another embodiment, a cell is contacted with one or more RNA effector molecules wherein the contacting modulates expression of a sialyltransferase. The sialyltransferase activity in a cell can be modulated by contacting the cell with a RNA effector molecule targeting at least one sialyltransferase gene. Table 7 lists some sialyltransferases that can be modulated, as well as the RNA effector molecules targeting sialyltransferases.

[00409] The RNA effector molecules targeting the sialyltransferases comprises an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence of the SEQ ID NOs

presented above (i.e., SEQ ID NOs:681105-681454, NOs:707535-707870, NOs:1131123-1131445, NOs:1155324-1155711, NOs:1391079-1391449, NOs:1435989-1436317).

[00410] In still another embodiment, a cell is contacted with at least one RNA effector molecule targeting one of FUT8, GMDS, and TSTA3, and another RNA effector molecule targeting one sialyltransferase. In a particular embodiment, a cell is contacted with RNA effector molecules targeting FUT8 and ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6.

[00411] Embodiments of the present invention modulated the activity of a transcript or a protein in a molecular pathway known to a skilled artisan or identified elsewhere in this specification. Such molecular pathways an cellular activities include, but are not limited to apoptosis, cell division, glycosylation, growth rate, a cellular productivity, a peak cell density, a sustained cell viability, a rate of ammonia production or consumption, or a rate of lactate production. Tables 10 to 16 identify example targets based on their function or role that they play in a cell:

Table 10. Lactate production (Chinese hamster)				
SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
3905	1573	lactate dehydrogenase A	1,468.00	1297283-1297604
8572	481	lactate dehydrogenase C	0.619	2887819-2888178
9187	343	lactate dehydrogenase A-like 6B	0.235	3064087-3064357
9600	207	lactate dehydrogenase B	0.216	3140011-3140113

Table 11. Proteases and Proteolysis related (Chinese hamster)				
SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
6	5005	carboxypeptidase D	5.679	11367-11661
23	4373	insulin degrading enzyme	24.134	16605-16843
151	3548	disintegrin & metallopeptidase domain 10	14.497	57423-57713
282	3138	YME1-like 1 (*S. cerevisiae*)	5.064	96707-96922
351	3031	SUMO/sentrin specific peptidase 6	10.532	116231-116447
360	3012	bone morphogenetic protein 1	14.594	118879-119164
367	3002	dipeptidylpeptidase 8	4.382	120799-121136
450	2894	tripeptidyl peptidase II	4.093	144491-144745
462	2883	nardilysin, N-Arg dibasic convertase, NRD convertase 1	23.889	147663-147880
483	2861	calpain 2	35.121	153383-153617
544	2789	N-ethylmaleimide sensitive fusion protein	30.345	170769-171035
557	2776	disintegrin & metallopeptidase domain 9 (meltrin γ)	15.711	174168-174399
582	2754	Zn metallopeptidase, STE24 homolog (*S. cerevisiae*)	5.717	181477-181863
677	2678	AE binding protein 1	54.178	210228-210444
816	2577	disintegrin and metallopeptidase domain 23	0.593	252647-252954

821	2575	a disintegrin and metallopeptidase domain 15 (metargidin)	11.757	254091-254472
940	2519	SUMO/sentrin specific peptidase 2	3.997	292258-292589
1012	2474	membrane-bound transcription factor peptidase, site 1	14.435	316272-316622
1064	2446	lon peptidase 1, mitochondrial	39.647	333731-334048
1108	2423	AFG3(ATPase family gene 3)-like 2 (yeast)	17.55	348153-348484
1137	2407	acylpeptide hydrolase	16.618	358347-358692
1153	2401	calpain 10	2.795	363875-364249
1194	2384	disintegrin-like & metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 7	8.75	377552-377859
1330	2323	complement component 1, r subcomponent	62.586	422509-422751
1331	2323	pitrilysin metallepetidase 1	16.737	422752-423147
1365	2304	X-prolyl aminopeptidase (aminopeptidase P) 1	34.448	434820-435212
1367	2303	neurolysin (metallopeptidase M3 family)	4.852	435611-435974
1423	2276	plasminogen activator, tissue	2.837	454515-454869
1462	2261	SUMO/sentrin specific peptidase 3	7.248	467735-468057
1488	2250	furin (paired basic aa cleaving enzyme)	14.282	476518-476914
1554	2228	SUMO/sentrin specific peptidase 5	1.726	498550-498878
1597	2208	aminopeptidase puromycin sensitive	4.993	513207-513606
1601	2208	complement component 1, s subcomponent	7.355	514675-514999
1703	2174	endoplasmic reticulum aminopeptidase 1	16.062	550016-550337
1828	2136	matrix metallopeptidase 9	16.328	593202-593492
1832	2133	endoplasmic reticulum metallopeptidase 1	3.502	594506-594744
1861	2124	spastic paraplegia 7 homolog (human)	8.718	604347-604631
1980	2085	complement component 1, r subcomponent B	28.837	644971-645023
1989	2082	thimet oligopeptidase 1	27.953	647877-648172
2005	2076	beta-site APP cleaving enzyme 1	3.234	653217-653567
2034	2066	intraflagellar transport 52 homolog (*Chlamydomonas*)	44.311	662569-662878
2060	2056	dihydrolipoamide dehydrogenase	39.837	671424-671769
2086	2048	methionyl aminopeptidase 1	16.104	680457-680813
2093	2046	cathepsin A	183.096	682818-683174
2109	2041	disintegrin-like & metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1	0.788	687923-688239
2352	1970	ATP/GTP binding protein-like 5	1.205	770448-770765
2369	1965	cathepsin D	167.968	776029-776328
2370	1965	methionine aminopeptidase 2	19.432	776329-776680
2440	1946	arginyl aminopeptidase (aminopeptidase B)	9.264	800159-800460
2473	1940	prolyl endopeptidase-like	2.435	811154-811532
2521	1929	dipeptidylpeptidase 9	4.703	827728-828118
2529	1926	AFG3 (ATPase family gene 3)-like 1 (yeast)	8.094	830536-830879
2549	1920	leukotriene A4 hydrolase	13.262	837346-837737
2627	1901	tubulointerstitial nephritis antigen-like 1	471.915	863337-863698
2688	1887	prolylcarboxypeptidase (angiotensinase C)	4.268	884238-884577
2726	1875	CNDP dipeptidase 2 (metallopeptidase M20 family)	17.92	897182-897473
2802	1857	legumain	105.23	923229-923566
2867	1840	cereblon	1.831	945414-945728
2888	1834	cathepsin F	27.16	952584-952981

2902	1830	proprotein convertase subtilisin/kexin type 7	5.151	957525-957819
2940	1818	OMA1 homolog, zinc metallopeptidase (*S. cerevisiae*)	10.717	970455-970848
2957	1814	disintegrin & metallopeptidase domain 22	6.245	976428-976826
2962	1812	bleomycin hydrolase	21.221	978233-978617
3044	1781	leucine aminopeptidase 3	53.967	1005879-1006172
3119	1765	prolyl endopeptidase	20.21	1031521-1031842
3129	1763	matrix metallopeptidase 3	44.776	1034832-1035193
3175	1751	disintegrin & metallopeptidase domain 8	3.157	1051064-1051435
3296	1720	suppression of tumorigenicity 14 (colon carcinoma)	2.378	1092011-1092357
3347	1706	LON peptidase N-terminal domain & ring finger 3	1.265	1109135-1109435
3515	1666	calpain 7	1.488	1165709-1166037
3553	1656	peptidase (mitochondrial processing) α	16.51	1178516-1178823
3565	1652	HtrA serine peptidase 1	42.699	1182505-1182824
3660	1631	aspartyl aminopeptidase	12.181	1214496-1214794
3685	1627	HtrA serine peptidase 2	11.095	1222907-1223252
3696	1623	intraflagellar transport 88 homolog (*Chlamydomonas*)	1.53	1226651-1227010
3770	1607	a disintegrin and metallopeptidase domain 12 (meltrin α)	0.371	1251949-1252245
3795	1599	ubiquinol-cytochrome c reductase core protein 1	109.161	1260523-1260890
3809	1594	matrix metallopeptidase 10	43.632	1265238-1265630
3832	1589	matrix metallopeptidase 14 (membrane-inserted)	5.689	1272953-1273286
3875	1579	peptidase (mitochondrial processing) β	37.799	1287161-1287545
3936	1565	predicted gene 5077	4.951	1307451-1307521
3940	1564	dipeptidylpeptidase 7	40.962	1308543-1308899
3951	1562	phosphatidylinositol glycan anchor biosynthesis, class K	26.236	1312259-1312656
4040	1540	cathepsin B	122.173	1342187-1342544
4112	1521	leucyl/cystinyl aminopeptidase	0.363	1366088-1366414
4134	1516	mitochondrial intermediate peptidase	1.762	1373601-1373949
4136	1515	calpain 1	1.667	1374276-1374636
4234	1494	WAP, FS, Ig, KU, and NTR-containing protein 1	1.307	1407418-1407713
4250	1492	caspase 9	1.769	1412589-1412860
4282	1485	matrix metallopeptidase 12	15.393	1423446-1423812
4320	1476	peptidase D	6.708	1436318-1436664
4345	1471	procollagen C-endopeptidase enhancer protein	38.334	1444649-1444973
4515	1433	ceroid lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease)	2.904	1500552-1500853
4548	1426	ubiquinol cytochrome c reductase core protein 2	74.045	1511637-1511998
4736	1385	cathepsin L	394.561	1574335-1574708
4999	1324	aminoacylase 1	16.465	1664426-1664734
5080	1303	protease, serine, 36	0.737	1691971-1692344
5266	1267	tripeptidyl peptidase I	0.706	1755385-1755682

5334	1251	O-sialoglycoprotein endopeptidase-like 1	1.425	1778801-1779170
5395	1238	SUMO/sentrin specific peptidase 8	1.488	1800688-1801060
5486	1216	glutaminyl-peptide cyclotransferase-like	2.05	1832626-1832993
5520	1207	carboxypeptidase X 1 (M14 family)	0.795	1844883-1845160
5529	1205	glutamyl aminopeptidase	0.69	1847806-1848189
5550	1200	disintegrin & metallopeptidase domain 17	1.374	1855220-1855596
5578	1195	proteasome (prosome, macropain) α type 1	94.105	1864684-1865015
5608	1188	caspase 12	0.856	1875252-1875646
5663	1175	CASP8 and FADD-like apoptosis regulator	4.448	1894743-1895132
5712	1164	ATP/GTP binding protein 1	0.455	1912461-1912860
5746	1157	caspase 3	11.813	1924836-1925195
5760	1154	archaelysin family metallopeptidase 2	3.826	1930073-1930404
5792	1147	matrix metallopeptidase 13	0.724	1941794-1942151
5854	1136	caspase 1	2.306	1964106-1964500
5905	1123	RAB23, member RAS oncogene family	1.099	1982920-1983307
5940	1116	cathepsin H	23.003	1995676-1996039
5976	1108	SEC11 homolog A (*S. cerevisiae*)	44.235	2008739-2009125
6015	1099	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	63.204	2022843-2023145
6033	1095	protease, serine 27	3.375	2029351-2029692
6044	1093	proteasome (prosome, macropain) α type 4	77.041	2033365-2033746
6101	1080	matrix metallopeptidase 23	2.487	2053947-2054295
6154	1068	cathepsin Z	400.641	2073581-2073970
6247	1047	ceroid-lipofuscinosis, neuronal 6	3.41	2107037-2107394
6327	1029	calpain 5	2.411	2135026-2135381
6344	1025	C2 calcium-dependent domain containing 3	0.136	2141185-2141522
6512	985	proteasome (prosome, macropain) α type 5	77.333	2200953-2201317
6552	976	endothelin converting enzyme 2	2.313	2215190-2215580
6611	966	proteasome (prosome, macropain) α type 3	3.156	2236096-2236486
6656	957	proteasome (prosome, macropain) α type 6	42.616	2251849-2252237
6686	950	apoptotic peptidase activating factor 1	0.325	2262408-2262743
6745	936	proteasome (prosome, macropain) β type 8 (large multifunctional peptidase 7)	32.531	2282619-2282981
6769	933	proteasome (prosome, macropain) β type 10	3.428	2291135-2291518
6798	926	caspase 7	0.436	2301618-2301960
6818	920	proteasome (prosome, macropain) β type 7	44.299	2308285-2308647
6848	914	proteasome (prosome, macropain) β type 4	25.753	2318721-2319092
6967	888	proteasome (prosome, macropain) β type 1	101.582	2357085-2357484
6999	880	caseinolytic peptidase, ATP-dependent, proteolytic subunit homolog (*E. coli*)	23.993	2368027-2368394
7109	858	matrix metallopeptidase 19	0.305	2404764-2405144
7120	855	caspase 6	4.965	2408466-2408843
7300	811	proteasome (prosome, macropain) α type 7	52.239	2467566-2467883
7433	780	proteasome (prosome, macropain) β type 5	25.65	2511900-2512253
7532	756	cathepsin O	0.321	2544359-2544680
7563	747	proteasome (prosome, macropain) α type 2	6.117	2554532-2554886
7620	734	proteasome (prosome, macropain) β type 3	8.915	2572635-2572964
7721	709	aurora kinase A interacting protein 1	9.974	2606127-2606501
7782	693	ATP/GTP binding protein-like 3	0.407	2627002-2627350
7940	648	matrix metallopeptidase 17	0.224	2680510-2680844

7948	646	pyroglutamyl-peptidase I	0.831	2683195-2683515
7979	638	protease, serine, 8 (prostasin)	0.479	2693206-2693562
8026	624	CASP2 and RIPK1 domain containing adaptor with death domain	1.176	2709036-2709355
8056	612	caspase 2	1.166	2718675-2719039
8255	558	matrix metallopeptidase 24	6.978	2781318-2781710
8290	549	proteasome (prosome, macropain) β type 2	3.953	2793443-2793832
8352	535	IMP1 inner mitochondrial membrane peptidase-like (*S. cerevisiae*)	6.039	2814696-2815033
8440	510	disintegrin-like & metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 4	0.139	2845165-2845528
8466	504	proteasome (prosome, macropain) β type 6	1.2	2853165-2853489
8547	487	small optic lobes homolog (*Drosophila*)	0.173	2879932-2880319
8577	481	calpain 11	0.187	2889008-2889328
8597	477	mannan-binding lectin serine peptidase 2	0.156	2896069-2896411
8653	465	membrane-bound transcription factor peptidase, site 2	0.105	2915060-2915410
8917	414	caspase 8	0.2	2995593-2995870
8935	409	carboxypeptidase N, polypeptide 1	0.233	3000705-3001032
8980	398	disintegrin & metallopeptidase domain 19 (meltrin β)	0.707	3012906-3013172
9067	373	proteasome (prosome, macropain) subunit, β type 9 (large multifunctional peptidase 2)	0.464	3035689-3035987
9119	360	SUMO1/sentrin specific peptidase 1	0.104	3048694-3048900
9253	329	phosphate regulating gene with homologies to endopeptidases on the X chromosome (hypophosphatemia, vitamin D resistant rickets)	0.053	3078631-3078850
9290	319	carboxypeptidase B2 (plasma)	0.216	3086591-3086854
9365	296	cathepsin W	0.241	3102885-3103082
9403	282	RIKEN cDNA 4930486L24 gene	0.203	3109975-3110173
9412	278	cDNA sequence BC039632	0.114	3111726-3111929
9418	275	IMP2 inner mitochondrial membrane peptidase-like (*S. cerevisiae*)	0.242	3112815-3113006
9498	244	calpain 12	0.103	3126461-3126617
9517	238	mucosa associated lymphoid tissue lymphoma translocation gene 1	0.359	3129264-3129311
9529	234	disintegrin & metallopeptidase domain 1a	0.077	3130955-3131114
9574	215	SUMO1/sentrin specific peptidase 7	0.045	3137116-3137276
9627	195	cathepsin 8	0.092	3142354-3142386
9644	188	proteasome (prosome, macropain) β type, 11	0.052	3143952-3143972
9647	187	disintegrin & metallopeptidase domain 28	0.137	3144200-3144221
9669	175	methionine aminopeptidase-like 1	0.139	3146223-3146337
3157186	770	SEC11 homolog C (*S. cerevisiae*)	22.702	3178484-3178583
3157231	468	macrophage stimulating 1 (hepatocyte growth factor-like)	0.205	3240817-3240916
3157254	428	transferrin receptor 2	0.148	3252917-3253016
3157343	370	predicted gene 1019	0.391	3193971-3194070
3157354	430	cathepsin K	0.29	3278249-3278348
3157355	419	calpain 8	0.461	3258905-3259004
3157374	287	carnosine dipeptidase 1 (metallopeptidase	0.102	3245017-3245116

		M20 family)		
3157412	788	dipeptidylpeptidase 10	0.189	3248617-3248716
3157448	1697	folate hydrolase	1.451	3185871-3185970
3157520	492	complement component 1, r subcomponent-like	0.264	3224791-3224890
3157628	194	disintegrin & metallopeptidase domain 33	0.061	3206058-3206157
3157660	369	echinoderm microtubule associated protein like 2	0.16	3266705-3266804
3157845	837	mast cell protease 8	4.869	3206558-3206657
3157898	422	disintegrin-like & metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 15	0.115	3193471-3193570
3157899	306	napsin A aspartic peptidase	0.207	3240917-3241016
3157906	387	cathepsin S	0.283	3272096-3272195
3157949	477	protein C	0.42	3271796-3271895
3158015	396	mast cell protease 4	0.405	3210058-3210157
3158034	923	HtrA serine peptidase 3	0.583	3258505-3258604
3158065	1746	WD repeat domain 7	1.717	3273496-3273595
3158090	371	secernin 2	0.243	3163021-3163120
3158135	418	mannan-binding lectin serine peptidase 1	0.152	3282249-3282348
3158156	463	NA	0.451	3181384-3181483
3158177	415	NA	0.13	3231817-3231916
3158199	521	hepatocyte growth factor	0.226	3253417-3253516
3158201	416	matrix metallopeptidase 21	0.224	3195471-3195570
3158231	385	matrix metallopeptidase 16	0.085	3174184-3174283
3158246	338	coagulation factor VII	0.181	3207558-3207657
3158294	648	matrix metallopeptidase 2	0.413	3214291-3214390
3158365	431	complement component factor i	0.209	3178584-3178683
3158378	492	alanyl (membrane) aminopeptidase	0.144	3228717-3228816

Table 12. Extracellular Space; External Region (Chinese hamster)				
SEQ ID NO:	cons L	Description	Avg Cov	siRNA SEQ ID NOs:
7	4892	collagen, type IV, α 2	29.59	11662-12024
10	4667	collagen, type V, α 1	22.034	12499-12766
40	4217	collagen, type IV, α 1	71.884	22106-22419
53	4076	laminin B1 subunit 1	72.723	26303-26608
68	3989	laminin, γ 1	8.547	31249-31602
72	3984	nidogen 1	31.556	32592-32943
98	3777	neural cell adhesion molecule 1	1.452	41193-41507
99	3776	inter- α (globulin) inhibitor H5	3.94	41508-41833
106	3741	latent TGF β binding protein 1	15.581	43659-44014
122	3653	laminin, α 5	10.318	48814-49139
150	3549	UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyl transferase 1	11.757	57147-57422
168	3455	activated leukocyte cell adhesion molecule	11.813	62634-62891
178	3411	UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyl transferase 2	22.835	65737-65999
188	3385	fibronectin 1	39.064	68761-69090
228	3262	collagen, type XII, α 1	0.842	80671-81033

Table 12. Extracellular Space; External Region (Chinese hamster)				
266	3179	vascular endothelial growth factor A	18.713	92246-92594
296	3122	calumenin	31.456	101047-101312
331	3068	collagen, type XVI, α 1	16.307	110363-110636
373	2991	CD44 antigen	11.502	122703-122982
374	2990	ring finger and SPRY domain containing 1	5.312	122983-123259
392	2965	lysyl oxidase-like 4	3.371	128072-128461
428	2922	coiled-coil domain containing 80	7.726	138093-138362
435	2913	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	3.732	140196-140578
546	2787	DnaJ (Hsp40) homolog, subfamily C, member 10	22.023	171304-171555
557	2776	disintegrin & metallopeptidase domain 9 (meltrin γ)	15.711	174168-174399
602	2739	lysyl oxidase-like 3	1.964	187446-187711
655	2695	perlecan (heparan sulfate proteoglycan 2)	13.274	203335-203554
677	2678	AE binding protein 1	54.178	210228-210444
679	2677	collagen, type VI, α 1	34.848	210698-211081
703	2663	RIKEN cDNA 2610507B11 gene	20.912	217294-217526
704	2662	serine (or cysteine) peptidase inhibitor, clade E, member 1	33.405	217527-217924
726	2641	collagen, type VI, α 2	42.145	224615-225009
798	2590	collagen & calcium binding EGF domains 1	2.683	246931-247299
816	2577	disintegrin & metallopeptidase domain 23	0.593	252647-252954
885	2543	platelet-derived growth factor, C polypeptide	3.586	273882-274243
941	2519	heat shock protein 5	729.81	292590-292837
956	2506	integrin α 5 (fibronectin receptor α)	13.30 8	297403-297671
968	2500	acid phosphatase-like 2	10.599	301329-301569
971	2499	WNT1 inducible signaling pathway protein 1	3.327	302229-302482
986	2492	thrombospondin 1	2.743	307445-307775
1014	2473	tissue inhibitor of metalloproteinase 2	22.337	317000-317395
1034	2463	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B	15.39	323916-324170
1059	2448	glypican 6	2.853	332251-332483
1079	2437	thrombospondin 3	16.07	338433-338822
1149	2404	MAM domain containing 2	23.86	362422-362815
1194	2384	disintegrin-like & metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 7	8.75	377552-377859
1216	2374	integrin α V	0.85	384630-384864
1274	2346	quiescin Q6 sulfhydryl oxidase 1	17.49	403798-404029
1307	2332	laminin, β 2	3.856	414909-415222
1382	2293	CD276 antigen	2.822	440554-440858
1408	2285	TBC1 domain family, member 15	5.501	449214-449575
1423	2276	plasminogen activator, tissue	2.837	454515-454869
1424	2276	connective tissue growth factor	6.301	454870-455117
1529	2238	interleukin 6 signal transducer	1.155	490131-490451
1583	2214	cleft lip & palate associated transmembrane protein 1	6.218	508317-508686
1587	2213	collagen, type XXVII, α 1	0.476	509761-510121
1662	2187	ecto-NOX disulfide-thiol exchanger 2	1.262	536177-536522

Table 12. Extracellular Space; External Region (Chinese hamster)				
1681	2181	brain derived neurotrophic factor	1.421	542519-542783
1694	2176	toll-like receptor 2	12.95	547130-547467
1700	2175	transforming growth factor, β receptor II	17.68	549106-549395
1713	2171	lysyl oxidase-like 1	27.43	553603-553837
1723	2167	prosaposin	159.42	556999-557313
1728	2165	leprecan 1	29.15	558793-559105
1785	2150	tuftelin 1	6.024	578466-578777
1792	2147	family with sequence similarity 108, member B	12.36	580929-581285
1801	2142	biglycan	335.92	584020-584336
1828	2136	matrix metallopeptidase 9	16.328	593202-593492
1831	2134	dystroglycan 1	3.205	594147-594505
1841	2131	glypican 1	9.404	597502-597879
1843	2130	lysosomal-associated membrane protein 1	239.94	598208-598530
1865	2124	secreted acidic cysteine rich glycoprotein	240.27	605640-606011
1902	2112	olfactomedin-like 2B	15.33	618054-618379
1934	2100	heparin-binding EGF-like growth factor	10.18	629091-629425
1990	2082	protein S (α)	10.73	648173-648463
2065	2055	integrin α FG-GAP repeat containing 1	7.636	673176-673566
2088	2048	ST3 β-galactoside α-2,3-sialyltransferase 1	5.651	681105-681454
2109	2041	disintegrin-like & metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1	0.788	687923-688239
2140	2029	colony stimulating factor 1 (macrophage)	2.182	698431-698749
2440	1946	arginyl aminopeptidase (aminopeptidase B)	9.264	800159-800460
2474	1940	epiregulin	9.501	811533-811821
2477	1938	complement component factor h	1.484	812520-812875
2542	1922	selenoprotein P, plasma, 1	49.03	835040-835364
2618	1903	granulin	165.89	860464-860761
2627	1901	tubulointerstitial nephritis antigen-like 1	471.92	863337-863698
2667	1890	family with sequence similarity 20, member C	2.956	876909-877243
2698	1885	insulin-like growth factor binding protein 4	45.48	887505-887820
2719	1879	extracellular matrix protein 1	8.456	894665-894972
2722	1877	calreticulin	630.60	895691-896051
2724	1876	cysteine rich protein 61	28.82	896415-896793
2755	1869	tenascin XB	0.354	907253-907581
2774	1862	glucose-fructose oxidoreductase domain containing 2	4.766	913662-913993
2782	1861	procollagen C-endopeptidase enhancer 2	46.63	916348-916725
2820	1853	biotinidase	6.907	929397-929702
2866	1840	milk fat globule-EGF factor 8 protein	184.99	945014-945413
2890	1833	coiled-coil domain containing 126	9.438	953382-953706
2960	1813	elastin microfibril interfacer 1	5.563	977509-977878
2980	1806	galactoside-binding lectin soluble 3	90.44	984430-984814
3067	1775	fibroblast growth factor 7	1.663	1013719-1014044
3118	1765	glucose phosphate isomerase 1	16.66	1031173-1031520
3129	1763	matrix metallopeptidase 3	44.78	1034832-1035193
3242	1737	arylsulfatase J	2.374	1073837-1074138
3284	1723	sushi-repeat-containing protein, X-linked 2	13.969	1087870-1088253
3296	1720	suppression of tumorigenicity 14 (colon carcinoma)	2.378	1092011-1092357

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3297	1719	four jointed box 1 (*Drosophila*)	3.328	1092358-1092750
3318	1714	ependymin related protein 1 (zebrafish)	16.418	1099390-1099766
3349	1706	RIKEN cDNA 4930503L19 gene	2.085	1109779-1110086
3370	1701	mannosidase 2, α B2	6.356	1116936-1117323
3410	1689	neuroblastoma, suppression of tumorigenicity 1	73.672	1130855-1131122
3509	1667	heparanase	5.741	1163608-1163901
3517	1666	aarF domain containing kinase 1	1.499	1166401-1166741
3565	1652	HtrA serine peptidase 1	42.699	1182505-1182824
3639	1636	chitinase domain containing 1	16.866	1207474-1207818
3673	1628	corneodesmosin	3.545	1218944-1219310
3727	1618	vascular endothelial growth factor C	10.284	1237351-1237686
3749	1612	ADAMTS-like 4	2.67	1244700-1245081
3783	1602	epidermal growth factor-containing fibulin-like extracellular matrix protein 1	6.911	1256425-1256734
3809	1594	matrix metallopeptidase 10	43.632	1265238-1265630
3879	1578	aldolase A, fructose-bisphosphate	476.31	1288654-1288987
3926	1567	clusterin	40.878	1304084-1304407
4064	1533	phospholipid transfer protein	39.57	1350158-1350474
4109	1521	glycosylphosphatidylinositol specific phospholipase D1	0.591	1365026-1365348
4177	1507	RIKEN cDNA A130022J15 gene	1.007	1387950-1388266
4188	1504	EGF-containing fibulin-like extracellular matrix protein 2	45.43	1391741-1392104
4234	1494	WAP, FS, Ig, KU, & NTR-containing protein 1	1.307	1407418-1407713
4240	1493	complement factor properdin	2.075	1409395-1409692
4245	1492	Ser (or Cys) peptidase inhibitor, clade I, member 1	0.687	1410934-1411281
4280	1485	glutathione reductase	6.516	1422793-1423122
4282	1485	matrix metallopeptidase 12	15.393	1423446-1423812
4319	1476	ST3 beta-galactoside α-2,3-sialyltransferase 2	1.043	1435989-1436317
4345	1471	procollagen C-endopeptidase enhancer protein	38.334	1444649-1444973
4362	1468	serum amyloid A-like 1	2.535	1450214-1450482
4405	1458	tsukushin	2.692	1464641-1464971
4410	1457	sodium channel, nonvoltage-gated 1 α	0.749	1466293-1466624
4417	1456	ADP-dependent glucokinase	1.872	1468606-1468902
4513	1433	leukemia inhibitory factor	2.095	1499872-1500182
4538	1428	RIKEN cDNA 3110057O12 gene	0.612	1508213-1508566
4576	1420	CD109 antigen	0.579	1521122-1521452
4614	1413	family with sequence similarity 3, member A	24.923	1533979-1534266
4627	1408	parathyroid hormone-like peptide	4.769	1537818-1538138
4767	1376	serine (or cysteine) peptidase inhibitor, clade F, member 1	20.015	1584786-1585074
4772	1374	annexin A2	701.66	1586334-1586631
4801	1368	cysteine-rich with EGF-like domains 2	53.263	1596381-1596717
4834	1362	hedgehog interacting protein-like 1	1.94	1607854-1608237
4843	1359	laminin, γ 2	0.673	1610932-1611257
4846	1358	family with sequence similarity 108, member A	22.48	1611921-1612236
4847	1358	secreted phosphoprotein 1	200.26	1612237-1612512
4878	1352	C1q and tumor necrosis factor related protein 4	48.396	1622523-1622869
4923	1344	Von Willebrand factor homolog	0.168	1638235-1638612

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4959	1336	paraoxonase 2	17.99	1650552-1650935
4965	1332	collagen, type III, α 1	0.44	1652715-1653073
4993	1326	collagen, type XVIII, α 1	0.529	1662476-1662775
4995	1325	Norrie disease (pseudoglioma) (human)	2.955	1663144-1663508
5017	1320	olfactomedin-like 3	1.465	1670554-1670828
5071	1308	endonuclease domain containing 1	1.415	1688826-1689139
5100	1301	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E	0.608	1698838-1699148
5102	1300	complement component (3b/4b) receptor 1-like	36.058	1699537-1699891
5103	1300	histocompatibility 2, D region locus 1	14.507	1699892-1699970
5145	1290	dehydrogenase/reductase (SDR family) member 13	3.209	1714013-1714298
5151	1288	cytokine receptor-like factor 1	35.42	1715952-1716278
5183	1283	acid phosphatase 6, lysophosphatidic	4.044	1727109-1727397
5231	1274	latent transforming growth factor β binding protein 2	0.288	1743609-1743996
5233	1274	histocompatibility 2, K1, K region	12.62	1744314-1744510
5244	1272	interleukin 4 receptor, α	1.087	1748021-1748398
5265	1268	interleukin 33	27.994	1755091-1755384
5270	1267	zona pellucida binding protein 2	8.813	1756658-1757006
5275	1265	family with sequence similarity 3, member C	6.069	1758518-1758803
5357	1245	transforming growth factor, β 1	13.689	1787146-1787456
5390	1239	N-acetylglucosamine-1-phosphotransferase, γ subunit	11.34	1799084-1799470
5400	1237	cartilage associated protein	24.359	1802419-1802805
5421	1232	intercellular adhesion molecule 1	3.334	1809854-1810180
5428	1230	calsyntenin 1	0.828	1812199-1812578
5435	1229	meteorin, glial cell differentiation regulator-like	5.487	1814631-1814930
5450	1225	wingless-related MMTV integration site 7B	0.932	1819882-1820264
5519	1207	glucose-fructose oxidoreductase domain containing 1	0.479	1844526-1844882
5520	1207	carboxypeptidase X 1 (M14 family)	0.795	1844883-1845160
5529	1205	glutamyl aminopeptidase	0.69	1847806-1848189
5537	1202	angiopoietin-like 4	0.987	1850651-1851035
5550	1200	a disintegrin and metallopeptidase domain 17	1.374	1855220-1855596
5556	1199	dickkopf homolog 3 (*Xenopus laevis*)	1.782	1857147-1857502
5644	1179	complement component 3	0.472	1888266-1888655
5682	1170	transforming growth factor, β receptor III	6.658	1901807-1902171
5694	1168	vascular endothelial growth factor B	11.401	1906017-1906367
5710	1164	decorin	1.4	1911705-1912079
5716	1164	cofilin 1, non-muscle	107.83	1914036-1914356
5718	1163	lysyl oxidase-like 2	0.322	1914742-1915076
5735	1160	thioredoxin domain containing 16	0.533	1920932-1921309
5752	1156	capping protein (actin filament), gelsolin-like	62.723	1927144-1927507
5783	1148	lectin, galactose binding, soluble 9	12.269	1938395-1938769
5792	1147	matrix metallopeptidase 13	0.724	1941794-1942151
5800	1145	multiple coagulation factor deficiency 2	5.202	1944542-1944919
5810	1144	Kazal-type serine peptidase inhibitor domain 1	37.259	1948146-1948458
5841	1138	collagen, type V, α 2	0.225	1959286-1959679

Table 12. Extracellular Space; External Region (Chinese hamster)				
5854	1136	caspase 1	2.306	1964106-1964500
5872	1132	γ-glutamyl hydrolase	9.842	1970781-1971062
5964	1111	colony stimulating factor 3 (granulocyte)	2.413	2004485-2004820
5967	1110	cellular repressor of E1A-stimulated genes 1	3.396	2005583-2005881
6004	1100	RIKEN cDNA 1600012H06 gene	1.469	2018789-2019169
6033	1095	protease, serine 27	3.375	2029351-2029692
6059	1090	torsin family 2, member A	4.118	2038737-2039067
6069	1087	DDRGK domain containing 1	25.9	2042411-2042776
6177	1063	dehydrogenase/reductase (SDR family) member 11	3.811	2081334-2081729
6185	1062	aminoacyl tRNA synthetase complex-interacting multifunctional protein 1	33.092	2084323-2084687
6208	1056	coiled-coil domain containing 134	4.556	2092810-2093167
6234	1050	plasminogen activator, urokinase receptor	78.786	2102477-2102872
6237	1049	phospholipase A2, group XV	1.496	2103576-2103969
6273	1039	nerve growth factor	9.393	2115896-2116286
6276	1038	wingless-related MMTV integration site 4	32.674	2116955-2117340
6296	1034	kelch-like 11 (*Drosophila*)	0.425	2124257-2124635
6328	1028	hydroxysteroid (17-β) dehydrogenase 11	4.421	2135382-2135767
6334	1028	chemokine (C-X-C motif) ligand 12	0.641	2137589-2137972
6363	1021	netrin 4	3.366	2148005-2148402
6385	1017	follistatin	0.853	2155919-2156270
6412	1009	GLI pathogenesis-related 2	2.074	2165641-2165996
6457	998	ecto-NOX disulfide-thiol exchanger 1	3.002	2181525-2181862
6493	989	collagen, type VII, α 1	0.344	2194670-2194969
6627	964	meteorin, glial cell differentiation regulator	3.641	2241580-2241948
6665	955	hyaluronic acid binding protein 4	2.739	2255107-2255429
6773	932	inhibin β-B	1.597	2292605-2292959
6787	928	wingless-related MMTV integration site 5B	0.458	2297590-2297892
6816	921	peroxidasin homolog (*Drosophila*)	0.334	2307638-2308007
6819	920	integrin α 2b	0.686	2308648-2308928
6830	918	interleukin 19	4.282	2312386-2312719
6900	903	phospholipase A2, group XIIA	11.576	2335117-2335473
6950	893	angiogenic factor with G patch and FHA domains 1	0.281	2351743-2352058
6964	889	Niemann Pick type C2	40.486	2356243-2356636
6974	887	apolipoprotein A-I binding protein	13.178	2359569-2359941
7015	877	TNF (ligand) superfamily, member 12	4.328	2373485-2373776
7019	876	Cys rich transmembrane BMP regulator 1 (chordin like)	0.287	2374809-2375187
7021	875	matrilin 4	7.832	2375566-2375930
7022	875	artemin	2.794	2375931-2376296
7109	858	matrix metallopeptidase 19	0.305	2404764-2405144
7125	853	profilin 1	11.177	2410108-2410492
7126	852	vasohibin 1	0.138	2410493-2410795
7142	849	Parkinson disease 7 domain containing 1	1.935	2415737-2416107
7156	846	intercellular adhesion molecule 4, Landsteiner-Wiener blood group	5.958	2420123-2420515
7158	845	c-fos induced growth factor	3.445	2420809-2421101
7185	839	leucine-rich repeats and calponin homology	0.206	2429731-2430059

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		(CH) domain containing 3		
7192	839	VGF nerve growth factor inducible	0.371	2432094-2432431
7199	838	transforming growth factor, β 3	1.124	2434410-2434754
7223	833	chemokine (C-X-C motif) ligand 1	3.826	2442608-2443003
7234	830	WNT1 inducible signaling pathway protein 2	1.032	2446311-2446606
7259	824	leucine-rich repeat LGI family, member 4	0.356	2454637-2454993
7279	817	follistatin-like 1	0.406	2460885-2461283
7305	810	tissue factor pathway inhibitor	4.848	2469295-2469576
7328	804	inhibin α	0.548	2477026-2477404
7360	796	placental specific protein 1	2.395	2487553-2487920
7380	793	stromal cell derived factor 2	6.558	2494318-2494652
7450	775	FMS-like tyrosine kinase 3 ligand	4.868	2517516-2517899
7454	774	platelet derived growth factor, α	4.859	2518844-2519200
7469	770	CD1d1 antigen	0.505	2523514-2523656
7475	769	tissue inhibitor of metalloproteinase 1	42.275	2525246-2525550
7484	767	UDP-Gal:betaGlcNAc β 1,4-galactosyltransferase, polypeptide 1	0.387	2528454-2528763
7624	733	sodium channel, nonvoltage-gated 1 β	0.301	2574019-2574393
7628	732	proline-rich Gla (G-carboxyglutamic acid) polypeptide 2	1.115	2575046-2575364
7658	724	hyaluronan and proteoglycan link protein 4	0.319	2584861-2585169
7676	720	chemokine (C-C motif) ligand 2	14.55	2590794-2591157
7707	713	intelectin 1 (galactofuranose binding)	1.888	2601763-2602070
7726	708	interleukin 17F	3.058	2607930-2608234
7758	700	bone morphogenetic protein 2	0.343	2618776-2619161
7770	697	olfactomedin 2	0.593	2622919-2623236
7789	692	collagen, type VIII, α 1	0.136	2629576-2629946
7810	688	mesencephalic astrocyte-derived neurotrophic factor	3.849	2636612-2636951
7820	685	integrin α X	0.229	2639993-2640227
7827	683	versican	0.055	2642303-2642596
7874	666	CD1d2 antigen	0.935	2658252-2658336
7903	658	interleukin 1 receptor accessory protein	0.254	2667913-2668256
7929	651	interleukin 23, α subunit p19	0.852	2676772-2677097
7935	649	follistatin-like 3	0.427	2678648-2679041
7938	649	stanniocalcin 2	0.821	2679803-2680201
7940	648	matrix metallopeptidase 17	0.224	2680510-2680844
7947	646	wingless-type MMTV integration site 9A	0.20	2682871-2683194
7979	638	protease, serine, 8 (prostasin)	0.479	2693206-2693562
8062	610	fibroblast growth factor 18	1.273	2720721-2721030
8066	610	ribonuclease, RNase A family 4	9.649	2721991-2722365
8108	598	thymosin, β 4, X chromosome	24.043	2734875-2735269
8119	595	serglycin	9.946	2738723-2739031
8138	590	RIKEN cDNA 1700040I03 gene	2.322	2744620-2744956
8146	588	cardiotrophin-like cytokine factor 1	1.757	2747178-2747573
8167	584	agouti related protein	1.444	2753704-2754040
8218	570	interleukin 18	2.856	2769797-2770097
8226	568	DNA segment, Chr 17, Wayne State University 104, expressed	3.239	2772236-2772535

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8244	562	interleukin 1 receptor-like 1	0.299	2777898-2778255
8255	558	matrix metallopeptidase 24	6.978	2781318-2781710
8257	558	elastin microfibril interfacer 3	0.17	2782095-2782379
8303	547	C1q and tumor necrosis factor related protein 1	0.218	2797989-2798315
8304	546	macrophage migration inhibitory factor	43.469	2798316-2798434
8332	540	twisted gastrulation homolog 1 (*Drosophila*)	0.318	2807636-2808031
8345	536	Fas (TNF receptor superfamily member 6)	0.501	2812206-2812506
8385	524	natriuretic peptide precursor type B	2.217	2825789-2826134
8387	523	suprabasin	2.479	2826504-2826901
8394	521	cystatin C	17.163	2828994-2829393
8410	516	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	0.212	2834784-2835155
8440	510	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 4	0.139	2845165-2845528
8500	495	natriuretic peptide precursor type A	1.563	2864212-2864568
8504	494	chemokine (C-X-C motif) ligand 10	1.586	2865648-2866015
8531	490	interleukin 15	1.901	2874576-2874952
8553	485	interleukin 11	0.384	2881854-2882091
8560	485	retinoic acid receptor responder (tazarotene induced) 2	0.687	2883778-2884132
8581	480	lectin, galactose binding, soluble 1	282.39	2890379-2890745
8597	477	mannan-binding lectin serine peptidase 2	0.156	2896069-2896411
8647	467	RIKEN cDNA 2300009A05 gene	0.768	2912945-2913330
8696	459	CSF 2 (granulocyte-macrophage)	1.109	2928757-2929061
8697	459	interleukin 18 binding protein	1.553	2929062-2929418
8698	459	prenylcysteine oxidase 1 like	0.228	2929419-2929743
8708	456	apolipoprotein O-like	0.456	2932503-2932836
8713	455	neuron derived neurotrophic factor	1.137	2933997-2934318
8746	450	TNF receptor superfamily, member 4	0.392	2944708-2945036
8753	449	sparc/osteonectin, cwcv & kazal-like domains proteoglycan 1	0.172	2946657-2946988
8756	449	integrin α 1	0.15	2947656-2948022
8777	444	laminin, α 2	0.046	2954307-2954650
8784	443	thyroglobulin	0.076	2956549-2956869
8821	437	apolipoprotein M	0.598	2967624-2967944
8871	423	spondin 2, extracellular matrix protein	0.189	2982359-2982686
8876	422	elastin microfibril interfacer 2	0.11	2983901-2984203
8916	414	anti-Mullerian hormone	0.248	2995308-2995592
8935	409	carboxypeptidase N, polypeptide 1	0.233	3000705-3001032
8945	407	insulin-like growth factor binding protein 6	0.548	3003421-3003704
9021	387	hemopexin	0.262	3023816-3024122
9063	374	periostin, osteoblast specific factor	0.118	3034618-3034877
9064	373	complement component 8, γ polypeptide	0.685	3034878-3035143
9079	370	neuregulin 3	0.146	3038641-3038935
9116	361	RIKEN cDNA 1190002N15 gene	0.094	3047961-3048223
9120	360	adrenomedullin	0.331	3048901-3049164
9131	357	apolipoprotein A-II	1.494	3051648-3051933
9136	356	nonagouti	0.963	3052970-3053198

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9151	352	TNF receptor superfamily, member 22	0.691	3056380-3056639
9164	348	TNF (ligand) superfamily, member 11	0.157	3058993-3059213
9185	344	Serine (or Cys) peptidase inhibitor, clade C (antithrombin), member 1	0.158	3063585-3063840
9207	339	RIKEN cDNA A430110N23 gene	0.132	3068647-3068843
9212	339	canopy 4 homolog (zebrafish)	0.335	3069460-3069696
9230	335	regenerating islet-derived 3 γ	0.43	3073532-3073815
9244	331	arylsulfatase K	0.177	3076784-3077031
9267	324	cerebral dopamine neurotrophic factor	0.109	3081521-3081786
9274	322	bone morphogenetic protein 6	0.219	3083187-3083415
9290	319	carboxypeptidase B2 (plasma)	0.216	3086591-3086854
9293	318	deoxyribonuclease 1-like 2	0.409	3087405-3087662
9295	318	apolipoprotein H	0.493	3087876-3088127
9307	312	growth hormone receptor	0.289	3090523-3090733
9325	307	transglutaminase 4 (prostate)	0.112	3094562-3094802
9363	296	oncostatin M	0.135	3102482-3102721
9366	295	osteomodulin	0.169	3103083-3103312
9367	295	Fc receptor, IgG, low affinity IIb	0.189	3103313-3103351
9368	295	DAN domain family, member 5	0.189	3103352-3103518
9375	293	antigen p97 (melanoma associated) identified by mAbs 133.2 and 96.5	0.073	3104582-3104752
9394	285	carboxylesterase 7	0.166	3108135-3108339
9402	282	ISG15 ubiquitin-like modifier	1.263	3109784-3109974
9403	282	RIKEN cDNA 4930486L24 gene	0.203	3109975-3110173
9404	281	transmembrane protein 25	0.122	3110174-3110389
9412	278	cDNA sequence BC039632	0.114	3111726-3111929
9431	270	GLI pathogenesis-related 1 (glioma)	0.512	3115200-3115432
9461	260	carbonic anhydrase 15	0.231	3120401-3120588
9518	237	cytotoxic T lymphocyte-associated protein 2 α	0.174	3129312-3129456
9536	233	laminin γ 3	0.04	3131997-3132159
9560	222	RIKEN cDNA 1110058L19 gene	0.33	3135368-3135519
9593	210	family with sequence similarity 20, member B	0.05	3139182-3139331
9604	205	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2	0.313	3140413-3140532
9611	202	chemokine (C-C motif) ligand 9	0.268	3141032-3141071
9654	185	cerebellin 3 precursor protein	0.051	3144853-3144886
9673	174	cellular repressor of E1A-stimulated genes 2	0.11	3146685-3146736
9694	166	histocompatibility 2, M region locus 3	0.309	NA-NA
9720	149	chemokine (C-X-C motif) ligand 3	0.148	3149776-3149850
9740	139	β cellulin, epidermal growth factor family member	0.073	3150839-3150877
9742	139	hyaluronoglucosaminidase 1	0.064	3150976-3151021
9756	131	glutathione peroxidase 3	0.087	3151589-3151685
3157149	488	tectorin β	0.18	3161121-3161220
3157152	479	angiogenin, ribonuclease, RNase A family, 5	0.895	3217891-3217990
3157165	234	surfactant associated protein D	0.176	3266005-3266104
3157173	1664	transcobalamin 2	5.78	3266205-3266304
3157204	1498	NA	0.661	3239917-3240016
3157207	463	epiphycan	0.269	3166484-3166583

Table 12. Extracellular Space; External Region (Chinese hamster)				
3157217	384	thrombospondin, type I, domain containing 4	0.044	3224191-3224290
3157225	705	renalase, FAD-dependent amine oxidase	2.03	3245517-3245616
3157231	468	macrophage stimulating 1 (hepatocyte growth factor-like)	0.205	3240817-3240916
3157234	711	neuregulin 4	1.009	3219591-3219690
3157276	1883	cell adhesion molecule w/ homology to L1CAM	0.289	3252517-3252616
3157279	427	ectonucleotide pyrophosphatase/ phosphodiesterase 3	0.153	3182184-3182283
3157283	323	NA	0.115	3279349-3279448
3157286	416	C1q-like 3	0.132	3208858-3208957
3157290	388	carbonic anhydrase 11	0.267	3238117-3238216
3157305	665	angiomotin	0.309	3173084-3173183
3157331	711	isthmin 1 homolog (zebrafish)	0.244	3172584-3172683
3157343	370	predicted gene 1019	0.391	3193971-3194070
3157352	311	killer cell lectin-like receptor, subfamily D, member 1	0.43	3221191-3221290
3157362	1350	immunoglobulin superfamily containing leucine-rich repeat	2.61	3279049-3279148
3157366	450	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	0.242	3260305-3260404
3157368	373	interleukin 16	0.075	3232717-3232816
3157372	584	lipase, family member N	0.389	3192971-3193070
3157373	339	angiopoietin 4	0.222	3239317-3239416
3157414	285	glycine receptor, β subunit	0.096	3187971-3188070
3157415	568	integrin α 6	0.213	3201597-3201696
3157422	1431	G protein-coupled receptor 125	1.185	3236817-3236916
3157455	494	dehydrogenase/reductase (SDR family) member 7C	0.832	3255005-3255104
3157459	250	chemokine (C-C motif) ligand 11	0.299	3199071-3199170
3157475	403	paraoxonase 3	0.226	3268005-3268104
3157481	804	follistatin-like 4	0.303	3183884-3183983
3157491	639	G protein-coupled receptor 98	0.033	3188771-3188870
3157500	458	seizure related gene 6	0.114	3189371-3189470
3157503	787	pentraxin related gene	1.801	3175884-3175983
3157510	700	secretory leukocyte peptidase inhibitor	7.778	3248817-3248916
3157516	361	roundabout homolog 4 (*Drosophila*)	0.098	3164884-3164983
3157520	492	complement component 1, r subcomponent-like	0.264	3224791-3224890
3157537	234	mucin 13, epithelial transmembrane	0.08	3203297-3203396
3157558	742	chemokine (C-C motif) ligand 7	6.395	3279849-3279948
3157590	520	interleukin 13 receptor, α 2	0.336	3213558-3213657
3157601	267	fukutin related protein	0.095	3212358-3212457
3157619	289	fin bud initiation factor homolog (zebrafish)	0.14	3185471-3185570
3157676	961	extracellular matrix protein 2, female organ and adipocyte specific	0.343	3256205-3256304
3157717	366	Fras1 related extracellular matrix protein 1	0.039	3271296-3271395
3157721	413	EGF-like module containing, mucin-like, hormone receptor-like sequence 1	0.249	3218391-3218490
3157729	356	tectorin α	0.049	3257705-3257804
3157760	967	interleukin 7 receptor	0.428	3216691-3216790
3157775	648	multiple EGF-like-domains 6	0.147	3174384-3174483

Table 12. Extracellular Space; External Region (Chinese hamster)				
3157796	402	secreted phosphoprotein 2	0.468	3270196-3270295
3157845	837	mast cell protease 8	4.869	3206558-3206657
3157850	577	collagen, type XV, α 1	0.108	3250617-3250716
3157858	323	apolipoprotein E	0.255	3172384-3172483
3157868	306	cathelicidin antimicrobial peptide	0.513	3234517-3234616
3157885	1542	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	0.705	3168184-3168283
3157898	422	disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 15	0.115	3193471-3193570
3157902	1558	fibrillin 1	0.197	3211258-3211357
3157936	2200	laminin, α 3	0.41	3160721-3160820
3157937	697	collagen, type XVII, α 1	0.131	3163384-3163483
3157938	372	secretagogin, EF-hand calcium binding protein	0.26	3258005-3258104
3157949	477	protein C	0.42	3271796-3271895
3157974	2507	thrombospondin 2	1.595	3265805-3265904
3157977	1031	interleukin 7	0.642	3242917-3243016
3158019	362	ABO blood group (transferase A, α 1-3-N-acetylgalactosaminyltransferase, transferase B, α 1-3-galactosyltransferase)	0.204	3185571-3185670
3158024	541	immunoglobulin superfamily, member 10	0.078	3194171-3194270
3158034	923	HtrA serine peptidase 3	0.583	3258505-3258604
3158038	176	Fc receptor, IgE, high affinity I, γpolypeptide	0.258	3201197-3201296
3158050	435	lumican	0.209	3262905-3263004
3158075	480	potassium inwardly-rectifying channel, subfamily J, member 3	0.297	3169184-3169283
3158077	496	fibulin 5	0.198	3239017-3239116
3158079	282	expressed sequence AI462493	0.577	3210858-3210957
3158107	484	scavenger receptor cysteine rich domain containing, group B (4 domains)	0.181	3161821-3161920
3158135	418	mannan-binding lectin serine peptidase 1	0.152	3282249-3282348
3158185	485	interleukin 1 family, member 9	2.527	3241217-3241316
3158191	197	dermatopontin	0.125	3210958-3211057
3158201	416	matrix metallopeptidase 21	0.224	3195471-3195570
3158209	1954	fibroblast growth factor receptor 2	2.109	3207458-3207557
3158212	2457	RIKEN cDNA 1300010F03 gene	0.56	3182084-3182183
3158227	235	bactericidal/permeability-increasing protein-like 2	0.101	3160521-3160620
3158236	1428	R-spondin 3 homolog (*Xenopus laevis*)	0.883	3261305-3261404
3158246	338	coagulation factor VII	0.181	3207558-3207657
3158249	442	amylase 1, salivary	0.247	3203097-3203196
3158274	393	C-type lectin domain family 18, member A	0.214	3219791-3219890
3158294	648	matrix metallopeptidase 2	0.413	3214291-3214390
3158295	426	stratifin	0.681	3216091-3216190
3158307	369	placental growth factor	0.923	3227817-3227916
3158309	408	adiponectin, C1Q and collagen domain containing	0.331	3225317-3225416
3158310	262	neuropeptide B	0.483	3278149-3278248
3158331	982	NEL-like 1 (chicken)	0.565	3163221-3163320
3158365	431	complement component factor i	0.209	3178584-3178683
3158373	246	pyroglutamylated RFamide peptide	0.172	3209458-3209557
3158381	762	CD24a antigen	0.906	3245917-3246016

Table 12. Extracellular Space; External Region (Chinese hamster)				
3158387	364	ladinin	0.193	3193271-3193370
3158415	552	growth differentiation factor 11	0.45	3178384-3178483
3158419	1567	NA	1.244	3273596-3273695

Table 13. Cell cycle/ Cell Division (Chinese hamster)				
SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
1	7293	ubiquitin specific peptidase 9, X chromosome	6.127	9772-10147
19	4458	platelet-activating factor acetylhydrolase, isoform 1b, subunit 1	4.915	15430-15711
25	4353	PDS5, regulator of cohesion maintenance, homolog B (*S. cerevisiae*)	2.006	17099-17460
81	3902	integrin β 1 (fibronectin receptor β)	126.69	35564-35891
126	3635	E2F transcription factor 3	7.133	50121-50455
146	3553	microtubule-actin crosslinking factor 1	3.329	56027-56372
149	3549	stromal antigen 1	5.503	56906-57146
189	3384	phosphatase and tensin homolog	0.633	69091-69404
214	3308	microtubule-associated protein, RP/EB family, member 2	9.685	76455-76767
236	3232	non-SMC condensin II complex, subunit D3	5.339	83095-83338
239	3230	septin 11	14.203	83878-84130
266	3179	vascular endothelial growth factor A	18.713	92246-92594
287	3132	splicing factor 1	10.149	98068-98328
304	3108	Nipped-B homolog (*Drosophila*)	1.896	103144-103477
317	3089	cytoskeleton associated protein 5	5.989	106729-106971
345	3034	glycogen synthase kinase 3 β	0.647	114424-114743
375	2989	RAD21 homolog (*S. pombe*)	34.322	123260-123508
378	2983	tousled-like kinase 1	3.811	124295-124551
382	2979	breakpoint cluster region	3.754	125289-125540
384	2977	transcriptional regulator, SIN3A (yeast)	3.56	125791-126119
426	2925	stromal antigen 2	1.018	137619-137852
431	2919	Tia1 cytotoxic granule-associated RNA binding protein-like 1	12.569	139041-139241
432	2919	cyclin D1	18.856	139242-139629
451	2894	kinetochore associated 1	2.501	144746-145029
477	2865	spindlin 1	18.581	151421-151677
486	2857	anaphase promoting complex subunit 1	2.309	154085-154328
510	2835	calcium/calmodulin-dependent protein kinase II γ	4.887	161048-161267
528	2814	spastin	4.005	166072-166288
540	2799	signal transducer & activator of transcription 5B	1.323	169415-169753
549	2785	AT hook containing transcription factor 1	2.992	172063-172296
573	2763	calmodulin 1	15.152	178775-179029
589	2746	nuclear protein in the AT region	2.695	183475-183690
644	2703	mitogen-activated protein kinase 6	18.977	200294-200550
658	2692	structural maintenace of chromosomes 3	18.331	204131-204513
662	2689	calcium/calmodulin-dependent protein kinase II, δ	5.415	205498-205717
689	2670	budding uninhibited by benzimidazoles 1	3.768	213750-213996

		homolog (*S. cerevisiae*)		
745	2630	minichromosome maintenance deficient 6 (MIS5 homolog, yeast)	38.269	230817-231043
800	2590	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor	1.877	247696-248086
811	2582	ajuba	12.735	251195-251502
825	2573	amyloid β (A4) precursor protein	165.22	255412-255644
838	2566	anaphase promoting complex subunit 4	10.429	259583-259826
866	2552	timeless homolog (Drosophila)	1.453	267981-268365
873	2550	cyclin G associated kinase	4.774	270072-270372
885	2543	platelet-derived growth factor, C polypeptide	3.586	273882-274243
889	2543	katanin p80 (WD40-containing) subunit B 1	12.112	275290-275634
891	2542	RB1-inducible coiled-coil 1	2.069	275944-276175
898	2540	kinesin family member 20B	10.559	278267-278603
899	2538	transformation related protein 53 binding protein 2	2.893	278604-278960
905	2536	ADP-ribosylation factor-like 8B	2.122	280457-280707
913	2532	proteaseome (prosome, macropain) 28 subunit, 3	21.397	283197-283568
965	2501	ubiquitin specific peptidase 16	11.237	300334-300663
990	2488	ubiquitin-conjugating enzyme E2I	38.98	308789-309160
1006	2477	large tumor suppressor 2	3.379	314156-314545
1009	2476	transcription factor Dp 2	2.614	315253-315631
1051	2450	anaphase-promoting complex subunit 5	60.895	329249-329648
1053	2449	polycystic kidney disease 1 homolog	1.249	330038-330429
1062	2447	septin 2	12.767	333080-333462
1068	2441	chromatin assembly factor 1, subunit A (p150)	6.127	334746-335135
1070	2440	promyelocytic leukemia	1.141	335490-335874
1082	2434	tousled-like kinase 2 (*Arabidopsis*)	5.586	339541-339778
1091	2431	ligase I, DNA, ATP-dependent	14.03	342515-342854
1102	2427	CTF18, chromosome transmission fidelity factor 18 homolog (*S. cerevisiae*)	3.974	346257-346598
1103	2426	dystonin	1.863	346599-346975
1188	2387	WEE 1 homolog 1 (*S. pombe*)	5.458	375593-375982
1208	2379	CDC14 cell division cycle 14 homolog A (*S. cerevisiae*)	2.141	381807-382191
1247	2359	microtubule-associated protein, RP/EB family, member 1	18.63	394632-394981
1255	2354	centrosomal protein 110	0.814	397494-397774
1261	2353	ligase III, DNA, ATP-dependent	1.44	399254-399624
1321	2325	beta-transducin repeat containing protein	2.152	419725-419957
1327	2324	centrosomal protein 55	19.363	421520-421872
1329	2323	adenomatosis polyposis coli	0.997	422123-422508
1341	2318	cell division cycle 73, Paf1/RNA polymerase II complex component, homolog (*S. cerevisiae*)	3.662	426333-426720
1353	2311	centrosomal protein 63	8.32	430642-430998
1354	2311	high mobility group box 1	4.567	430999-431370
1369	2302	protein phosphatase 1, catalytic subunit, γ isoform	113.24	436277-436523
1403	2287	structural maintenance of chromosomes 1A	13.394	447520-447805

1425	2276	minichromosome maintenance deficient 5, cell division cycle 46 (*S. cerevisiae*)	20.01	455118-455499
1438	2270	cysteine and glycine-rich protein 2 binding protein	2.431	459534-459931
1505	2243	growth arrest-specific 2 like 1	14.15	482255-482606
1523	2239	TSPY-like 2	4.364	487980-488352
1532	2236	CDC16 cell division cycle 16 homolog (*S. cerevisiae*)	61.55	491125-491521
1537	2234	anaphase promoting complex subunit 2	8.972	492880-493248
1542	2232	Jun oncogene	5.841	494469-494742
1554	2228	SUMO/sentrin specific peptidase 5	1.726	498550-498878
1557	2227	annexin A11	55.57	499580-499921
1560	2227	SET domain containing (lysine methyltransferase) 8	16.79	500465-500805
1562	2226	small G protein signaling modulator 3	9.371	501162-501548
1565	2224	ZW10 homolog (*Drosophila*), centromere/ kinetochore protein	12.63	502292-502621
1571	2221	RAD17 homolog (S. pombe)	7.172	504416-504768
1582	2214	family with sequence similarity 83, member D	12.85	508106-508316
1593	2210	rho/rac guanine nucleotide exchange factor (GEF) 2	3.451	511846-512237
1608	2206	minichromosome maintenance deficient 3 (*S. cerevisiae*)	24.19	517207-517557
1638	2194	polo-like kinase 2 (*Drosophila*)	4.793	527681-527996
1706	2173	catalase	18.084	551058-551444
1716	2169	cyclin G2	4.918	554595-554969
1724	2167	E4F transcription factor 1	4.358	557314-557678
1726	2166	cyclin I	14.85	558041-558430
1741	2160	non-SMC condensin I complex, subunit D2	12.081	563227-563611
1743	2159	polymerase (DNA directed) sigma	11.13	563897-564261
1744	2159	RIKEN cDNA 2400003C14 gene	16.24	564262-564570
1746	2159	transformation/transcription domain-associated protein	0.661	564955-565345
1749	2158	minichromosome maintenance deficient 7 (*S. cerevisiae*)	52.55	566044-566427
1750	2158	retinoblastoma 1	1.741	566428-566760
1758	2157	protein phosphatase 1G (formerly 2C), Mg-dependent, γ isoform	65.51	569118-569459
1767	2154	programmed cell death 6 interacting protein	24.67	572196-572546
1822	2137	polo-like kinase 1 (*Drosophila*)	42.62	591133-591528
1829	2135	amyloid β (A4) precursor protein-binding, family B, member 1	13.93	593493-593882
1837	2132	polycystic kidney disease 2	2.329	596164-596507
1838	2132	proviral integration site 3	16.75	596508-596892
1849	2128	NIMA (never in mitosis gene a)-related expressed kinase 6	11.135	600327-600624
1856	2126	SEH1-like (*S. cerevisiae*)	6.521	602767-603120
1860	2124	cyclin G1	3.56	603997-604346
1874	2121	NIMA (never in mitosis gene a)-related expressed kinase 9	5.452	608758-609143
1882	2118	ubiquitin-like modifier activating enzyme 3	26.578	611535-611917

1897	2113	RIKEN cDNA 2010005J08 gene	3.915	616258-616623
1910	2110	macrophage erythroblast attacher	48.23	620748-621108
1939	2098	leucine zipper, putative tumor suppressor 2	14.19	630655-630915
1944	2097	cell division cycle 42 homolog (*S. cerevisiae*)	189.61	632324-632630
1972	2086	protein phosphatase 1, catalytic subunit, β isoform	1.708	642111-642462
2029	2068	heat shock protein 8	891.02	660889-661277
2078	2050	cyclin F	3.468	677909-678208
2094	2045	polo-like kinase 3 (*Drosophila*)	7.762	683175-683550
2105	2042	CD2-associated protein	0.744	686855-687170
2111	2040	cyclin D binding myb-like transcription factor 1	1.893	688585-688896
2121	2035	Fanconi anemia, complementation group D2	1.038	691993-692390
2131	2032	minichromosome maintenance deficient 2 mitotin (*S. cerevisiae*)	14.00	695280-695591
2139	2030	multiple endocrine neoplasia 1	2.911	698091-698430
2182	2017	inhibitor of growth family, member 1	6.197	712451-712798
2235	2001	septin 7	3.112	730587-730976
2257	1993	cell division cycle 27 homolog (*S. cerevisiae*)	0.583	738313-738671
2283	1987	MAP-kinase activating death domain	1.589	747015-747324
2293	1985	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1	0.781	750597-750920
2297	1984	protein phosphatase 3, catalytic subunit, α isoform	4.715	751950-752267
2346	1973	calmodulin 3	14.01	768392-768693
2378	1963	ubiquitin-like, containing PHD & RING finger domains 2	7.038	778921-779204
2379	1963	protein regulator of cytokinesis 1	14.63	779205-779513
2381	1963	retinoblastoma binding protein 8	4.133	779852-780237
2416	1954	kinesin family member C1	16.34	792040-792370
2426	1951	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 2	2.172	795330-795651
2430	1949	anillin, actin binding protein	2.848	796726-797054
2441	1946	CLIP associating protein 2	1.013	800461-800731
2455	1943	host cell factor C1	2.096	805085-805458
2471	1940	mutS homolog 2 (*E. coli*)	6.134	810424-810813
2474	1940	epiregulin	9.501	811533-811821
2505	1931	septin 8	0.895	822293-822664
2513	1930	DnaJ (Hsp40) homolog, subfamily C, member 2	34.4	825067-825402
2515	1929	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	22.655	825796-826120
2531	1925	NDC80 homolog, kinetochore complex component (*S. cerevisiae*)	20.308	831233-831608
2534	1925	signal-induced proliferation associated gene 1	3.696	832257-832632
2547	1921	cell division cycle and apoptosis regulator 1	1.757	836705-837044
2562	1916	septin 5	22.256	841871-842174
2569	1914	cyclin-dependent kinase 7 (homolog of *Xenopus* MO15 cdk-activating kinase)	1.788	844194-844512
2582	1911	non-SMC condensin I complex, subunit H	14.505	848672-848987

2583	1910	inner centromere protein	4.499	848988-849386
2586	1910	par-3 partitioning defective 3 homolog B (*C. elegans*)	0.422	850130-850455
2593	1909	BTG3 associated nuclear protein	4.134	852510-852846
2595	1909	DBF4 homolog (*S. cerevisiae*)	8.657	853157-853542
2608	1906	E2F transcription factor 1	7.007	857154-857487
2621	1902	Rac GTPase-activating protein 1	19.316	861408-861766
2634	1899	ubiquitin specific peptidase 22	1.692	865729-866104
2644	1897	protein phosphatase 2 (formerly 2A), catalytic subunit, α isoform	46.955	869071-869380
2691	1887	growth arrest specific 2	2.282	885284-885579
2693	1886	ring finger protein 2	1.202	885899-886287
2707	1882	fizzy/cell division cycle 20 related 1 (*Drosophila*)	24.719	890466-890779
2728	1875	STE20-related kinase adaptor α	12.387	897852-898184
2745	1872	mitotic arrest deficient 1-like 1	4.132	903571-903958
2781	1861	histone deacetylase 3	24.855	916015-916347
2792	1859	Mdm2, transformed 3T3 cell double minute p53 binding protein	1.49	919781-920087
2793	1858	non-SMC condensin II complex, subunit G2	2.181	920088-920444
2809	1855	cell division cycle 25 homolog A (*S. pombe*)	1.851	925695-926050
2817	1854	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1	4.485	928459-928777
2834	1851	neuroblastoma *ras* oncogene	2.46	934198-934494
2844	1847	large tumor suppressor	0.394	937654-937969
2848	1847	RAD9 homolog (*S. pombe*)	13.395	938950-939251
2896	1832	centromere protein E	1.871	955437-955745
2904	1829	breast cancer 1	7.497	958124-958436
2910	1827	cyclin D2	1.579	960077-960401
2925	1823	cell division cycle 45 homolog (*S. cerevisiae*-like)	5.32	965312-965711
2968	1810	E2F transcription factor 6	4.213	980320-980709
2971	1808	E2F transcription factor 4	11.352	981429-981759
2984	1804	Jun-B oncogene	63.645	985798-986175
3006	1794	retinoblastoma binding protein 4	5.65	993294-993657
3033	1784	3-phosphoglycerate dehydrogenase	126.19	1002179-1002496
3034	1784	cell division cycle 20 homolog (*S. cerevisiae*)	79.792	1002497-1002849
3039	1783	vacuolar protein sorting 4b (yeast)	2.342	1004233-1004573
3051	1779	suppressor of variegation 3-9 homolog 1 (*Drosophila*)	2.513	1008311-1008610
3066	1776	mitogen-activated protein kinase 3	37.586	1013377-1013718
3067	1775	fibroblast growth factor 7	1.663	1013719-1014044
3081	1772	septin 6	16.844	1018327-1018620
3110	1766	protein kinase, membrane associated tyrosine/ threonine 1	10.224	1028441-1028755
3145	1758	cyclin D3	23.86	1040554-1040910
3149	1757	retinoblastoma-like 2	1.946	1041915-1042243
3152	1756	lin-9 homolog (*C. elegans*)	0.83	1042878-1043200
3161	1755	E2F transcription factor 8	1.759	1046151-1046504

3171	1752	chromatin assembly factor 1, subunit B (p60)	14.978	1049710-1050012
3177	1750	CDC23 (cell division cycle 23, yeast homolog)	2.323	1051775-1052083
3214	1742	RAD50 interactor 1	2.415	1064421-1064789
3215	1742	c-abl oncogene 1, receptor tyrosine kinase	0.436	1064790-1065134
3238	1738	high mobility group AT-hook 2	0.823	1072519-1072837
3256	1733	potassium channel tetramerisation domain containing 11	2.201	1078388-1078757
3283	1723	protein phosphatase 1D magnesium-dependent, δ isoform	2.77	1087491-1087869
3289	1721	menage a trois 1	12.96	1089606-1089959
3301	1718	peripheral myelin protein 22	9.401	1093771-1094161
3306	1717	CLIP associating protein 1	0.948	1095379-1095748
3338	1709	NEDD8 activating enzyme E1 subunit 1	9.826	1106097-1106429
3390	1696	cell division cycle 2-like 1	17.014	1124002-1124331
3419	1688	bladder cancer associated protein homolog (human)	4.537	1133723-1134082
3426	1687	regulator of chromosome condensation 1	4.314	1136021-1136304
3474	1673	cyclin A2	5.366	1151948-1152332
3505	1668	katanin p60 (ATPase-containing) subunit A1	32.182	1162218-1162611
3551	1656	RIKEN cDNA B230120H23 gene	0.667	1177903-1178190
3559	1654	SKI-like	1.243	1180446-1180768
3574	1650	cell division cycle 6 homolog (*S. cerevisiae*)	2.478	1185367-1185759
3577	1650	cell division cycle 25 homolog B (*S. pombe*)	1.866	1186395-1186715
3583	1649	checkpoint kinase 1 homolog (*S. pombe*)	3.146	1188354-1188736
3598	1645	cyclin-dependent kinase 2	16.205	1193336-1193684
3604	1644	excision repair cross-complementing rodent repair deficiency complementation group 6 - like	3.307	1195379-1195725
3605	1644	vacuolar protein sorting 24 (yeast)	5.661	1195726-1196052
3652	1633	minichromosome maintenance deficient 8 (*S. cerevisiae*)	2.747	1211842-1212151
3699	1623	transforming, acidic coiled-coil containing protein 3	13.073	1227651-1228044
3705	1622	seven in absentia 2	1.664	1229814-1230210
3727	1618	vascular endothelial growth factor C	10.284	1237351-1237686
3736	1616	cullin 7	1.583	1240268-1240610
3743	1614	thioredoxin interacting protein	5.1	1242664-1242964
3761	1609	ataxia telangiectasia mutated homolog (human)	0.181	1248864-1249255
3768	1607	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1	5.639	1251267-1251627
3773	1605	inhibitor of growth family, member 4	12.81	1252896-1253239
3787	1601	transcription factor Dp 1	6.434	1257788-1258139
3792	1600	salt inducible kinase 1	0.413	1259549-1259840
3804	1596	RIKEN cDNA 6720463M24 gene	2.973	1263541-1263924
3828	1591	cyclin K	1.622	1271584-1271845
3855	1584	activating transcription factor 5	9.537	1280625-1280989
3865	1582	nuclear autoantigenic sperm protein (histone-binding)	31.057	1283868-1284213
3885	1577	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b,	11.687	1290692-1291012

		member 1		
3907	1573	Zwilch, kinetochore associated, homolog (*Drosophila*)	1.026	1297895-1298179
3910	1572	cyclin B1	25.641	1298863-1299236
3913	1571	signal transducer & activator of transcription 5A	1.268	1299843-1300222
3921	1568	zinc finger protein 369	5.039	1302401-1302734
3969	1558	chromatin modifying protein 1A	7.377	1318357-1318651
4008	1547	Fanconi anemia, complementation group I	1.721	1331437-1331720
4010	1547	septin 9	27.144	1332075-1332392
4016	1545	aryl-hydrocarbon receptor	0.43	1334066-1334367
4023	1544	Wilms' tumour 1-associating protein	2.862	1336382-1336718
4069	1531	ubiquitin-like, containing PHD & RING finger domains, 1	6.026	1351856-1352193
4071	1530	NIMA-related expressed kinase 2	2.858	1352509-1352861
4090	1525	zinc finger, C3HC type 1	17.029	1358571-1358886
4097	1523	RuvB-like protein 1	55.736	1360967-1361271
4103	1522	HAUS augmin-like complex, subunit 4	20.991	1362890-1363204
4140	1514	E2F transcription factor 5	2.277	1375653-1375938
4154	1511	transformed mouse 3T3 cell double minute 2	3.215	1380172-1380483
4156	1511	EP300 interacting inhibitor of differentiation 1	21.285	1380867-1381243
4160	1510	fibronectin type 3 & SPRY domain-containing protein	2.066	1382212-1382607
4171	1508	casein kinase 2, α prime polypeptide	16.889	1385888-1386249
4193	1502	mitogen-activated protein kinase 1	15.004	1393467-1393856
4199	1500	cytoskeleton associated protein 2	1.674	1395624-1396011
4233	1494	protein phosphatase 6, catalytic subunit	9.673	1407109-1407417
4255	1491	budding uninhibited by benzimidazoles 1 homolog, β (*S. cerevisiae*)	2.264	1414236-1414628
4266	1488	tumor susceptibility gene 101	23.4	1417992-1418306
4268	1487	STE20-related kinase adaptor β	1.082	1418669-1418996
4290	1482	mutL homolog 1 (*E. coli*)	5.514	1426359-1426686
4304	1480	KH domain containing, RNA binding, signal transduction associated 1	4.254	1431183-1431494
4339	1472	helicase, lymphoid specific	0.521	1442541-1442877
4380	1463	pelota homolog (*Drosophila*)	13.919	1456293-1456635
4414	1456	cyclin-dependent kinase 5	3.895	1467595-1467925
4476	1442	ring finger protein 8	3.436	1488202-1488477
4480	1441	cyclin B2	64.86	1489394-1489722
4491	1439	ADP-ribosylation factor-like 8A	11.733	1492911-1493304
4537	1428	dual specificity phosphatase 1	8.225	1507891-1508212
4554	1425	growth arrest and DNA-damage-inducible, β interacting protein 1	3.26	1513621-1513922
4632	1407	cell division cycle 7 (*S. cerevisiae*)	2.07	1539427-1539781
4685	1394	annexin A1	186.99	1557035-1557427
4702	1391	chromatin licensing and DNA replication factor 1	5.76	1563109-1563436
4728	1387	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	140.45	1571589-1571985
4729	1387	regulator of chromosome condensation 2	9.39	1571986-1572324
4732	1386	sirtuin 2 (silent mating type information regulation 2, homolog) (*S. cerevisiae*)	9.325	1573015-1573411

4747	1383	seven in absentia 1A	1.166	1578078-1578382
4775	1373	ecotropic viral integration site 5	1.536	1587335-1587660
4777	1373	zinc finger protein 830	2.475	1587986-1588305
4792	1371	protein phosphatase 1, catalytic subunit, α isoform	294.16	1593376-1593702
4811	1366	coiled-coil domain containing 99	1.214	1599899-1600288
4839	1360	cyclin-dependent kinase 4	100.24	1609522-1609852
4882	1350	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	0.584	1623871-1624266
4893	1348	vacuolar protein sorting 4a (yeast)	1.43	1627799-1628173
4897	1348	anaphase promoting complex subunit 7	3.347	1629252-1629559
4957	1336	transformation related p53	6.608	1649857-1650157
4969	1332	TGFβ-regulated gene 1	16.316	1654114-1654473
4976	1330	nucleoporin 214	0.854	1656631-1657026
4978	1330	homeo box B4	1.659	1657427-1657747
5039	1316	S-phase kinase-associated protein 2 (p45)	0.814	1678054-1678363
5104	1300	nuclear distribution gene C homolog (*Aspergillus*)	102.46	1699971-1700369
5201	1281	cyclin D-type binding-protein 1	14.126	1733399-1733721
5208	1279	nucleolar and spindle associated protein 1	2.386	1735724-1736042
5221	1275	growth arrest and DNA-damage-inducible 45 β	21.495	1740423-1740753
5268	1267	F-box protein 5	1.752	1756030-1756337
5277	1265	COP9 (constitutive photomorphogenic) homolog, subunit 5 (*Arabidopsis*)	22.12	1759189-1759545
5287	1263	nucleophosmin 1	155.72	1762731-1763125
5319	1255	chromatin modifying protein 1B	4.81	1773630-1773932
5357	1245	TGFβ1	13.689	1787146-1787456
5370	1243	HAUS augmin-like complex, subunit 7	59.234	1791926-1792280
5373	1242	H2A histone family, member X	35.377	1792920-1793310
5389	1239	high mobility group 20 B	18.123	1798703-1799083
5399	1238	RAN, member RAS Oncogene family	61.23	1802120-1802418
5401	1237	nucleoporin 37	8.371	1802806-1803091
5443	1227	CHK2 checkpoint homolog (*S. pombe*)	1.749	1817364-1817648
5448	1226	RIKEN cDNA F630043A04 gene	2.085	1819143-1819511
5459	1223	BRCA2 and CDKN1A interacting protein	22.32	1823218-1823604
5476	1218	cell division cycle 123 homolog (*S. cerevisiae*)	19.04	1829272-1829545
5513	1209	NIMA-related expressed kinase 1	0.751	1842362-1842733
5531	1204	DNA cross-link repair 1A, PSO2 homolog (*S. cerevisiae*)	0.722	1848560-1848902
5560	1198	forkhead box N3	0.714	1858644-1859006
5569	1196	nibrin	0.874	1861722-1862120
5580	1194	cell division cycle 2 homolog A (*S. pombe*)	43.513	1865374-1865693
5609	1188	F-box protein 31	1.331	1875647-1875991
5636	1182	mitogen-activated protein kinase 7	1.049	1885325-1885696
5653	1178	apoptosis antagonizing transcription factor	19.78	1891250-1891647
5667	1173	reprimo, TP53 dependent G2 arrest mediator candidate	2.891	1896225-1896560
5676	1171	cell growth regulator with ring finger domain 1	8.143	1899574-1899946
5694	1168	vascular endothelial growth factor B	11.40	1906017-1906367

5698	1166	aurora kinase A	16.86	1907469-1907831
5701	1166	telomeric repeat binding factor 1	2.789	1908582-1908967
5729	1161	MAD2 mitotic arrest deficient-like 2 (yeast)	23.02	1918682-1919036
5746	1157	caspase 3	11.813	1924836-1925195
5773	1151	protein tyrosine phosphatase 4a1	0.279	1934685-1935079
5774	1151	centrobin, centrosomal BRCA2 interacting protein	1.021	1935080-1935410
5787	1148	mitochondrial tumor suppressor 1	0.27	1939909-1940301
5828	1140	growth arrest and DNA-damage-inducible 45 α	21.77	1954514-1954899
5833	1139	cyclin H	10.28	1956302-1956671
5869	1132	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	13.76	1969649-1970047
5877	1129	E2F transcription factor 7	0.593	1972492-1972861
5881	1129	mediator of DNA damage checkpoint 1	0.237	1974024-1974400
5882	1129	calmodulin 2	263.81	1974401-1974748
5899	1124	cyclin E1	1.228	1980613-1981009
5902	1124	cell cycle related kinase	3.686	1981792-1982170
5927	1118	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	10.528	1990790-1991181
5933	1117	thioredoxin-like 4A	29.973	1993063-1993439
5997	1101	NUF2, NDC80 kinetochore complex component, homolog (*S. cerevisiae*)	1.166	2016390-2016751
6008	1100	DSN1, MIND kinetochore complex component, homolog (*S. cerevisiae*)	2.499	2020156-2020546
6049	1092	RIKEN cDNA 2610002M06 gene	2.988	2035042-2035392
6060	1089	cell division cycle associated 8	7.204	2039068-2039461
6065	1088	asp (abnormal spindle)-like, microcephaly associated (*Drosophila*)	0.54	2040964-2041345
6084	1083	bridging integrator 3	4.997	2047682-2048036
6119	1075	ankyrin repeat domain 54	3.785	2060520-2060872
6130	1072	proline/serine-rich coiled-coil 1	1.861	2064611-2064994
6141	1070	aurora kinase B	6.311	2068620-2068994
6153	1068	max binding protein	0.824	2073201-2073580
6173	1064	CDK2 (cyclin-dependent kinase 2)-associated protein 1	21.227	2079920-2080306
6246	1047	CDK5 and Abl enzyme substrate 1	0.472	2106649-2107036
6309	1031	CDK5 and Abl enzyme substrate 2	3.54	2128521-2128907
6318	1030	centrin 2	4.69	2131765-2132103
6434	1005	telomeric repeat binding factor 2	1.302	2173556-2173867
6480	992	cyclin-dependent kinase 6	1.042	2189891-2190242
6534	981	discs, large (*Drosophila*) homolog-associated protein 5	3.759	2208846-2209155
6553	976	RIKEN cDNA 2810433K01 gene	2.289	2215581-2215976
6574	973	checkpoint w/ forkhead & ring finger domains	0.59	2222821-2223198
6581	971	HAUS augmin-like complex, subunit 1	5.105	2225453-2225779
6647	960	Bmi1 polycomb ring finger oncogene	0.42	2248765-2249118
6664	956	par-6 (partitioning defective 6,) homolog α (*C. elegans*)	1.905	2254717-2255106
6669	955	*ras* homolog gene family, member U	0.296	2256530-2256915
6678	952	BCL2-antagonist/killer 1	3.0	2259855-2260161

6713	943	centrosomal protein 250	0.433	2271720-2272085
6714	942	centromere protein O	0.733	2272086-2272464
6729	939	kinesin family member 11	1.155	2277436-2277733
6782	929	nuclear distribution gene E homolog 1 (*A. nidulans*)	7.884	2295836-2296146
6812	922	forkhead box O4	1.102	2306279-2306609
6827	918	protein kinase inhibitor α	0.376	2311372-2311759
6833	917	septin 3	0.248	2313405-2313686
6882	908	aurora kinase C	14.22	2329723-2330035
6898	903	spindle assembly 6 homolog (*C. elegans*)	0.224	2334515-2334801
6909	902	septin 10	4.725	2337987-2338293
6952	892	timeless interacting protein	1.598	2352399-2352710
7003	880	neural precursor cell expressed, developmentally down-regulated gene 1	0.33	2369333-2369684
7010	877	proteasome (prosome, macropain) assembly chaperone 2	9.024	2371767-2372110
7115	858	centromere protein H	4.014	2406674-2407073
7126	852	vasohibin 1	0.138	2410493-2410795
7151	847	germ cell-specific gene 2	0.717	2418878-2419222
7158	845	c-fos induced growth factor	3.445	2420809-2421101
7159	845	MAD2 mitotic arrest deficient-like 1 (yeast)	8.32	2421102-2421467
7175	841	baculoviral IAP repeat-containing 5	0.966	2426437-2426713
7199	838	TGFβ3	1.124	2434410-2434754
7208	836	Leu rich repeat & coiled-coil domain containing 1	0.248	2437517-2437910
7216	834	suppressor of variegation 3-9 homolog 2 (*Drosophila*)	0.735	2440171-2440490
7224	833	NIMA (never in mitosis gene a)-related expressed kinase 4	0.325	2443004-2443301
7228	832	cell division cycle 25 homolog C (*S. pombe*)	1.85	2444341-2444625
7249	826	RIKEN cDNA 4922501C03 gene	0.438	2451461-2451761
7283	816	ribosomal protein S6	18.875	2462245-2462567
7291	813	HAUS augmin-like complex, subunit 2	3.496	2464662-2464966
7330	803	MAD2L1 binding protein	3.685	2477746-2478077
7365	796	cDNA sequence BC023882	0.603	2489301-2489640
7405	788	cell division cycle associated 3	10.276	2502489-2502808
7439	778	B-cell leukemia/lymphoma 2	0.149	2513854-2514170
7444	776	cell division cycle associated 2	0.252	2515580-2515941
7454	774	platelet derived growth factor, α	4.859	2518844-2519200
7551	749	expressed sequence C79407	0.187	2550344-2550743
7554	749	enhancer of rudimentary homolog (*Drosophila*)	3.142	2551508-2551815
7565	747	CDC28 protein kinase 1b	22.475	2555228-2555394
7603	738	SPC24, NDC80 kinetochore complex component, homolog (*S. cerevisiae*)	1.038	2567431-2567712
7630	732	serine/threonine kinase 11	0.449	2575716-2576017
7633	731	anaphase promoting complex subunit 10	0.787	2576622-2576942
7674	720	malignant T cell amplified sequence 1	1.817	2590027-2590422
7720	710	arginine vasopressin-induced 1	19.275	2605845-2606126
7756	700	Rap1 interacting factor 1 homolog (yeast)	0.083	2618117-2618471
7781	693	proviral integration site 1	0.392	2626615-2627001

7795	691	pituitary tumor-transforming gene 1	3.612	2631430-2631804
7803	689	breast cancer 2	0.07	2634236-2634594
7838	679	par-6 (partitioning defective 6) homolog β (*C. elegans*)	0.235	2645826-2646140
7841	678	NIMA (never in mitosis gene a)-related expressed kinase 3	0.716	2646895-2647246
7864	669	amyloid beta (A4) precursor protein-binding, family B, member 2	0.212	2654750-2655139
7879	666	cyclin-dependent kinase inhibitor 1A (P21)	3.252	2659502-2659871
7888	664	StAR-related lipid transfer (START) domain containing 13	0.12	2662630-2662978
7899	659	ADP-ribosylation factor-like 3	2.999	2666529-2666853
7957	644	RIKEN cDNA 2810452K22 gene	4.522	2686201-2686541
8038	618	polyamine-modulated factor 1	5.098	2712675-2712999
8046	615	cell division cycle associated 5	1.215	2715194-2715557
8079	606	ADP-ribosylation factor-like 2	4.584	2726337-2726723
8095	601	cyclin-dependent kinase inhibitor 1B	0.381	2731076-2731440
8100	601	E2F transcription factor 2	0.204	2732428-2732782
8123	594	citron	0.131	2740025-2740319
8155	587	sphingomyelin phosphodiesterase 3, neutral	0.179	2750331-2750645
8174	583	mitochondrial ribosomal protein L41	0.749	2755819-2756155
8176	583	dynactin 3	1.37	2756466-2756744
8209	573	CDC28 protein kinase regulatory subunit 2	0.994	2767357-2767753
8220	569	geminin	1.653	2770484-2770876
8281	552	ubiquitin-conjugating enzyme E2C	2.402	2790466-2790755
8283	551	SPC25, NDC80 kinetochore complex component, homolog (*S. cerevisiae*)	4.035	2791059-2791454
8300	548	MIS12 homolog (yeast)	0.199	2796988-2797361
8308	544	NSL1, MIND kinetochore complex component, homolog (*S. cerevisiae*)	0.644	2799438-2799722
8335	539	par-3 (partitioning defective 3) homolog (*C. elegans*)	0.154	2808716-2809107
8348	536	myeloid leukemia factor 1	0.454	2813229-2813621
8349	535	DNA-damage inducible transcript 3	4.982	2813622-2813956
8378	526	RIKEN cDNA 2610039C10 gene	2.336	2823614-2823897
8390	522	RAD50 homolog (*S. cerevisiae*)	0.102	2827676-2828022
8393	522	proline rich 5 (renal)	0.462	2828643-2828993
8398	520	cell division cycle 26	6.939	2830505-2830878
8402	519	ciliary rootlet coiled-coil, rootletin	0.102	2831925-2832268
8429	512	ligase IV, DNA, ATP-dependent	0.41	2841502-2841815
8594	478	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	2.07	2895015-2895359
8620	473	cyclin E2	0.159	2904183-2904530
8643	468	RIKEN cDNA 9130404D08 gene	0.284	2912093-2912444
8680	462	4HAUS augmin-like complex, subunit 8	1.099	2923769-2924049
8765	447	tet oncogene family member 2	0.115	2950714-2950987
8776	445	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor	1.068	2953967-2954306
8808	439	centrin 3	1.05	2963461-2963764
8817	437	S100 calcium binding protein A6 (calcyclin)	10.781	2966362-2966657

8877	422	thioredoxin-like 4B	0.561	2984204-2984528
8905	416	K(lysine) acetyltransferase 2B	0.097	2992304-2992627
8932	409	SAC3 domain containing 1	0.452	2999825-3000109
8933	409	ZW10 interactor	0.203	3000110-3000428
8954	403	junction-mediating and regulatory protein	0.09	3005715-3006035
8964	401	establishment of cohesion 1 homolog 2 (*S. cerevisiae*)	0.202	3008545-3008860
9019	388	BCL2-associated X protein	1.131	3023234-3023515
9107	363	growth arrest-specific 2 like 3	0.094	3045621-3045858
9152	352	anaphase promoting complex subunit 13	0.808	3056640-3056878
9161	349	RAB GTPase activating protein 1	0.083	3058415-3058689
9360	297	structural maintenance of chromosomes 4	0.073	3101933-3102112
9392	286	cyclin T1	0.131	3107706-3107919
9409	280	anaphase promoting complex subunit 11	2.158	3111178-3111374
9434	270	growth arrest specific 1	0.093	3115802-3115978
9477	255	shugoshin-like 1 (*S. pombe*)	0.109	3123096-3123285
9504	241	protein tyrosine phosphatase, receptor type, V	0.04	3127383-3127553
9512	239	G protein-coupled receptor 132	0.098	3128448-3128607
9665	179	regulator of G-protein signaling 2	0.059	3145905-3146047
9740	139	betacellulin, EGF family member	0.073	3150839-3150877
3157247	594	endoplasmic reticulum to nucleus signaling 1	0.18	3179284-3179383
3157294	353	family with sequence similarity 33, member A	0.31	3266905-3267004
3157319	402	HAUS augmin-like complex, subunit 5	0.183	3232617-3232716
3157349	680	NA	7.561	3271596-3271695
3157464	268	TMF1-regulated nuclear protein 1	0.324	3280149-3280248
3157487	308	NIMA (never in mitosis gene a)-related expressed kinase 11	0.493	3167184-3167283
3157523	803	centromere protein V	3.696	3267205-3267304
3157530	446	adenylate kinase 1	0.22	3212658-3212757
3157631	3098	establishment of cohesion 1 homolog 1 (*S. cerevisiae*)	4.952	3198571-3198670
3157646	403	hepatic nuclear factor 4, α	0.092	3262105-3262204
3157712	3480	structural maintenance of chromosomes 2	1.498	3189471-3189570
3157780	1357	PEST proteolytic signal containing nuclear protein	2.21	3191171-3191270
3157798	765	speedy homolog A (*Xenopus*)	0.756	3226217-3226316
3157809	1876	NA	0.817	3262505-3262604
3157812	168	sestrin 2	0.071	3260905-3261004
3157837	1573	caspase 8 associated protein 2	0.5	3184971-3185070
3157862	2352	retinoblastoma-like 1 (p107)	2.545	3265505-3265604
3157928	393	NA	0.096	3213958-3214057
3157931	840	podoplanin	8.076	3202997-3203096
3157962	4088	NA	15.347	3158921-3159020
3157993	162	epidermal growth factor receptor	0.048	3166784-3166883
3158035	383	septin 1	0.279	3259205-3259304
3158037	432	phospholipase A2, group XVI	0.124	3230917-3231016
3158121	3735	p53-inducible nuclear protein 1	2.567	3197071-3197170
3158132	612	RIKEN cDNA 4632434I11 gene	0.26	3275096-3275195
3158184	776	calcium/calmodulin-dependent protein kinase II α	0.183	3257905-3258004
3158209	1954	fibroblast growth factor receptor 2	2.109	3207458-3207557

3158213	203	deleted in bladder cancer 1 (human)	0.113	3194971-3195070
3158218	4830	NA	2.47	3259005-3259104
3158295	426	stratifin	0.681	3216091-3216190
3158307	369	placental growth factor	0.923	3227817-3227916
3158328	1531	RAB11 family interacting protein 3 (class II)	0.975	3221991-3222090

Table 14. Apoptosis (Chinese hamster)				
SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
16	4536	homeodomain interacting protein kinase 1	5.166	14439-14801
21	4379	feminization 1 homolog b (*C. elegans*)	5.83	15971-16283
31	4290	nuclear receptor subfamily 3, group C, member 1	6.926	19057-19428
44	4201	SH3-domain kinase binding protein 1	6.615	23443-23756
73	3972	cell adhesion molecule 1	13.147	32944-33332
102	3754	neurofibromatosis 1	1.523	42422-42742
104	3746	PHD finger protein 17	2.772	43019-43313
111	3699	intersectin 1 (SH3 domain protein 1A)	3.481	45218-45546
131	3611	mitogen-activated protein kinase 9	5.629	51635-51907
170	3445	RING1 and YY1 binding protein	15.89	63269-63644
189	3384	phosphatase and tensin homolog	0.633	69091-69404
199	3345	protein kinase C, α	2.25	72112-72439
204	3339	sphingosine phosphate lyase 1	2.842	73601-73949
205	3337	unc-5 homolog B (*C. elegans*)	15.951	73950-74213
218	3290	alanyl-tRNA synthetase	25.07	77662-77970
243	3224	Fas-associated factor 1	10.626	85018-85295
266	3179	vascular endothelial growth factor A	18.713	92246-92594
272	3152	Rho-associated coiled-coil containing protein kinase 1	3.17	94052-94292
279	3139	methyl CpG binding protein 2	1.23	95910-96141
293	3127	SAFB-like, transcription modulator	10.672	100152-100477
300	3115	nischarin	3.465	102105-102309
345	3034	glycogen synthase kinase 3 β	0.647	114424-114743
366	3003	cullin 1	25.78	120499-120798
375	2989	RAD21 homolog (*S. pombe*)	34.322	123260-123508
384	2977	transcriptional regulator, SIN3A (yeast)	3.56	125791-126119
386	2976	cytotoxic granule-associated RNA binding protein 1	1.496	126356-126593
390	2967	tumor necrosis factor receptor superfamily, member 21	22.566	127481-127779
394	2960	apoptosis inhibitor 5	2.055	128748-129043
419	2931	dedicator of cytokinesis 1	4.621	135539-135925
431	2919	Tia1 cytotoxic granule-associated RNA binding protein-like 1	12.569	139041-139241
434	2913	mitogen-activated protein kinase kinase kinase 7	2.174	139905-140195
511	2835	hypoxia inducible factor 1, α subunit	6.799	161268-161478
525	2821	BCL2-like 13 (apoptosis facilitator)	7.089	165351-165590
540	2799	signal transducer and activator of	1.323	169415-169753

		transcription 5B		
543	2791	Janus kinase 2	4.149	170408-170768
562	2773	uveal autoantigen with coiled-coil domains and ankyrin repeats	14.96	175535-175851
609	2735	activity-dependent neuroprotective protein	6.52	189603-189839
621	2718	catenin	30.996	192742-193116
670	2686	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	4.948	208013-208351
710	2658	TNF receptor-associated factor 3	1.284	219648-219935
729	2640	homeodomain interacting protein kinase 3	0.784	225660-225908
732	2639	transforming growth factor, β receptor I	4.064	226652-227037
825	2573	amyloid β (A4) precursor protein	165.22	255412-255644
867	2552	sphingomyelin synthase 1	9.259	268366-268630
891	2542	RB1-inducible coiled-coil 1	2.069	275944-276175
899	2538	p53-binding protein 2	2.893	278604-278960
901	2538	zinc finger matrin type 3	0.701	279250-279506
913	2532	proteaseome (prosome, macropain) 28 subunit, 3	21.397	283197-283568
933	2523	phosphatidylinositol 3-kinase, catalytic, α polypeptide	1.26	290027-290396
994	2485	Tax1 (human T-cell leukemia virus type I) binding protein 1	26.472	310231-310562
1001	2480	myeloid cell leukemia sequence 1	11.498	312684-312913
1046	2453	TNF receptor-associated factor 7	17.763	327682-328074
1070	2440	promyelocytic leukemia	1.141	335490-335874
1096	2428	synovial apoptosis inhibitor 1, synoviolin	3.957	344178-344523
1116	2418	mutS homolog 6 (*E. coli*)	11.162	350996-351268
1121	2417	ubiquitin-conjugating enzyme E2Z (putative)	3.951	352601-352956
1230	2367	mitogen-activated protein kinase 8	0.908	388975-389185
1237	2364	rabaptin, RAB GTPase binding effector protein 1	1.86	391313-391594
1285	2341	D4, zinc and double PHD fingers family 2	14.055	407477-407781
1286	2340	RNA binding motif protein 5	6.953	407782-408116
1329	2323	adenomatosis polyposis coli	0.997	422123-422508
1340	2318	GRAM domain containing 4	3.878	426012-426332
1381	2294	Vac14 homolog (*S. cerevisiae*)	7.275	440226-440553
1386	2293	serine incorporator 3	64.3	441950-442265
1398	2290	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 α)	0.933	445880-446276
1430	2274	ring finger protein 216	2.663	456856-457171
1458	2262	Alstrom syndrome 1 homolog (human)	0.712	466342-466731
1468	2259	HLA-B-associated transcript 3	18.8	469774-470120
1469	2258	RIKEN cDNA 5730403B10 gene	2.351	470121-470460
1491	2250	BCL2-like 2	6.539	477629-477999
1520	2239	optic atrophy 1 homolog (human)	2.52	487010-487405
1547	2230	mitogen-activated protein kinase 8 interacting protein 1	5.814	496124-496454
1561	2227	autophagy/beclin 1 regulator 1	1.709	500806-501161
1572	2221	glutaminyl-tRNA synthetase	17.276	504769-505049
1596	2209	Kruppel-like factor 11	2.24	512866-513206

1610	2205	ankyrin 2, brain	0.639	517928-518264
1615	2204	interleukin-1 receptor-associated kinase 2	7.953	519606-519900
1617	2203	BCL2/adenovirus E1B interacting protein 3-like	4.764	520293-520639
1623	2201	v-raf-leukemia viral Oncogene 1	11.737	522454-522805
1625	2201	carbohydrate sulfotransferase 11	1.436	523162-523531
1640	2194	p21 protein (Cdc42/Rac)-activated kinase 2	12.908	528351-528713
1670	2185	GATA zinc finger domain containing 2A	6.186	538783-539093
1681	2181	brain derived neurotrophic factor	1.421	542519-542783
1745	2159	huntingtin interacting protein 1	2.993	564571-564954
1767	2154	programmed cell death 6 interacting protein	24.67	572196-572546
1793	2146	thymoma viral proto-oncogene 1	55.121	581286-581643
1807	2140	prion protein	10.293	586022-586407
1814	2138	autophagy-related 7 (yeast)	3.031	588504-588828
1828	2136	matrix metallopeptidase 9	16.33	593202-593492
1829	2135	amyloid beta (A4) precursor protein-binding, family B, member 1	13.93	593493-593882
1849	2128	NIMA (never in mitosis gene a)-related expressed kinase 6	11.135	600327-600624
1866	2123	huntingtin	0.879	606012-606402
1913	2108	apoptosis-inducing factor, mitochondrion-associated 1	114.54	621815-622188
1925	2104	DnaJ (Hsp40) homolog, subfamily A, member 3	15.15	625909-626254
1943	2097	chromodomain helicase DNA binding protein 8	3.526	631928-632323
1963	2088	tumor necrosis factor receptor superfamily, member 1b	0.748	638890-639228
1967	2087	serum/glucocorticoid regulated kinase 1	4.001	640401-640729
1971	2086	Scl/Tal1 interrupting locus	0.813	641737-642110
1974	2086	lymphotoxin B receptor	20.795	642821-643161
1985	2083	serine/threonine kinase 4	2.64	646540-646922
2003	2079	X-ray repair complementing defective repair in CHO cells 5	5.752	652584-652920
2017	2073	myocyte enhancer factor 2D	8.208	657055-657357
2021	2072	B-cell translocation gene 2, anti-proliferative	5.326	658375-658645
2024	2071	K(lysine) acetyltransferase 2A	4.934	659254-659597
2040	2064	STE20-like kinase (yeast)	10.30 6	664580-664973
2050	2061	engulfment and cell motility 2, ced-12 homolog (*C. elegans*)	7.176	668000-668354
2054	2059	phosphoprotein enriched in astrocytes 15A	14.429	669292-669690
2080	2049	CLPTM1-like	89.279	678524-678834
2083	2049	ADP-ribosylation factor 6	4.368	679540-679784
2124	2034	ras homolog gene family, member A	135.6 12	693012-693333
2139	2030	multiple endocrine neoplasia 1	2.911	698091-698430
2185	2015	myelocytomatosis oncogene	119.45	713438-713745
2193	2012	THO complex 1	2.149	716160-716525

2199	2011	autophagy-related 5 (yeast)	7.623	718183-718508
2227	2003	smoothened homolog (*Drosophila*)	2.634	727770-728086
2230	2002	BCL2-like 1	9.446	728838-729216
2242	1999	sequestosome 1	51.17	733070-733459
2252	1996	mitogen-activated protein kinase 5	1.378	736639-737018
2283	1987	MAP-kinase activating death domain	1.589	747015-747324
2284	1987	TNF receptor associated factor 4	7.889	747325-747659
2305	1982	thymoma viral proto-oncogene 1 interacting protein	21.705	754612-754878
2364	1967	protein disulfide isomerase associated 3	173.82	774355-774677
2367	1966	TSC22 domain family, member 3	5.809	775361-775690
2400	1959	phosphofurin acidic cluster sorting protein 2	2.811	786380-786716
2403	1958	DnaJ (Hsp40) homolog, subfamily C, member 5	5.417	787385-787676
2450	1945	receptor (TNFRSF)-interacting serine-threonine kinase 1	0.965	803414-803712
2471	1940	mutS homolog 2 (*E. coli*)	6.134	810424-810813
2496	1934	Kv channel interacting protein 3, calsenilin	1.03	819220-819569
2515	1929	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	22.655	825796-826120
2547	1921	cell division cycle and apoptosis regulator 1	1.757	836705-837044
2599	1908	tripartite motif-containing 39	1.032	854385-854718
2608	1906	E2F transcription factor 1	7.007	857154-857487
2660	1891	TGFβ-regulated gene 4	10.934	874486-874847
2668	1890	apoptotic chromatin condensation inducer 1	3.906	877244-877643
2670	1890	BCL2-associated athanogene 3	5.061	878043-878361
2691	1887	growth arrest specific 2	2.282	885284-885579
2749	1871	protein phosphatase 1, regulatory (inhibitor) subunit 13B	2.369	905145-905540
2790	1859	excision repair cross-complementing rodent repair deficiency, complementation group 2	1.408	919056-919386
2811	1854	retinoic acid receptor, gamma	2.638	926437-926742
2815	1854	serine/threonine kinase 3 (Ste20, yeast homolog)	4.084	927749-928072
2831	1851	aldehyde dehydrogenase family 1, subfamily A1	40.058	933071-933460
2838	1850	catenin, beta like 1	20.124	935528-935906
2848	1847	RAD9 homolog (S. pombe)	13.395	938950-939251
2904	1829	breast cancer 1	7.497	958124-958436
2965	1810	protein kinase, DNA activated, catalytic polypeptide	0.793	979242-979576
3042	1782	sphingosine-1-phosphate phosphatase 1	3.922	1005199-1005578
3054	1777	death effector domain-containing	1.323	1009277-1009659
3073	1775	vanin 1	20.503	1015567-1015901
3078	1773	zinc finger CCCH type containing 12A	3.152	1017198-1017589
3094	1769	TRAF3 interacting protein 2	4.391	1022836-1023187
3096	1769	MKL (megakaryoblastic leukemia)/myocardin-like 1	1.041	1023531-1023903
3234	1738	FAST kinase domains 5	2.617	1071097-1071485
3268	1729	B-cell leukemia/lymphoma 6	8.467	1082762-1083124

3273	1726	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	62.681	1084449-1084755
3275	1726	ubiquitin-conjugating enzyme E2B, RAD6 homology (*S. cerevisiae*)	13.78	1085017-1085315
3289	1721	menage a trois 1	12.96	1089606-1089959
3300	1718	TNF receptor-associated factor 5	3.925	1093396-1093770
3324	1712	poly-U binding splicing factor 60	14.514	1101460-1101740
3326	1711	RIKEN cDNA 1200009F10 gene	3.501	1102067-1102381
3338	1709	NEDD8 activating enzyme E1 subunit 1	9.826	1106097-1106429
3342	1708	phosphatidylinositol glycan anchor biosynthesis, class T	24.872	1107410-1107750
3358	1704	DNA-damage regulated autophagy modulator 1	2.146	1112807-1113187
3382	1699	major facilitator superfamily domain containing 10	17.753	1121263-1121574
3390	1696	cell division cycle 2-like 1	17.014	1124002-1124331
3419	1688	bladder cancer associated protein homolog (human)	4.537	1133723-1134082
3448	1680	family with sequence similarity 188, member A	2.812	1143475-1143791
3499	1670	SAP30 binding protein	3.008	1160338-1160643
3524	1664	integral membrane protein 2B	103.29	1168940-1169261
3540	1661	superoxide dismutase 2, mitochondrial	2.559	1174163-1174529
3559	1654	SKI-like	1.243	1180446-1180768
3651	1633	FK506 binding protein 8	53.498	1211464-1211841
3654	1632	glutamate-cysteine ligase, catalytic subunit	12.64	1212479-1212769
3685	1627	HtrA serine peptidase 2	11.095	1222907-1223252
3692	1625	family with sequence similarity 82, member A2	4.761	1225295-1225616
3693	1624	BCL2-associated athanogene 5	26.647	1225617-1225987
3695	1623	pleiomorphic adenoma gene-like 2	0.74	1226344-1226650
3705	1622	seven in absentia 2	1.664	1229814-1230210
3710	1621	voltage-dependent anion channel 1	35.606	1231561-1231854
3736	1616	cullin 7	1.583	1240268-1240610
3749	1612	ADAMTS-like 4	2.67	1244700-1245081
3761	1609	ataxia telangiectasia mutated homolog (human)	0.181	1248864-1249255
3776	1605	death associated protein 3	18.724	1253963-1254317
3787	1601	transcription factor Dp 1	6.434	1257788-1258139
3806	1595	adenosine deaminase	19.88	1264324-1264663
3837	1587	modulator of apoptosis 1	2.395	1274626-1274921
3855	1584	activating transcription factor 5	9.537	1280625-1280989
3913	1571	signal transducer and activator of transcription 5A	1.268	1299843-1300222
3926	1567	clusterin	40.878	1304084-1304407
3983	1553	RAS p21 protein activator 1	0.463	1323060-1323449
3994	1550	caspase recruitment domain family, member 10	3.045	1326706-1327065
4014	1545	protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	82.16 2	1333415-1333732

4041	1540	presenilin 1	3.007	1342545-1342881
4052	1537	BCL2-associated athanogene 4	0.353	1346314-1346657
4085	1525	RELT tumor necrosis factor receptor	2.067	1356880-1357195
4090	1525	zinc finger, C3HC type 1	17.03	1358571-1358886
4106	1522	TNF receptor-associated factor 2	7.2	1363971-1364287
4128	1517	programmed cell death 11	1.078	1371711-1372000
4152	1512	cytokine induced apoptosis inhibitor 1	6.495	1379554-1379805
4165	1510	nuclear receptor subfamily 4, group A, member 1	3.433	1383906-1384203
4166	1509	bifunctional apoptosis regulator	2.213	1384204-1384477
4199	1500	cytoskeleton associated protein 2	1.674	1395624-1396011
4201	1500	eukaryotic translation initiation factor 2 alpha kinase 3	2.46	1396283-1396617
4202	1500	intraflagellar transport 57 homolog (*Chlamydomonas*)	4.102	1396618-1396929
4247	1492	B-cell receptor-associated protein 29	2.19	1411569-1411898
4250	1492	caspase 9	1.769	1412589-1412860
4252	1491	RRN3 RNA polymerase I transcription factor homolog (yeast)	2.225	1413234-1413535
4255	1491	budding uninhibited by benzimidazoles 1 homolog, β(*S. cerevisiae*)	2.264	1414236-1414628
4268	1487	STE20-related kinase adaptor beta	1.082	1418669-1418996
4275	1486	FAST kinase domains 2	4.522	1421149-1421474
4290	1482	mutL homolog 1 (E. coli)	5.514	1426359-1426686
4322	1476	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 2 (p85 beta)	3.629	1436979-1437294
4325	1476	eukaryotic translation elongation factor 1 α 2	3.269	1437945-1438305
4327	1475	Notch gene homolog 2 (Drosophila)	0.347	1438663-1438970
4339	1472	helicase, lymphoid specific	0.521	1442541-1442877
4348	1470	Ras-related GTP binding A	46.31	1445616-1445968
4379	1464	SH3-domain GRB2-like B1 (endophilin)	13.153	1455957-1456292
4383	1463	tripartite motif-containing 35	1.003	1457309-1457624
4414	1456	cyclin-dependent kinase 5	3.895	1467595-1467925
4421	1455	ring finger protein 34	7.18	1469632-1469965
4433	1453	reticulon 4	53.172	1473726-1474051
4434	1453	protein kinase, interferon inducible double stranded RNA dependent activator	5.527	1474052-1474353
4461	1446	DNA-damage-inducible transcript 4	3.353	1483293-1483590
4478	1441	CCAAT/enhancer binding protein (C/EBP), β	11.321	1488766-1489110
4504	1435	polycomb group ring finger 2	3.603	1496993-1497354
4515	1433	ceroid lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease)	2.904	1500552-1500853
4525	1431	GATA binding protein 6	1.073	1503752-1504126
4568	1422	WW domain-containing oxidoreductase	2.113	1518412-1518773
4594	1416	transmembrane BAX inhibitor motif containing 6	16.969	1527288-1527665
4606	1414	cold shock domain protein A	171.461	1531366-1531649
4642	1405	shisa homolog 5 (Xenopus laevis)	11.181	1542721-1542999
4668	1399	testis expressed gene 261	22.005	1551436-1551738
4682	1396	protein phosphatase 1, regulatory	1.002	1556095-1556385

		(inhibitor) subunit 13 like		
4705	1391	pleckstrin homology-like domain, family A, member 3	17.062	1564100-1564401
4744	1384	fibroblast growth factor receptor 1	0.422	1577052-1577365
4747	1383	seven in absentia 1A	1.166	1578078-1578382
4764	1376	jumonji domain containing 6	14.926	1583786-1584134
4831	1362	integrator complex subunit 1	1.012	1606795-1607183
4859	1355	myocyte enhancer factor 2A	0.685	1616416-1616715
4904	1346	FAST kinase domains 3	2.435	1631671-1632058
4905	1346	mitochondrial carrier homolog 1 (C. elegans)	54.765	1632059-1632447
4912	1345	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	3.151	1634477-1634773
4929	1342	tectonic family member 3	1.25	1640228-1640526
4941	1339	catenin (cadherin associated protein), β1	0.495	1644372-1644747
4944	1339	B-cell leukemia/lymphoma 10	9.013	1645462-1645856
4952	1337	tribbles homolog 3 (Drosophila)	7.419	1648199-1648515
4953	1337	mitochondrial ubiquitin ligase activator of NFKB 1	2.065	1648516-1648850
4957	1336	transformation related protein 53	6.608	1649857-1650157
4963	1334	amyloid β(A4) precursor protein-binding, family B, member 3	1.378	1651979-1652331
4993	1326	collagen, type XVIII, α1	0.529	1662476-1662775
5016	1320	RIKEN cDNA 4930453N24 gene	3.737	1670187-1670553
5046	1313	deoxyribonuclease II α	31.897	1680451-1680725
5047	1313	estrogen receptor-binding fragment-associated gene 9	21.721	1680726-1681024
5056	1311	BCL2-associated athanogene 1	11.445	1683576-1683895
5079	1304	baculoviral IAP repeat-containing 3	7.2	1691584-1691970
5081	1303	family with sequence similarity 176, member A	3.606	1692345-1692702
5111	1298	brain & reproductive organ-expressed protein	57.864	1702336-1702627
5112	1297	tumor necrosis factor, α-induced protein 8	5.97	1702628-1702979
5120	1295	eukaryotic translation initiation factor 5A	661.40	1705373-1705736
5131	1292	presenilin 2	2.55	1709139-1709525
5139	1291	BCL2 binding component 3	1.503	1712045-1712425
5140	1291	WD repeat domain 92	1.995	1712426-1712738
5168	1285	sphingosine kinase 2	1.151	1721818-1722158
5174	1285	death inducer-obliterator 1	1.104	1723982-1724350
5221	1275	growth arrest & DNA-damage-inducible 45 β	21.495	1740423-1740753
5222	1275	BCL2/adenovirus E1B interacting protein 3	9.252	1740754-1741152
5260	1269	receptor (TNFRSF)-interacting serine-threonine kinase 2	2.702	1753377-1753673
5295	1259	nuclear receptor subfamily 4, group A, member 2	0.73	1765734-1766070
5318	1255	DNA fragmentation factor, beta subunit	1.315	1773243-1773629
5328	1252	rhotekin	2.833	1776824-1777199
5357	1245	transforming growth factor, beta 1	13.689	1787146-1787456
5379	1240	baculoviral IAP repeat-containing 2	1.473	1795149-1795509
5479	1217	cytochrome c, somatic	5.321	1830214-1830597
5501	1212	microphthalmia-associated transcription factor	0.4	1838060-1838448
5506	1211	craniofacial development protein 1	17.159	1839938-1840310

5512	1210	pleckstrin homology-like domain, family A, member 1	6.85	1841998-1842361
5532	1204	B-cell receptor-associated protein 31	116.56	1848903-1849265
5537	1202	angiopoietin-like 4	0.987	1850651-1851035
5550	1200	disintegrin & metallopeptidase domain 17	1.374	1855220-1855596
5567	1197	X-linked inhibitor of apoptosis	0.297	1861051-1861417
5568	1197	ring finger protein 130	5.397	1861418-1861721
5589	1192	Werner syndrome homolog (human)	0.711	1868474-1868871
5608	1188	caspase 12	0.856	1875252-1875646
5633	1182	Harvey rat sarcoma virus oncogene 1	4.391	1884273-1884616
5636	1182	mitogen-activated protein kinase 7	1.049	1885325-1885696
5641	1180	death effector domain-containing DNA binding protein 2	1.463	1887105-1887480
5649	1178	STAM binding protein	2.283	1889758-1890088
5663	1175	CASP8 & FADD-like apoptosis regulator	4.448	1894743-1895132
5671	1173	programmed cell death 7	1.96	1897662-1898025
5711	1164	leucine-rich & death domain containing	2.507	1912080-1912460
5746	1157	caspase 3	11.813	1924836-1925195
5771	1151	TNFRSF1A-associated via death domain	11.061	1934043-1934332
5775	1151	cell death-inducing DFFA-like effector c	55.287	1935411-1935807
5791	1147	microtubule-associated protein 1S	6.328	1941401-1941793
5844	1137	BCL2-like 11 (apoptosis facilitator)	0.584	1960442-1960764
5854	1136	caspase 1	2.306	1964106-1964500
5862	1133	zinc finger, DHHC domain containing 16	4.4	1967129-1967439
5883	1129	X-ray repair complementing defective repair in CHO cells 4	6.458	1974749-1975138
5906	1123	sphingosine kinase 1	15.987	1983308-1983651
5931	1117	Fas death domain-associated protein	2.242	1992296-1992675
5946	1115	diablo homolog (Drosophila)	10.353	1997873-1998247
5968	1110	amiloride-sensitive cation channel 1, neuronal (degenerin)	1.444	2005882-2006234
5987	1104	ceroid-lipofuscinosis, neuronal 8	0.372	2012719-2013104
6050	1092	Sp110 nuclear body protein	2.119	2035393-2035780
6051	1092	phosducin-like 3	12.75	2035781-2036142
6055	1091	LPS-induced TN factor	4.202	2037259-2037644
6056	1091	programmed cell death 6	53.378	2037645-2037947
6153	1068	max binding protein	0.824	2073201-2073580
6165	1066	G2/M-phase specific E3 ubiquitin ligase	0.358	2077605-2078002
6185	1062	aminoacyl tRNA synthetase complex-interacting multifunctional protein 1	33.092	2084323-2084687
6223	1053	glutamate-Cys ligase, modifier subunit	22.216	2098389-2098782
6239	1049	myocyte enhancer factor 2C	0.524	2104360-2104732
6252	1045	TM2 domain containing 1	2.155	2108754-2109139
6273	1039	nerve growth factor	9.393	2115896-2116286
6295	1034	forkhead box C1	0.44	2123858-2124256
6305	1031	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	0.52	2127434-2127800
6312	1030	programmed cell death 2	6.216	2129627-2130022
6369	1020	programmed cell death 4	0.953	2150178-2150561
6396	1013	DNA fragmentation factor, alpha subunit	2.45	2159909-2160294
6445	1003	aminoacyl tRNA synthetase complex-	12.784	2177473-2177849

		interacting multifunctional protein 2		
6481	991	polymerase (DNA directed), beta	1.632	2190243-2190641
6522	984	endonuclease G	23.832	2204505-2204896
6557	975	B-cell CLL/lymphoma 7C	6.467	2216903-2217199
6572	973	transcription factor 7, T-cell specific	1.383	2222093-2222467
6624	964	tumor necrosis factor receptor superfamily, member 12a	58.921	2240612-2240962
6644	960	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	0.16	2247664-2248042
6647	960	Bmi1 polycomb ring finger oncogene	0.42	2248765-2249118
6678	952	BCL2-antagonist/killer 1	3	2259855-2260161
6686	950	apoptotic peptidase activating factor 1	0.325	2262408-2262743
6710	944	BCL2/adenovirus E1B interacting protein 2	15.617	2270556-2270934
6736	938	TNF receptor-associated factor 1	1.035	2279878-2280163
6786	928	steroid receptor RNA activator 1	9.006	2297190-2297589
6798	926	caspase 7	0.436	2301618-2301960
6804	924	GLI-Kruppel family member GLI2	0.489	2303659-2303992
6806	924	purine-nucleoside phosphorylase 1	10.99	2304356-2304474
6807	923	tumor necrosis factor receptor superfamily, member 1a	2.901	2304475-2304854
6813	922	TNF, α-induced protein 3	0.517	2306610-2306966
6830	918	interleukin 19	4.282	2312386-2312719
6858	913	nucleotide-binding oligomerization domain containing 2	0.461	2322123-2322429
6866	911	GLI-Kruppel family member GLI3	1.434	2324663-2324995
6958	890	BCL2-like 12 (proline rich)	18.291	2354097-2354391
6975	887	yippee-like 3 (Drosophila)	1.989	2359942-2360263
7010	877	proteasome (prosome, macropain) assembly chaperone 2	9.024	2371767-2372110
7015	877	TNF (ligand) superfamily, member 12	4.328	2373485-2373776
7067	866	HIV-1 tat interactive protein 2, homolog (human)	7.75	2391070-2391405
7082	863	pleckstrin homology domain containing, family F (with FYVE domain) member 1	2.804	2395849-2396175
7092	861	sirtuin 1 (silent mating type information regulation 2, homolog) 1 (*S. cerevisiae*)	0.22	2399178-2399470
7120	855	caspase 6	4.965	2408466-2408843
7124	853	homeodomain interacting protein kinase 2	0.328	2409808-2410107
7144	849	serum/glucocorticoid regulated kinase 3	0.553	2416403-2416787
7167	843	fibroblast growth factor receptor 3	0.243	2423777-2424112
7175	841	baculoviral IAP repeat-containing 5	0.966	2426437-2426713
7187	839	nucleotide-binding oligomerization domain containing 1	0.746	2430407-2430803
7196	838	transformation related protein 63	0.317	2433439-2433750
7199	838	transforming growth factor, beta 3	1.124	2434410-2434754
7209	836	ras homolog gene family, member B	0.721	2437911-2438277
7213	835	glutathione peroxidase 1	10.976	2439217-2439612
7244	828	cysteine-serine-rich nuclear protein 2	0.278	2449829-2450156
7283	816	ribosomal protein S6	18.875	2462245-2462567
7297	811	TNF receptor-associated factor 6	1.188	2466579-2466938
7320	807	C1D nuclear receptor co-repressor	0.376	2474231-2474564

7349	800	nucleolar protein 3 (apoptosis repressor with CARD domain)	2.282	2484064-2484342
7374	794	ceroid-lipofuscinosis, neuronal 5	1.261	2492286-2492578
7418	785	myeloid differentiation primary response gene 116	2.514	2506840-2507215
7424	782	RIKEN cDNA 1110007C09 gene	3.301	2508903-2509183
7426	782	engulfment and cell motility 1, ced-12 homolog (*C. elegans*)	0.528	2509515-2509793
7439	778	B-cell leukemia/lymphoma 2	0.149	2513854-2514170
7484	767	UDP-Gal:βGlcNAc β1,4-galactosyltransferase, polypeptide 1	0.387	2528454-2528763
7498	765	sodium channel, voltage-gated, type II, α1	0.184	2533197-2533494
7504	763	interferon activated gene 204	9.678	2535305-2535372
7506	762	apoptosis enhancing nuclease	1.126	2535692-2536051
7528	756	transmembrane protein 85	25.649	2543334-2543651
7579	744	etoposide induced 2.4 mRNA	0.629	2559503-2559877
7584	743	apoptosis-inducing factor, mitochondrion-associated 2	0.88	2561258-2561555
7596	740	tumor protein, translationally-controlled 1	10.23	2565288-2565685
7631	732	methyl-CpG binding domain protein 4	0.522	2576018-2576339
7651	725	BH3 interacting domain death agonist	13.705	2582517-2582823
7664	723	distal-less homeobox 1	0.37	2586625-2586998
7672	721	xeroderma pigmentosum, complementation group A	1.337	2589348-2589735
7675	720	eukaryotic translation elongation factor 1 ε1	1.403	2590423-2590793
7689	717	BCL2/adenovirus E1B interacting protein 1	1.953	2595342-2595666
7749	702	peroxiredoxin 2	15.903	2616024-2616366
7784	693	Ser/Thr kinase 17b (apoptosis-inducing)	0.603	2627742-2628087
7794	691	giant axonal neuropathy	0.587	2631132-2631429
7803	689	breast cancer 2	0.07	2634236-2634594
7864	669	amyloid beta (A4) precursor protein-binding, family B, member 2	0.212	2654750-2655139
7879	666	cyclin-dependent kinase inhibitor 1A (P21)	3.252	2659502-2659871
7880	666	protein phosphatase 1F (PP2C domain containing)	2.902	2659872-2660259
7924	652	excision repair cross-complementing rodent repair deficiency, complementation group 1	21.84	2675071-2675432
7978	639	BCL2 modifying factor	0.17	2692923-2693205
8013	630	TCF3 (E2A) fusion partner	4.464	2704602-2704917
8026	624	CASP2 and RIPK1 domain containing adaptor with death domain	1.176	2709036-2709355
8030	623	ring finger and FYVE like domain containing protein	0.192	2710236-2710629
8056	612	caspase 2	1.166	2718675-2719039
8065	610	testis expressed gene 11	0.205	2721707-2721990
8095	601	cyclin-dependent kinase inhibitor 1B	0.381	2731076-2731440
8100	601	E2F transcription factor 2	0.204	2732428-2732782
8116	595	inhibitor of DNA binding 1	1.398	2737742-2738071
8119	595	serglycin	9.946	2738723-2739031
8133	591	defender against cell death 1	4.551	2742894-2743239
8174	583	mitochondrial ribosomal protein L41	0.749	2755819-2756155

8191	580	RIKEN cDNA 2810002N01 gene	1.368	2761213-2761609
8218	570	interleukin 18	2.856	2769797-2770097
8241	562	BCL2-associated athanogene 2	1.083	2776948-2777283
8282	551	programmed cell death 5	3.991	2790756-2791058
8328	540	FAST kinase domains 1	0.298	2806153-2806512
8345	536	Fas (TNF receptor superfamily member 6)	0.501	2812206-2812506
8349	535	DNA-damage inducible transcript 3	4.982	2813622-2813956
8369	530	superoxide dismutase 1, soluble	9.577	2820605-2820925
8381	524	nuclear protein 1	26.14	2824647-2825002
8386	523	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 13	1.74	2826135-2826503
8429	512	ligase IV, DNA, ATP-dependent	0.41	2841502-2841815
8473	502	programmed cell death 10	0.375	2855519-2855901
8508	493	serine (or cysteine) peptidase inhibitor, clade B, member 9	0.146	2867128-2867490
8543	488	NLR family, apoptosis inhibitory protein 1	0.091	2878738-2879123
8562	484	calcium and integrin binding 1 (calmyrin)	2.049	2884444-2884809
8595	478	death-associated protein	6.602	2895360-2895710
8608	475	BCL2-interacting killer	1.02	2899985-2900289
8633	470	SIVA1, apoptosis-inducing factor	2.357	2908717-2909086
8662	464	death-associated protein kinase 3	0.33	2918007-2918383
8746	450	tumor necrosis factor receptor superfamily, member 4	0.392	2944708-2945036
8762	448	RIKEN cDNA 1700020C11 gene	0.321	2949726-2950061
8776	445	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor	1.068	2953967-2954306
8785	442	zinc finger protein 346	0.244	2956870-2957191
8833	434	tumor necrosis factor (ligand) superfamily, member 10	0.089	2971279-2971604
8911	415	vitamin D receptor	0.096	2993954-2994263
8917	414	caspase 8	0.2	2995593-2995870
8946	407	G protein-coupled receptor kinase 1	0.1	3003705-3003945
8950	405	baculoviral IAP repeat-containing 6	0.047	3004660-3004919
8954	403	junction-mediating and regulatory protein	0.09	3005715-3006035
8970	400	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta	0.2	3010046-3010308
8989	396	nudix (nucleoside diphosphate linked moiety X)-type motif 2	0.696	3015481-3015727
8998	393	BCL2-associated transcription factor 1	0.506	3017654-3017919
9019	388	BCL2-associated X protein	1.131	3023234-3023515
9047	379	cell death-inducing DNA fragmentation factor, alpha subunit-like effector B	0.326	3030361-3030636
9061	375	X-ray repair complementing defective repair in Chinese hamster cells 2	0.116	3034073-3034352
9110	362	PRKC, apoptosis, WT1, regulator	0.2	3046374-3046623
9122	360	BCL2-associated agonist of cell death	0.429	3049436-3049721
9125	359	ring finger protein 7	0.318	3050245-3050522
9151	352	tumor necrosis factor receptor superfamily, member 22	0.691	3056380-3056639
9168	347	ribonucleotide reductase M2 B (TP53 inducible)	0.11	3059844-3060049

9232	334	apoptosis-associated tyrosine kinase	0.065	3074031-3074270
9276	322	purine rich element binding protein B	0.763	3083608-3083822
9291	319	TP53 regulated inhibitor of apoptosis 1	3.404	3086855-3087130
9321	307	cysteine-serine-rich nuclear protein 1	0.109	3093672-3093894
9351	299	caspase recruitment domain family, member 14	0.076	3100085-3100282
9363	296	oncostatin M	0.135	3102482-3102721
9386	291	BCL2/adenovirus E1B 19kD interacting protein like	0.168	3106657-3106876
9434	270	growth arrest specific 1	0.093	3115802-3115978
9436	269	Fas apoptotic inhibitory molecule	0.408	3116150-3116343
9440	160	NLR family, apoptosis inhibitory protein 5	0.618	3116945-3116985
9464	259	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20	0.096	3120923-3121116
9466	258	post-GPI attachment to proteins 2	0.218	3121170-3121374
9473	256	engulfment and cell motility 3, ced-12 homolog (*C. elegans*)	0.119	3122400-3122588
9504	241	protein Tyr phosphatase, receptor type, V	0.04	3127383-3127553
9508	240	fission 1 (mitochondrial outer membrane) homolog (yeast)	0.298	3127965-3128127
9516	238	nerve growth factor receptor (TNFRSF16) associated protein 1	0.256	3129086-3129263
9517	238	mucosa associated lymphoid tissue lymphoma translocation gene 1	0.359	3129264-3129311
9526	234	NUAK family, SNF1-like kinase, 2	0.077	3130443-3130616
9547	229	Ras association (RalGDS/AF-6) domain family member 5	0.163	3133777-3133906
9576	215	tumor necrosis factor receptor superfamily, member 10b	0.089	3137352-3137413
9587	211	tensin 4	0.089	3138556-3138633
9679	173	heat shock protein 1B	0.091	3147029-3147080
9740	139	betacellulin, epidermal growth factor family member	0.073	3150839-3150877
9741	139	NLR family, pyrin domain containing 3	0.035	3150878-3150975
3157184	1487	retinoic acid receptor, beta	1.024	3177484 - 3177583
3157219	274	eyes absent 1 homolog (*Drosophila*)	0.064	3260105 - 3260204
3157247	594	endoplasmic reticulum (ER) to nucleus signalling 1	0.18	3179284 - 3179383
3157277	397	cell death-inducing DNA fragmentation factor, α-like effector A	0.341	3274796 - 3274895
3157296	3494	RNA binding motif protein 25	4.319	3267605 - 3267704
3157366	450	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	0.242	3260305 - 3260404
3157479	733	ELL associated factor 2	0.451	3264005 - 3264104
3157505	644	crystallin, alpha B	0.99	3280749 - 3280848
3157518	901	ectodysplasin A2 isoform receptor	0.239	3181584 - 3181683
3157545	387	death-associated protein kinase 2	0.216	3254417 - 3254516
3157559	371	XIAP associated factor 1	0.143	3203397 - 3203496
3157562	1064	NLR family, pyrin domain containing 1A	0.283	3194871 - 3194970
3157570	321	relaxin/insulin-like family peptide receptor 2	0.161	3227917 - 3228016
3157594	236	LIM homeobox transcription factor 1 beta	0.194	3202097 - 3202196

3157643	549	zinc finger CCCH type containing 8	0.373	3219691 - 3219790
3157762	794	APAF1 interacting protein	6.754	3227717 - 3227816
3157765	1398	twist homolog 1 (*Drosophila*)	3.622	3160121 - 3160220
3157772	2098	RIKEN cDNA 2610301G19 gene	2.281	3173184 - 3173283
3157807	762	src homology 2 domain-containing transforming protein B	0.391	3186971 - 3187070
3157837	1573	caspase 8 associated protein 2	0.5	3184971 - 3185070
3157885	1542	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	0.705	3168184 - 3168283
3157890	1059	angiotensin II receptor, type 2	0.668	3167484 - 3167583
3157913	2302	topoisomerase I binding, arginine/serine-rich	2.01	3173284 - 3173383
3157926	837	NA	0.212	3253017 - 3253116
3157949	477	protein C	0.42	3271796 - 3271895
3157952	795	homeobox, msh-like 1	0.717	3279749 - 3279848
3157977	1031	interleukin 7	0.642	3242917 - 3243016
3157980	428	phospholipase C, gamma 2	0.099	3169484 - 3169583
3157993	162	epidermal growth factor receptor	0.048	3166784 - 3166883
3158019	362	ABO blood group (transferase A, α1-3-N-acetylgalactosaminyltransferase, transferase B, α1-3-galactosyltransferase)	0.204	3185571 - 3185670
3158038	176	Fc receptor, IgE, high affinity I, γpolypeptide	0.258	3201197 - 3201296
3158091	478	NLR family, CARD domain containing 4	0.179	3216191 - 3216290
3158094	886	forkhead box O3	0.446	3175484 - 3175583
3158120	566	gasdermin A	0.477	3209058 - 3209157
3158121	3735	transformation related protein 53 inducible nuclear protein 1	2.567	3197071 - 3197170
3158129	525	protein Tyr phosphatase, receptor type, F	0.136	3255205 - 3255304
3158132	612	RIKEN cDNA 4632434I11 gene	0.26	3275096 - 3275195
3158149	629	Src homology 2 domain containing F	0.416	3221791 - 3221890
3158154	347	microtubule-associated protein tau	0.08	3245217 - 3245316
3158175	190	excision repair cross-complementing rodent repair deficiency, complementation group 6	0.023	3230317 - 3230416
3158199	521	hepatocyte growth factor	0.226	3253417 - 3253516
3158202	2263	GULP, engulfment adaptor PTB domain containing 1	3.671	3167784 - 3167883
3158294	648	matrix metallopeptidase 2	0.413	3214291 - 3214390
3158322	490	NLR family, apoptosis inhibitory protein 2	0.102	3179584 - 3179683
3158324	937	apoptosis, caspase activation inhibitor	1.828	3272696 - 3272795
3158331	982	NEL-like 1 (chicken)	0.565	3163221 - 3163320
3158359	394	angiotensin II receptor, type 1a	0.175	3213058 - 3213157
3158381	762	CD24a antigen	0.906	3245917 - 3246016

Table 15. Protein folding (Chinese hamster)

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
91	3840	peptidyl-prolyl isomerase G (cyclophilin G)	10.266	38781-39067
164	3470	calnexin	23.27	61559-61785

218	3290	alanyl-tRNA synthetase	25.07	77662-77970
412	2946	DnaJ (Hsp40) homolog, subfamily C, member 14	7.271	133746-134002
476	2865	heat shock 105kDa/110kDa protein 1	19.863	151195-151420
546	2787	DnaJ (Hsp40) homolog, subfamily C, member 10	22.023	171304-171555
579	2758	heat shock protein 90, beta (Grp94), member 1	606.207	180574-180954
594	2744	heat shock protein 90, alpha (cytosolic), class A member 1	93.844	184698-184927
827	2572	heat shock protein 9	28.56	255926-256325
893	2541	DnaJ (Hsp40) homolog, subfamily A, member 2	15.853	276519-276904
977	2496	heat shock protein 90 alpha (cytosolic), class B member 1	609.471	304274-304591
1048	2451	RAN binding protein 2	3.802	328313-328601
1078	2437	ERO1-like (S. cerevisiae)	6.094	338047-338432
1097	2428	sarcolemma associated protein	1.377	344524-344917
1254	2355	expressed sequence C80913	4.935	397171-397493
1384	2293	TNF receptor-associated protein 1	66.179	441242-441639
1543	2232	heat shock protein 1 (chaperonin)	134.366	494743-495086
1679	2181	FK506 binding protein 4	66.756	541802-542184
1925	2104	DnaJ (Hsp40) homolog, subfamily A, member 3	15.15	625909-626254
1932	2102	DnaJ (Hsp40) homolog, subfamily A, member 1	18.764	628385-628725
1948	2092	t-complex protein 1	67.336	633771-634149
1960	2089	DnaJ (Hsp40) homolog, subfamily C, member 16	1.225	637892-638209
2029	2068	heat shock protein 8	891.015	660889-661277
2076	2052	DnaJ (Hsp40) homolog, subfamily B, member 1	9.75	677203-677558
2198	2012	FK506 binding protein 9	6.327	717817-718182
2403	1958	DnaJ (Hsp40) homolog, subfamily C, member 5	5.417	787385-787676
2408	1957	chaperonin containing Tcp1, subunit 3 (γ)	229.706	789130-789474
2502	1933	chaperonin containing Tcp1, subunit 2 (β)	197.327	821357-821658
2610	1905	FK506 binding protein 10	11.722	857806-858195
2671	1890	chaperonin containing Tcp1, 4 (δ)	106.158	878362-878726
2722	1877	calreticulin	630.596	895691-896051
2995	1797	chaperonin containing Tcp1, 6a (zeta)	101.293	989555-989847
3064	1776	chaperonin containing Tcp1, 7 (eta)	197.813	1012622-1013001
3202	1747	chaperonin containing Tcp1, 8 (theta)	46.504	1060416-1060692
3243	1737	peptidylprolyl isomerase (cyclophilin)-like 4	2.479	1074139-1074475
3263	1730	tubulin-specific chaperone E	13.488	1080945-1081272
3269	1729	chaperonin containing Tcp1, subunit 5 (ε)	174.058	1083125-1083449
3276	1726	peptidylprolyl isomerase domain and WD repeat containing 1	1.901	1085316-1085607
3399	1693	peptidylprolyl isomerase (cyclophilin)-like 2	8.8	1127061-1127426
3651	1633	FK506 binding protein 8	53.498	1211464-1211841

3768	1607	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1	5.639	1251267-1251627
3893	1575	FK506 binding protein 1a	280.554	1293334-1293698
4000	1549	von Hippel-Lindau binding protein 1	35.144	1328790-1329108
4159	1510	DnaJ (Hsp40) homolog, subfamily C, member 1	3.933	1381932-1382211
4267	1487	STIP1 homology and U-Box containing protein 1	36.452	1418307-1418668
4379	1464	SH3-domain GRB2-like B1 (endophilin)	13.153	1455957-1456292
4393	1460	caseinolytic peptidase X (*E.coli*)	1.978	1460653-1461024
4429	1454	GrpE-like 1, mitochondrial	12.051	1472389-1472681
4545	1426	GrpE-like 2, mitochondrial	1.493	1510687-1510976
4697	1393	torsin family 1, member A (torsin A)	20.451	1561330-1561725
4955	1336	peptidylprolyl isomerase D (cyclophilin D)	17.796	1649170-1649515
5149	1289	DnaJ (Hsp40) homolog, subfamily B, member 9	0.929	1715305-1715623
5217	1277	FK506 binding protein 5	0.441	1738906-1739301
5227	1274	selenoprotein	61.456	1742333-1742644
5347	1248	DnaJ (Hsp40) homolog, subfamily B, member 12	3.209	1783440-1783810
5350	1247	DnaJ (Hsp40) homolog, subfamily B, member 11	17.061	1784585-1784897
5405	1236	DnaJ (Hsp40) homolog, subfamily B, member 4	1.568	1804161-1804465
5852	1136	aryl-hydrocarbon receptor-interacting protein	21.695	1963346-1963707
5965	1111	natural killer tumor recognition sequence	0.378	2004821-2005182
6059	1090	torsin family 2, member A	4.118	2038737-2039067
6183	1062	FK506 binding protein 14	2.059	2083548-2083925
6388	1016	serologically defined colon cancer antigen 10	3.1	2157023-2157404
6631	962	DnaJ (Hsp40) homolog, subfamily C, member 17	1.346	2243108-2243387
6640	960	calreticulin 3	3.271	2246344-2246668
6648	959	DnaJ (Hsp40) homolog, subfamily C, member 30	1.456	2249119-2249439
6662	956	peptidylprolyl isomerase C	21.193	2253978-2254373
6684	951	peptidylprolyl isomerase B	30.861	2261765-2262058
6723	941	peptidylprolyl isomerase E (cyclophilin E)	11.137	2275330-2275633
7277	817	DnaJ (Hsp40) homolog, subfamily C, member 18	0.36	2460206-2460591
7348	800	DnaJ (Hsp40) homolog, subfamily C, member 4	4.236	2483678-2484063
7499	764	FK506 binding protein 11	6.2	2533495-2533867
7597	740	prefoldin 2	8.764	2565686-2566071
7599	740	FK506 binding protein 7	1.092	2566115-2566476
7642	729	peptidylprolyl isomerase A	86.046	2579547-2579908
7643	729	FK506 binding protein 3	38.663	2579909-2580256
7889	664	ubiquitously expressed transcript	1.147	2662979-2663371
8128	593	DnaJ (Hsp40) homolog, subfamily B, member 5	0.47	2741242-2741540

8339	538	FK506 binding protein 2	5.81	2810112-2810427
8366	531	prefoldin 5	2.394	2819456-2819825
8398	520	cell division cycle 26	6.939	2830505-2830878
8405	517	heat shock protein 1 (chaperonin 10)	4.477	2833031-2833420
8480	501	peptidylprolyl isomerase (cyclophilin)-like 1	0.94	2857424-2857802
8689	461	prefoldin 1	2.791	2926689-2926987
8788	442	tetratricopeptide repeat domain 9C	0.133	2957757-2958131
8881	421	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	1.474	2985485-2985777
8886	420	H2-K region expressed gene 2	4.724	2986944-2987208
8901	416	RIKEN cDNA A830007P12 gene	0.129	2991112-2991407
8963	401	FK506 binding protein 1b	0.504	3008274-3008544
9430	271	peptidyl prolyl isomerase H	0.124	3115010-3115199
3157256	387	peptidylprolyl isomerase (cyclophilin)-like 3	1.624	3262205 - 3262304
3157418	441	DnaJ (Hsp40) homolog, subfamily B, member 14	0.238	3228617 - 3228716
3157499	462	FK506 binding protein 6	0.331	3177284 - 3177383
3157505	644	crystallin, alpha B	0.99	3280749 - 3280848
3157831	1176	FK506 binding protein 15	0.713	3167384 - 3167483
3157871	528	DnaJ (Hsp40) homolog, subfamily A, member 4	0.656	3215391 - 3215490
3158190	691	histocompatibility 2, class II, locus Mb2	2.388	3199171 - 3199196
3158259	974	histocompatibility 2, class II, locus Mb1	2.25	3256605 - 3256704
3158293	407	chaperonin containing Tcp1, 6b (zeta)	0.228	3166684 - 3166783

Table 16. Immune response (Chinese hamster)

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
73	3972	cell adhesion molecule 1	13.147	32944-33332
78	3935	strawberry notch homolog 2 (Drosophila)	39.592	34611-34972
440	2902	toll interacting protein	9.02	141719-141960
680	2676	polymerase (RNA) III (DNA directed) polypeptide E	5.84	211082-211316
1175	2393	inositol polyphosphate phosphatase-like 1	3.628	371083-371386
1299	2335	toll-like receptor 4	2.692	412131-412513
1330	2323	complement component 1, r subcomponent	62.58 6	422509-422751
1382	2293	CD276 antigen	2.822	440554-440858
1440	2270	TANK-binding kinase 1	3.946	460287-460685
1490	2250	transcription factor E3	4.882	477308-477628
1601	2208	complement component 1, s subcomponent	7.355	514675-514999
1694	2176	toll-like receptor 2	12.948	547130-547467
1703	2174	endoplasmic reticulum aminopeptidase 1	16.062	550016-550337
1718	2169	MAD homolog 3 (Drosophila)	1.913	555364-555694
1873	2121	protein kinase C, delta	15.233	608454-608757
1885	2116	interleukin-1 receptor-associated kinase 1	6.896	612534-612817
1980	2085	complement component 1, r subcomponent B	28.837	644971-645023
2234	2001	signal transducer and activator of	2.945	730267-730586

		transcription 6		
2242	1999	sequestosome 1	51.17	733070-733459
2471	1940	mutS homolog 2 (*E. coli*)	6.134	810424-810813
2474	1940	epiregulin	9.501	811533-811821
2477	1938	complement component factor h	1.484	812520-812875
2520	1929	drebrin-like	40.69	827385-827727
2525	1927	myxovirus (influenza virus) resistance 2	8.118	829145-829432
2627	1901	tubulointerstitial nephritis antigen-like 1	471.92	863337-863698
2876	1838	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	14.82	948495-948800
3073	1775	vanin 1	20.50	1015567-1015901
3094	1769	TRAF3 interacting protein 2	4.391	1022836-1023187
3179	1750	polymerase (RNA) III (DNA directed) polypeptide D	5.685	1052412-1052729
3259	1732	polymerase (RNA) III (DNA directed) polypeptide C	15.023	1079448-1079786
3268	1729	B-cell leukemia/lymphoma 6	8.467	1082762-1083124
3603	1645	Fc receptor, IgG, alpha chain transporter	84.176	1195070-1195378
3771	1606	ectonucleotide pyrophosphatase/phosphodiesterase 1	1.076	1252246-1252538
3936	1565	predicted gene 5077	4.951	1307451-1307521
4041	1540	presenilin 1	3.007	1342545-1342881
4063	1533	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	4.595	1349852-1350157
4126	1519	avian reticuloendotheliosis viral (v-rel) oncogene related B	4.305	1371109-1371427
4240	1493	complement factor properdin	2.075	1409395-1409692
4256	1491	polymerase (RNA) III (DNA directed) polypeptide B	1.005	1414629-1414949
4290	1482	mutL homolog 1 (E. coli)	5.514	1426359-1426686
4513	1433	leukemia inhibitory factor	2.095	1499872-1500182
4578	1419	2'-5' oligoadenylate synthetase-like 2	1.78	1521814-1522122
4619	1411	major facilitator superfamily domain containing 6	1.657	1535249-1535610
4662	1400	transcription factor EB	2.445	1549445-1549837
4780	1372	CCAAT/enhancer binding protein (C/EBP), γ	0.522	1588969-1589358
4832	1362	mitochondrial antiviral signaling protein	1.615	1607184-1607527
4944	1339	B-cell leukemia/lymphoma 10	9.013	1645462-1645856
4957	1336	transformation related protein 53	6.608	1649857-1650157
5102	1300	complement component (3b/4b) receptor 1-like	36.058	1699537-1699891
5103	1300	histocompatibility 2, D region locus 1	14.507	1699892-1699970
5114	1296	ECSIT homolog (Drosophila)	34.83	1703363-1703719
5131	1292	presenilin 2	2.55	1709139-1709525
5154	1287	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	2.617	1716973-1717346
5189	1282	OTU domain, ubiquitin aldehyde binding 1	6.598	1729190-1729552
5233	1274	histocompatibility 2, K1, K region	12.62	1744314-1744510
5244	1272	interleukin 4 receptor, alpha	1.087	1748021-1748398
5260	1269	receptor (TNFRSF)-interacting serine-threonine kinase 2	2.702	1753377-1753673

5436	1229	polymerase (RNA) III (DNA directed) polypeptide F	0.45	1814931-1815240
5532	1204	B-cell receptor-associated protein 31	116.56	1848903-1849265
5598	1190	parathymosin	27.95	1871721-1872006
5618	1187	myeloid differentiation primary response gene 88	1.629	1878827-1879137
5644	1179	complement component 3	0.472	1888266-1888655
5825	1141	ORAI calcium release-activated calcium modulator 1	3.196	1953406-1953799
5948	1114	interferon regulatory factor 7	2.718	1998635-1999022
5964	1111	colony stimulating factor 3 (granulocyte)	2.413	2004485-2004820
6050	1092	Sp110 nuclear body protein	2.119	2035393-2035780
6073	1086	histocompatibility 2, Q region locus 10	6.325	2043884-2044062
6124	1073	linker for activation of T cells	2.661	2062427-2062767
6240	1048	canopy 3 homolog (zebrafish)	15.161	2104733-2105122
6334	1028	chemokine (C-X-C motif) ligand 12	0.641	2137589-2137972
6418	1008	histocompatibility 2, T region locus 23	35.314	2167964-2168216
6454	999	toll-interleukin 1 receptor (TIR) domain-containing adaptor protein	0.575	2180459-2180745
6507	986	acid phosphatase 5, tartrate resistant	9.561	2199344-2199734
6550	978	Nedd4 family interacting protein 1	41.452	2214566-2214874
6615	966	histocompatibility 2, Q region locus 7	6.966	2237589-2237640
6647	960	Bmi1 polycomb ring finger oncogene	0.42	2248765-2249118
6745	936	proteasome (prosome, macropain) subunit, β type 8 (large multifunctional peptidase 7)	32.531	2282619-2282981
6858	913	nucleotide-binding oligomerization domain containing 2	0.461	2322123-2322429
6916	900	membrane-associated ring finger (C3HC4) 8	0.75	2340263-2340589
7015	877	tumor necrosis factor (ligand) superfamily, member 12	4.328	2373485-2373776
7039	872	Fc receptor, IgG, low affinity III	2.956	2381692-2381999
7128	852	SAM domain and HD domain, 1	0.214	2411159-2411550
7135	850	DNA cross-link repair 1C, PSO2 homolog (*S. cerevisiae*)	0.264	2413358-2413664
7223	833	chemokine (C-X-C motif) ligand 1	3.826	2442608-2443003
7260	823	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	0.166	2454994-2455378
7283	816	ribosomal protein S6	18.875	2462245-2462567
7297	811	TNF receptor-associated factor 6	1.188	2466579-2466938
7469	770	CD1d1 antigen	0.505	2523514-2523656
7586	743	phosphoprotein associated with glycosphingolipid microdomains 1	0.439	2561944-2562307
7670	721	myxovirus (influenza virus) resistance 1	0.687	2588615-2588951
7676	720	chemokine (C-C motif) ligand 2	14.55	2590794-2591157
7683	718	toll-like receptor 3	0.226	2593179-2593525
7716	710	polymerase (RNA) III (DNA directed) polypeptide H	2.352	2604412-2604804
7754	701	hemochromatosis	0.638	2617430-2617793
7764	698	polymerase (RNA) III (DNA directed) polypeptide G	0.231	2620918-2621272
7874	666	CD1d2 antigen	0.935	2658252-2658336

7903	658	interleukin 1 receptor accessory protein	0.254	2667913-2668256
7929	651	interleukin 23, alpha subunit p19	0.852	2676772-2677097
7943	647	proteasome maturation protein	19.088	2681546-2681896
8097	601	histocompatibility 2, Q region locus 2	1.764	2731750-2731823
8129	592	exonuclease 1	0.312	2741541-2741842
8218	570	interleukin 18	2.856	2769797-2770097
8244	562	interleukin 1 receptor-like 1	0.299	2777898-2778255
8245	562	calcitonin gene-related peptide-receptor component protein	0.987	2778256-2778534
8304	546	macrophage migration inhibitory factor	43.469	2798316-2798434
8312	543	immunoglobulin joining chain	0.441	2800818-2801142
8318	541	T-cell specific GTPase	0.193	2802893-2803167
8345	536	Fas (TNF receptor superfamily member 6)	0.501	2812206-2812506
8495	496	SH2B adaptor protein 2	0.174	2862373-2862711
8504	494	chemokine (C-X-C motif) ligand 10	1.586	2865648-2866015
8531	490	interleukin 15	1.901	2874576-2874952
8597	477	mannan-binding lectin serine peptidase 2	0.156	2896069-2896411
8616	474	Src-like-adaptor 2	1.772	2902824-2903199
8663	464	chemokine (C-C motif) receptor 7	0.236	2918384-2918739
8696	459	CSF 2 (granulocyte-macrophage)	1.109	2928757-2929061
8719	455	histocompatibility 28	0.469	2936057-2936444
8794	441	histocompatibility 2, Q region locus 1	1.023	2959862-2959912
8812	439	TNF (ligand) superfamily, member 9	11.755	2964694-2965039
8833	434	TNF (ligand) superfamily, member 10	0.089	2971279-2971604
8871	423	spondin 2, extracellular matrix protein	0.189	2982359-2982686
9014	389	polymerase (RNA) III (DNA directed) polypeptide K	0.509	3021834-3022134
9021	387	hemopexin	0.262	3023816-3024122
9064	373	complement component 8, gamma polypeptide	0.685	3034878-3035143
9067	373	proteasome (prosome, macropain), β type 9 (large multifunctional peptidase 2)	0.464	3035689-3035987
9135	356	interleukin 1 receptor, type I	0.507	3052757-3052969
9164	348	TNF (ligand) superfamily, member 11	0.157	3058993-3059213
9204	341	POU domain, class 2, transcription factor 2	0.107	3068222-3068455
9363	296	oncostatin M	0.135	3102482-3102721
9367	295	Fc receptor, IgG, low affinity IIb	0.189	3103313-3103351
9389	289	TNF (ligand) superfamily, member 4	0.18	3107093-3107318
9395	285	2'-5' oligoadenylate synthetase 1B	0.156	3108340-3108557
9517	238	mucosa associated lymphoid tissue lymphoma translocation gene 1	0.359	3129264-3129311
9611	202	chemokine (C-C motif) ligand 9	0.268	3141032-3141071
9624	196	toll-like receptor 13	0.061	3142028-3142161
9667	178	chemokine (C-C motif) receptor 2	0.144	3146072-3146098
9670	175	histocompatibility 2, Q region locus 8	0.302	3146338-3146451
9720	149	chemokine (C-X-C motif) ligand 3	0.148	3149776-3149850
3157279	427	ectonucleotide pyrophosphatase/ phosphodiesterase 3	0.153	3182184 - 3182283
3157459	250	chemokine (C-C motif) ligand 11	0.299	3199071 - 3199170
3157520	492	complement component 1, r subcomponent-like	0.264	3224791 - 3224890
3157558	742	chemokine (C-C motif) ligand 7	6.395	3279849 - 3279948

3157639	212	spleen tyrosine kinase	0.041	3259705 - 3259804
3157663	437	interleukin 1 receptor-like 2	0.224	3176584 - 3176683
3157759	437	toll-like receptor 1	0.309	3203997 - 3204096
3157859	973	Casitas B-lineage lymphoma b	0.221	3172084 - 3172183
3157977	1031	interleukin 7	0.642	3242917 - 3243016
3158027	1030	akirin 2	2.138	3188971 - 3189070
3158038	176	Fc receptor, IgE, high affinity I, γ polypeptide	0.258	3201197 - 3201296
3158135	418	mannan-binding lectin serine peptidase 1	0.152	3282249 - 3282348
3158169	681	TBK1 binding protein 1	0.203	3175184 - 3175283
3158197	284	MHC, class I-related	0.114	3163484 - 3163583
3158259	974	histocompatibility 2, class II, locus Mb1	2.25	3256605 - 3256704
3158365	431	complement component factor i	0.209	3178584 - 3178683
3158381	762	CD24a antigen	0.906	3245917 - 3246016

V. RNA effector modification

[00412] In some embodiments of the present invention, an oligonucleotide (e.g., a RNA effector molecule) is chemically modified to enhance stability or other beneficial characteristics. In one embodiment the RNA effector molecule is not chemically modified.

[00413] Oligonucleotides can be modified to prevent rapid degradation of the oligonucleotides by endo- and exo-nucleases and avoid undesirable off-target effects. The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in CURRENT PROTOCOLS IN NUCLEIC ACID CHEMISTRY (Beaucage et al., eds., John Wiley & Sons, Inc., NY). Modifications include, for example, (a) end modifications, e.g., 5' end modifications (phosphorylation, conjugation, inverted linkages, etc.), or 3' end modifications (conjugation, DNA nucleotides, inverted linkages, etc.); (b) base modifications, e.g., replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; (c) sugar modifications (e.g., at the 2' position or 4' position) or replacement of the sugar; as well as (d) internucleoside linkage modifications, including modification or replacement of the phosphodiester linkages. Specific examples of oligonucleotide compounds useful in this invention include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. Specific examples of oligonucleotide compounds useful in this invention include, but are not limited to oligonucleotides containing modified or non-natural internucleoside linkages. Oligonucleotides having modified internucloside linkages include, among others, those that do not have a phosphorus atom in the internucleoside linkage.

[00414] For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside linkage(s)

can also be considered to be oligonucleosides. In particular embodiments, the modified oligonucleotides will have a phosphorus atom in its internucleoside linkage(s). For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In particular embodiments, the modified RNA will have a phosphorus atom in its internucleoside backbone.

[00415] Modified internucleoside linkages include (e.g., RNA backbones) include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those) having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[00416] Representative patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patents No. 3,687,808; No. 4,469,863; No. 4,476,301; No. 5,023,243; No. 5,177,195; No. 5,188,897; No. 5,264,423; No. 5,276,019; No. 5,278,302; No. 5,286,717; No. 5,321,131; No. 5,399,676; No. 5,405,939; No. 5,453,496; No. 5,455,233; No. 5,466,677; No. 5,476,925; No. 5,519,126; No. 5,536,821; No. 5,541,316; No. 5,550,111; No. 5,563,253; No. 5,571,799; No. 5,587,361; No. 5,625,050; No. 6,028,188; No. 6,124,445; No. 6,160,109; No. 6,169,170; No. 6,172,209; No. 6, 239,265; No. 6,277,603; No. 6,326,199; No. 6,346,614; No. 6,444,423; No. 6,531,590; No. 6,534,639; No. 6,608,035; No. 6,683,167; No. 6,858,715; No. 6,867,294; No. 6,878,805; No. 7,015,315; No. 7,041,816; No. 7,273,933; No. 7,321,029; and No. RE39464.

[00417] Modified oligonucleotide internucleoside linakges (e.g., RNA backbones) that do not include a phosphorus atom therein have internucleoside linkages that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino

backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[00418] Representative patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patents No. 5,034,506; No. 5,166,315; No. 5,185,444; No. 5,214,134; No. 5,216,141; No. 5,235,033; No. 5,64,562; No. 5,264,564; No. 5,405,938; No. 5,434,257; No. 5,466,677; No. 5,470,967; No. 5,489,677; No. 5,541,307; No. 5,561,225; No. 5,596,086; No. 5,602,240; No. 5,608,046; No. 5,610,289; No. 5,618,704; No. 5,623,070; No. 5,663,312; No. 5,633,360; No. 5,677,437; and No. 5,677,439.

[00419] In other modified oligonucleotides suitable or contemplated for use in RNA effector molecules, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, a RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of a RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patents No. 5,539,082; No. 5,714,331; and No. 5,719,262. Further teaching of PNA compounds can be found, for example, in Nielsen et al., 254 Science 1497-1500 (1991).

[00420] Some embodiments featured in the invention include oligonucleotides with phosphorothioate internucleoside linkages and oligonucleosides with heteroatom backbones, and in particular $-CH_2-NH-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$[known as a methylene (methylimino) or MMI backbone], $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$ and $-N(CH_3)-CH_2-CH_2-$ [wherein the native phosphodiester internucleoside linkage is represented as $-O-P-O-CH_2-$] (*see* U.S. Patent No. 5,489,677), and amide backbones (*see* U.S. Patent No. 5,602,240). In some embodiments, the oligonucleotides featured herein have morpholino backbone structures (*see* U.S. Patent No. 5,034,506).

[00421] Modified oligonucleotides can also contain one or more substituted sugar moieties. The RNA effector molecules, e.g., dsRNAs, featured herein can include one of the following at the 2' position: H (deoxyribose); OH (ribose); F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$,

$O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to 10, inclusive. In some embodiments, oligonucleotides 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, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, a RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide (e.g., a RNA effector molecule), or a group for improving the pharmacodynamic properties of an oligonucleotide (e.g., a RNA effector molecule), 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., 78 Helv. Chim. Acta 486-504 (1995)), i.e., an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-CH_2-O-CH_2-$N(CH_2)_2$.

[00422] Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotide and the 5' position of 5' terminal nucleotide. Oligonucletodides can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Patents No. 4,981,957; No. 5,118,800; No. 5,319,080; No. 5,359,044; No. 5,393,878; No. 5,446,137; No. 5,466,786; No. 5,514,785; No. 5,519,134; No. 5,567,811; No. 5,576,427; No. 5,591,722; No. 5,597,909; No. 5,610,300; No. 5,627,053; No. 5,639,873; No. 5,646,265; No. 5,658,873; No. 5,670,633; and No. 5,700,920, certain of which are commonly owned with the instant application.

[00423] An oligonucleotide (e.g., a RNA effector molecule) can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as as inosine, xanthine, hypoxanthine, nubularine, isoguanisine, tubercidine, 2-(halo)adenine, 2-(alkyl)adenine, 2-(propyl)adenine, 2 (amino)adenine, 2-(aminoalkyll)adenine, 2 (aminopropyl)adenine, 2 (methylthio) N6 (isopentenyl)adenine, 6 (alkyl)adenine, 6 (methyl)adenine, 7 (deaza)adenine, 8 (alkenyl)adenine, 8-(alkyl)adenine, 8 (alkynyl)adenine, 8 (amino)adenine, 8-(halo)adenine, 8-(hydroxyl)adenine,

8 (thioalkyl)adenine, 8-(thiol)adenine, N6-(isopentyl)adenine, N6 (methyl)adenine, N6, N6 (dimethyl)adenine, 2-(alkyl)guanine,2 (propyl)guanine, 6-(alkyl)guanine, 6 (methyl)guanine, 7 (alkyl)guanine, 7 (methyl)guanine, 7 (deaza)guanine, 8 (alkyl)guanine, 8-(alkenyl)guanine, 8 (alkynyl)guanine, 8-(amino)guanine, 8 (halo)guanine, 8-(hydroxyl)guanine, 8 (thioalkyl)guanine, 8-(thiol)guanine, N (methyl)guanine, 2-(thio)cytosine, 3 (deaza) 5 (aza)cytosine, 3-(alkyl)cytosine, 3 (methyl)cytosine, 5-(alkyl)cytosine, 5-(alkynyl)cytosine, 5 (halo)cytosine, 5 (methyl)cytosine, 5 (propynyl)cytosine, 5 (propynyl)cytosine, 5 (trifluoromethyl)cytosine, 6-(azo)cytosine, N4 (acetyl)cytosine, 3 (3 amino-3 carboxypropyl)uracil, 2-(thio)uracil, 5 (methyl) 2 (thio)uracil, 5 (methylaminomethyl)-2 (thio)uracil, 4-(thio)uracil, 5 (methyl) 4 (thio)uracil, 5 (methylaminomethyl)-4 (thio)uracil, 5 (methyl) 2,4 (dithio)uracil, 5 (methylaminomethyl)-2,4 (dithio)uracil, 5 (2-aminopropyl)uracil, 5-(alkyl)uracil, 5-(alkynyl)uracil, 5-(allylamino)uracil, 5 (aminoallyl)uracil, 5 (aminoalkyl)uracil, 5 (guanidiniumalkyl)uracil, 5 (1,3-diazole-1-alkyl)uracil, 5-(cyanoalkyl)uracil, 5-(dialkylaminoalkyl)uracil, 5 (dimethylaminoalkyl)uracil, 5-(halo)uracil, 5-(methoxy)uracil, uracil-5 oxyacetic acid, 5 (methoxycarbonylmethyl)-2-(thio)uracil, 5 (methoxycarbonyl-methyl)uracil, 5 (propynyl)uracil, 5 (propynyl)uracil, 5 (trifluoromethyl)uracil, 6 (azo)uracil, dihydrouracil, N3 (methyl)uracil, 5-uracil (i.e., pseudouracil), 2 (thio)pseudouracil,4 (thio)pseudouracil,2,4-(dithio)psuedouracil,5-(alkyl)pseudouracil, 5-(methyl)pseudouracil, 5-(alkyl)-2-(thio)pseudouracil, 5-(methyl)-2-(thio)pseudouracil, 5-(alkyl)-4 (thio)pseudouracil, 5-(methyl)-4 (thio)pseudouracil, 5-(alkyl)-2,4 (dithio)pseudouracil, 5-(methyl)-2,4 (dithio)pseudouracil, 1 substituted pseudouracil, 1 substituted 2(thio)-pseudouracil, 1 substituted 4 (thio)pseudouracil, 1 substituted 2,4-(dithio)pseudouracil, 1 (aminocarbonylethylenyl)-pseudouracil, 1 (aminocarbonylethylenyl)-2(thio)-pseudouracil, 1 (aminocarbonylethylenyl)-4 (thio)pseudouracil, 1 (aminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-pseudouracil, 1 (aminoalkylamino-carbonylethylenyl)-2(thio)-pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-4 (thio)pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-substituted 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-substituted 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl,

7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-(guanidiniumalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-(guanidiniumalkyl-hydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 1,3,5-(triaza)-2,6-(dioxa)-naphthalene, inosine, xanthine, hypoxanthine, nubularine, tubercidine, isoguanisine, inosinyl, 2-aza-inosinyl, 7-deaza-inosinyl, nitroimidazolyl, nitropyrazolyl, nitrobenzimidazolyl, nitroindazolyl, aminoindolyl, pyrrolopyrimidinyl, 3-(methyl)isocarbostyrilyl, 5-(methyl)isocarbostyrilyl, 3-(methyl)-7-(propynyl)isocarbostyrilyl, 7-(aza)indolyl, 6-(methyl)-7-(aza)indolyl, imidizopyridinyl, 9-(methyl)-imidizopyridinyl, pyrrolopyrizinyl, isocarbostyrilyl, 7-(propynyl)isocarbostyrilyl, propynyl-7-(aza)indolyl, 2,4,5-(trimethyl)phenyl, 4-(methyl)indolyl, 4,6-(dimethyl)indolyl, phenyl, napthalenyl, anthracenyl, phenanthracenyl, pyrenyl, stilbenyl, tetracenyl, pentacenyl, difluorotolyl, 4-(fluoro)-6-(methyl)benzimidazole, 4-(methyl)benzimidazole, 6-(azo)thymine, 2-pyridinone, 5 nitroindole, 3 nitropyrrole, 6-(aza)pyrimidine, 2 (amino)purine, 2,6-(diamino)purine, 5 substituted pyrimidines, N2-substituted purines, N6-substituted purines, O6-substituted purines, substituted 1,2,4-triazoles, pyrrolo-pyrimidin-2-on-3-yl, 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, para-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, bis-ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, para-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, ortho-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, bis-ortho-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, pyridopyrimidin-3-yl, 2-oxo-7-amino-pyridopyrimidin-3-yl, 2-oxo-pyridopyrimidine-3-yl, or any O-alkylated or N-alkylated derivatives thereof. Modified nucleobases also include natural bases that comprise conjugated moieties, e.g., a ligand.

[00424] Further nucleobases include those disclosed in U.S. Patent No. 3,687,808; MODIFIED NUCLEOSIDES BIOCHEM., BIOTECH. & MEDICINE (Herdewijn, ed., Wiley-VCH, 2008); WO 2009/120878; CONCISE ENCYCLOPEDIA OF POLYMER SCIENCE & ENGIN. 858-59 (Kroschwitz ed., John Wiley & Sons, 1990); Englisch et al., 30 Angewandte Chemie, Intl. Ed. 613 (1991); Sanghvi, 15 DSRNA RESEARCH & APPLICATIONS 289-302 (Crooke & Lebleu, eds., CRC Press, Boca Raton, FL, 1993). Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi,

at 276-78), and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[00425] Representative patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patents No. 3,687,808; No. 4,845,205; No. 5,130,30; No. 5,134,066; No. 5,175,273; No. 5,367,066; No. 5,432,272; No. 5,457,191No. 5,457,187; No. 5,459,255; No. 5,484,908; No. 5,502,177; No. 5,525,711; No. 5,552,540; No. 5,587,469; No. 5,594,121, No. 5,596,091; No. 5,614,617; No. 5,681,941; No. 6,015,886; No. 6,147,200; No. 6,166,197; No. 6,222,025; No. 6,235,887; No. 6,380,368; No. 6,528,640; No. 6,639,062; No. 6,617,438; No. 7,045,610; No. 7,427,672; and No. 7,495,088; and No. 5,750,692.

[00426] The oligonucleotides can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to oligonucleotide molecules has been shown to increase oligonucleotide molecule stability in serum, and to reduce off-target effects. Elmen et al., 33 Nucl. Acids Res. 439-47 (2005); Mook et al., 6 Mol. Cancer Ther. 833-43 (2007); Grunweller et al., 31 Nucl. Acids Res. 3185-93 (2003); U.S. Patents No. 6,268,490; No. 6,670,461; No. 6,794,499; No. 6,998,484; No. 7,053,207; No. 7,084,125; and No. 7,399,845.

[00427] In certain instances, the oligonucleotides of a RNA effector molecule can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution or cellular uptake of the oligonucleotides, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo et al., 365 Biochem. Biophys. Res. Comm. 54-61 (2007)); Letsinger et al., 86 PNAS 6553 (1989)); cholic acid (Manoharan et al., 1994); a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., 1992; Manoharan et al., 1993); a thiocholesterol (Oberhauser et al., 1992); an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., 1991; Kabanov et al., 259 FEBS Lett. 327 (1990); Svinarchuk et al., 75 Biochimie 75 (1993)); a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., 1995); Shea et al., 18 Nucl. Acids Res. 3777 (1990)); a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995); or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995); a palmityl moiety (Mishra et al., 1995); or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., 1996).

Representative United States patents that teach the preparation of such RNA conjugates have been listed herein. Typical conjugation protocols involve the synthesis of an oligonucleotide bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

[00428] Nucleic acid sequences of exemplary RNA effector molecules are represented below using standard nomenclature, and specifically the abbreviations of Table 17, as follows:

Table 17. Abbreviations of nucleotide monomers used in nucleic acid sequence representation.

Abbreviation	Nucleotide(s)
A	adenosine
C	cytidine
G	guanosine
T	thymidine
U	uridine
N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine
c	2'-O-methylcytidine
g	2'-O-methylguanosine
u	2'-O-methyluridine
dT	2'-deoxythymidine
s	phosphorothioate linkage

These monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Ligands

[00429] Another modification of the oligonucleotides (e.g., of a RNA effector molecule) featured in the invention involves chemically linking to the oligonucleotide one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., 86 PNAS 6553-56 (1989); cholic acid (Manoharan et al., 4 Biorg. Med. Chem. Let. 1053-60 (1994)); a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., 660 Ann. NY Acad. Sci. 306309 (1992); Manoharan et al., 3 Biorg. Med. Chem. Let. 2765-70 (1993)); a thiocholesterol (Oberhauser et al., 20 Nucl. Acids Res. 533-38 (1992)); an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., 10 EMBO J. 1111-18 (1991); Kabanov et al., 259 FEBS Lett. 327-30 (1990); Svinarchuk et al., 75 Biochimie 49-54 (1993)); a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-

glycero-3-phosphonate (Manoharan et al., 36 Tetrahedron Lett. 3651-54 (1995); Shea et al., 18 Nucl. Acids Res. 3777-83 (1990)); a polyamine or a polyethylene glycol chain (Manoharan et al., 14 Nucleosides & Nucleotides 969-73 (1995)); or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995); a palmityl moiety (Mishra et al., 1264 Biochim. Biophys. Acta 229-37 (1995)); or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., 227 J. Pharmacol. Exp. Ther. 923-37 (1996)).

[00430] In one embodiment, a ligand alters the distribution, targeting or lifetime of a RNA effector molecule agent into which it is incorporated. In some embodiments a ligand provides an enhanced affinity for a selected target, e.g., molecule, cell or cell type, compartment, e.g., a cellular or organ compartment, tissue, organ or region of the body, as, e.g., compared to a species absent such a ligand. Ideally, ligands will not take part in duplex pairing in a duplexed nucleic acid.

[00431] Ligands can include a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid. 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-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example 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 α-helical peptide.

[00432] Ligands 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 B12, biotin, or an RGD peptide or RGD peptide mimetic.

[00433] Other examples of ligands include 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), lipophilic molecules, e.g, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (e.g., antennapedia peptide, Tat peptide), 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 (e.g., imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu^{3+} complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

[00434] Ligands 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. Ligands can 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, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

[00435] The ligand can be a substance, e.g., a drug, which can increase the uptake of the RNA effector molecule agent into the cell, for example, by disrupting the cell's cytoskeleton, e.g., by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxol, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

[00436] An example ligand is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, e.g., human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, e.g., a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, Naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) can be used to adjust binding to a serum protein, e.g., HSA.

[00437] A lipid based ligand can be used to modulate, e.g., control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the embryo. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney. For example, the lipid based ligand binds HSA, or it binds HSA with a sufficient affinity such that the conjugate will be distributed to a non-kidney tissue but also be reversible. Alternatively, the lipid-based ligand binds HSA weakly or not at all, such that the conjugate will be distributed to the kidney. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid-based ligand.

[00438] In another aspect, the ligand is a moiety, e.g., a vitamin, that is taken up by an embryonic cell, e.g., a proliferating cell. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, e.g., folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by embryonic cells. Also included are HSA and low density lipoproteins.

[00439] In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent can be an α-helical agent, and can include a lipophilic and a lipophobic phase.

[00440] The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined 3-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to RNA effector molecule agents can affect pharmacokinetic distribution of the RNA effector molecule, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5 to 50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long (*see* Table 18, for example).

Table 18. Exemplary Cell Permeation Peptides

Cell Permeation Peptide	Amino acid Sequence	SEQ ID NO:	Reference
Penetratin	RQIKIWFQNRRMKWKK	3284943	Derossi et al., 269 J. Biol. Chem. 10444 (1994)
Tat fragment (48-60)	GRKKRRQRRRPPQC	3284944	Vives et al., 272 J. Biol. Chem. 16010 (1997)

Table 18. Exemplary Cell Permeation Peptides			
Cell Permeation Peptide	Amino acid Sequence	SEQ ID NO:	Reference
Signal Sequence-based peptide	GALFLGWLGAAGSTMGAWSQPKKKRKV	3284945	Chaloin et al., 243 Biochem. Biophys. Res. Commun. 601 (1998)
PVEC	LLIILRRRIRKQAHAHSK	3284946	Elmquist et al., 269 Exp. Cell Res. 237 (2001)
Transportan	GWTLNSAGYLLKINLKALAALAKKIL	3284947	Pooga et al., 12 FASEB J. 67 (1998)
Amphiphilic model peptide	KLALKLALKALKAALKLA	3284948	Oehlke et al., 2 Mol. Ther. 339 (2000)
Arg_9	RRRRRRRRR	3284949	Mitchell et al., 56 J. Pept. Res. 318 (2000)
Bacterial cell wall permeating	KFFKFFKFFK	3284950	
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRN LVPRTES	3284951	
Cecropin P1	SWLSKTAKKLENSAKKRISEGIAIAIQGGP R	3284952	
α-defensin	ACYCRIPACIAGERRYGTCIYQGRLWAFC C	3284953	
b-defensin	DHYNCVSSGGQCLYSACPIFTKIQGTCYR GKAKCCK	3284954	
Bactenecin	RKCRIVVIRVCR	3284955	
PR-39	RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPP RFPPRFPGKR-NH_2	3284956	
Indolicidin	ILPWKWPWWPWRR-NH_2	3284957	

[00441] A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (e.g., consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO:3284958) An RFGF analogue (e.g., amino acid sequence AALLPVLLAAP (SEQ ID NO:3284959) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide that carres large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ [SEQ

ID NO:3284960]) and the *Drosophila* antennapedia protein (RQIKIWFQNRRMKWKK [SEQ ID NO:284961]) can function as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library. Lam et al., 354 Nature 82-84 (1991). The peptide or peptidomimetic can be tethered to a dsRNA agent via an incorporated monomer unit is a cell targeting peptide such as an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. As noted, the peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described herein can be utilized.

[00442] An RGD peptide moiety can be used to target a tumor cell, such as an endothelial tumor cell or a breast cancer tumor cell. Zitzmann et al., 62 Cancer Res. 5139-43 (2002). An RGD peptide can facilitate targeting of an dsRNA agent to tumors of a variety of other tissues, including the lung, kidney, spleen, or liver. Aoki et al., 8 Cancer Gene Ther. 783-87 (2001). Preferably, the RGD peptide will facilitate targeting of a RNA effector molecule agent to the kidney. The RGD peptide can be linear or cyclic, and can be modified, e.g., glycosylated or methylated to facilitate targeting to specific tissues. For example, a glycosylated RGD peptide can deliver a RNA effector molecule agent to a tumor cell expressing $\alpha V\beta 3$. Haubner et al., 42 J. Nucl. Med. 326-36 (2001).

[00443] A "cell permeation peptide" is capable of permeating a cell. It can be, for example, an α-helical linear peptide (e.g., LL-37 or Ceropin P1), a disulfide bond-containing peptide (e.g., α-defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (e.g., PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen. Simeoni et al., 31 Nucl. Acids Res. 2717-24 (2003).

[00444] Representative patents that teach the preparation of oligonucleotide conjugates include, but are not limited to, U.S. Patents No. 4,828,979; No. 4,948,882; No. 5,218,105; No. 5,525,465; No. 5,541,313; No. 5,545,730; No. 5,552,538; No. 5,578,717, No. 5,580,731; No. 5,591,584; No. 5,109,124; No. 5,118,802; No. 5,138,045; No. 5,414,077; No. 5,486,603; No. 5,512,439; No. 5,578,718; No. 5,608,046; No. 4,587,044; No. 4,605,735; No. 4,667,025; No. 4,762,779; No. 4,789,737; No. 4,824,941; No. 4,835,263; No. 4,876,335; No. 4,904,582; No. 4,958,013; No. 5,082,830; No. 5,112,963; No. 5,214,136; No. 5,082,830; No. 5,112,963; No. 5,214,136; No. 5,245,022; No. 5,254,469; No. 5,258,506; No. 5,262,536; No. 5,272,250; No. 5,292,873; No. 5,317,098; No. 5,371,241, No. 5,391,723; No. 5,416,203, No. 5,451,463;

No. 5,510,475; No. 5,512,667; No. 5,514,785; No. 5,565,552; No. 5,567,810; No. 5,574,142; No. 5,585,481; No. 5,587,371; No. 5,595,726; No. 5,597,696; No. 5,599,923; No. 5,599,928; No. 5,688,941; No. 6,294,664; No. 6,320,017; No. 6,576,752; No. 6,783,931; No. 6,900,297; and No. 7,037,646.

[00445] It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within sn oligonucleotide. The present invention also includes oligonucleotide molecule compounds which are chimeric compounds. "Chimeric" RNA effector molecule compounds or "chimeras," in the context of this invention, are oligonucleotide compounds, such as dsRNAs, that contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of a dsRNA compound. These RNA effector molecules typically contain at least one region wherein the RNA is modified so as to confer upon the RNA effector molecule increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of a RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of RNA effector molecule inhibition of gene expression. Consequently, comparable results can often be obtained with shorter RNA effector molecules when chimeric dsRNAs are used, compared to phosphorothioate deoxydsRNAs hybridizing to the same target region. Cleavage of the oligonucleotide can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

VI. Introduction/Delivery of RNA Effector Molecules

[00446] The delivery of an oligonucleotide (e.g., a RNA effector molecule) to cells according to methods provided herein can be achieved in a number of different ways. For example, delivery can be performed directly by administering a composition comprising a RNA effector molecule, e.g., a dsRNA, into cell culture. Alternatively, delivery can be performed indirectly by administering into the cell one or more vectors that encode and direct the expression of the RNA effector molecule. These alternatives are discussed further herein.

[00447] In some embodiments, the RNA effector molecule is a siRNA or shRNA effector molecule introduced into a cell by introducing into the cell an invasive bacterium containing one or more siRNA or shRNA effector molecules or DNA encoding one or more siRNA or shRNA

effector molecules (a process sometimes referred to as transkingdom RNAi (tkRNAi)). The invasive bacterium can be an attenuated strain of *Listeria*, *Shigella*, *Salmonella*, *E. coli*, or *Bifidobacteriae*, or a non-invasive bacterium that has been genetically modified to increase its invasive properties, e.g., by introducing one or more genes that enable invasive bacteria to access the cytoplasm of cells. Examples of such cytoplasm-targeting genes include listeriolysin O of *Listeria* and the invasin protein of *Yersinia pseudotuberculosis*. Methods for delivering RNA effector molecules to animal cells to induce transkingdom RNAi (tkRNAi) are known in the art. *See, e.g.*, U.S. Patent Pubs. No. 2008/0311081 and No. 2009/0123426. In one embodiment, the RNA effector molecule is a siRNA molecule. In one embodiment, the RNA effector molecule is not a shRNA molecule.

[00448] As noted herein, oligonucleotides can be modified to prevent rapid degradation of the dsRNA by endo- and exo-nucleases and avoid undesirable off-target effects. For example, RNA effector molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. In one embodiment, the RNA effector molecule is not modified by chemical conjugation to a lipophilic group, e.g., cholesterol.

[00449] In an alternative embodiment, RNA effector molecules can be delivered using a drug delivery system such as a nanoparticle, a dendrimer, a polymer, a liposome, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of a RNA effector molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient cellular uptake. Cationic lipids, dendrimers, or polymers can either be bound to RNA effector molecules, or induced to form a vesicle or micelle that encases the RNA effector molecule. *See, e.g.*, Kim et al., 129 J. Contr. Release 107-16 (2008). Methods for making and using cationic-RNA effector molecule complexes are well within the abilities of those skilled in the art. *See e.g.,* Sorensen et al 327 J. Mol. Biol. 761-66 (2003); Verma et al., 9 Clin. Cancer Res. 1291-1300 (2003); Arnold et al., 25 J. Hypertens. 197-205 (2007).

[00450] Where the RNA effector molecule is a double-stranded molecule, such as a small interfering RNA (siRNA), comprising a sense strand and an antisense strand, the sense strand and antisense strand can be separately and temporally exposed to a cell, cell lysates, tissue, or cell culture. The phrase “separately and temporally” refers to the introduction of each strand of a double-stranded RNA effector molecule to a cell, cell lysates, tissue or cell culture in a single-stranded form, e.g., in the form of a non-annealed mixture of both strands or as separate, i.e., unmixed, preparations of each strand. In some embodiments, there is a time interval between the introduction of each strand which can range from seconds to several minutes to about an hour or

more, e.g., 12 hr, 24 hr, 48 hr, 72 hr, 84 hr, 96 hr, or 108 hr, or more. Separate and temporal administration can be performed with canonical or non-canonical RNA effector molecules.

[00451] It is also contemplated herein that a plurality of RNA effector molecules are administered in a separate and temporal manner. Thus, each of a plurality of RNA effector molecules can be administered at a separate time or at a different frequency interval to achieve the desired average percent inhibition for the target gene. For example, RNA effector molecules targeting Bak can be administered more frequently tha RNA effector molecule targeting LDH, as the expression of Bak recovers faster following treatment with a Bak RNA effector molecule. In one embodiment, the RNA effector molecules are added at a concentration from approximately 0.01 nM to 200 nM. In another embodiment, the RNA effector molecules are added at an amount of approximately 50 molecules per cell up to and including 500,000 molecules per cell. In another embodiment, the RNA effector molecules are added at a concentration from about 0.1 fmol/10^6 cells to about 1 pmol/10^6 cells.

[00452] In another aspect, a RNA effector molecule for modulating expression of a target gene can be expressed from transcription units inserted into DNA or RNA vectors. *See, e.g.,* Couture et al., 12 TIG 5-10 (1996); WO 00/22113; WO 00/22114; U.S. Patent No. 6,054,299. Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extra chromosomal plasmid. Gassmann, et al., 92 PNAS 1292 (1995).

[00453] The individual strand or strands of a RNA effector molecule can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate , for example, a dsRNA, two separate expression vectors can be co-introduced (e.g., by transfection or infection) into a target cell. Alternatively each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

[00454] RNA effector molecule expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, such as those compatible with vertebrate cells, insect cells, or yeast cells can be used to produce recombinant constructs for the expression of a RNA effector molecule as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired

nucleic acid segment. RNA effector molecule expressing vectors can be delivered directly to target cells using standard transfection and transduction methods.

[00455] RNA effector molecule expression plasmids can be transfected into target cells as a complex with cationic lipid carriers (e.g., OLIGOFECTAMINE™ transfection reagent) or non-cationic lipid-based carriers (e.g., TRANSIT-TKO® transfection reagent, Mirus Bio LLC, Madison, WI). Multiple lipid transfections for RNA effector molecule-mediated knockdowns targeting different regions of a target RNA over a period of a week or more are also contemplated by the invention. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells ex vivo can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (e.g., antibiotics and drugs), such as hygromycin B resistance. RNA effector molecule expression plasmids can be transfected into target cells as a complex with cationic lipid carriers (e.g., OLIGOFECTAMINE™ reagent) or non-cationic lipid-based carriers (e.g., TRANSIT-TKO® transfection reagent). Multiple lipid transfections for RNA effector molecule-mediated knockdowns targeting different regions of a target RNA over a period of a week or more are also contemplated by the invention. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as GFP. Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (e.g., antibiotics and drugs), such as hygromycin B resistance.

[00456] Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, etc.; (c) adeno-associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, e.g., vaccinia virus vectors or avipox, e.g. canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, e.g., EPV and EBV vectors. Constructs for the recombinant expression of a RNA effector molecule will generally require regulatory elements, e.g., promoters, enhancers, etc., to ensure the expression of the RNA

effector molecule in target cells. Other aspects to consider for vectors and constructs are further described herein.

[00457] Vectors useful for the delivery of a RNA effector molecule will include regulatory elements (promoter, enhancer, etc.) sufficient for expression of the RNA effector molecule in the desired target cell or tissue. The regulatory elements can be chosen to provide either constitutive or regulated/inducible expression.

[00458] Expression of the RNA effector molecule can be precisely regulated, for example, by using an inducible regulatory sequence that is sensitive to certain physiological regulators, e.g., glucose levels. Docherty et al., 8 FASEB J. 20-24 (1994). Such inducible expression systems, suitable for the control of dsRNA expression in cells include, for example, regulation by ecdysone, estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-β-D1 -thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the RNA effector molecule transgene.

[00459] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding a RNA effector molecule can be used. For example, a retroviral vector can be used. *See* Miller et al., 217 Meth. Enzymol. 581-99 (1993); U.S. Patent No. 6,949,242. Retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding a RNA effector molecule are cloned into one or more vectors, which facilitates delivery of the nucleic acid into a cell. More detail about retroviral vectors can be found, for example, in Boesen et al., 6 Biotherapy 291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy include Clowes et al., 93 J. Clin. Invest. 644-651 (1994); Kiem et al., 83 Blood 1467-73 (1994); Salmons & Gunzberg, 4 Human Gene Ther. 129-11 (1993); Grossman & Wilson, 3 Curr. Opin. Genetics Devel. 110-14 (1993). Lentiviral vectors contemplated for use include, for example, the HIV based vectors described in U.S. Patents No 6,143,520; No. 5,665,557; and No. 5,981,276.

[00460] It should be noted, as discussed herein, that host cell-surface receptors for retroviral entry can be inhabited by ERV Env proteins (virus interference). *See* Miller, 93 PNAS 11407-13 (1996). The retroviral envelope (Env) protein mediates the binding of virus particles to their cellular receptors, enabling virus entry: the first step in a new replication cycle. If an ERV is expressed in a cell, re-infection by a related exogenous retrovirus is prevented through interference (also called receptor interference): the Env protein of an ERV that is

inserted into the cell membrane will interfere with the corresponding exogenous virus by receptor competition. This protects the cell from being overloaded with retroviruses. For example, enJSRVs can block the entry of exogenous JSRVs because they all utilize the cellular hyaluronidase-2 as a receptor. Spencer et al., 77 J. Virol. 5749-53 (2003). It is noteworthy that defective ERVs are no less interfering. Two enJSRVs, enJS56A1 and enJSRV-20, contain a mutant Gag polyprotein that can interfere with the late stage replication of exogenous JSRVs. Arnaud et al., 2 PLoS e170 (2007). Thus, interference between defective and replication-competent retroviruses provides an important mechanism of ERV copy number control. Receptor interference by ERV-expressed Env molecules (e.g., expressed by the HERV-H family) can hinder transfection or re-infection of cells intended to produce recombinant proteins. Such effects can explain low copy number or low expression in retroviral vector-mediated recombinant host cells, such as host cells transfected with two retroviral vectors, each encoding a single, complementary antibody chain. Hence, a target gene of the present embodiments that inhibits expression of ERV Env protein(s) provides for increasing retroviral vector multiplicity in host cells and increased yield of immunogenic agent.

[00461] Adenoviruses are also contemplated for use in delivery of RNA effector molecules. A suitable AV vector for expressing a RNA effector molecule featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia et al., 20 Nat. Biotech. 1006-10 (2002).

[00462] Use of Adeno-associated virus (AAV) vectors is also contemplated (Walsh et al., 204 Proc. Soc. Exp. Biol. Med. 289-300 (1993); U.S. Patent No. 5,436,146. In one embodiment, the RNA effector molecule can be expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector having, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter. Suitable AAV vectors for expressing the dsRNA featured in the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski et al., 61 J. Virol. 3096-101 (1987); Fisher et al., 70 J. Virol, 70: 520-32 (1996); Samulski et al., 63 J. Virol. 3822-26 (1989); U.S. Patents No 5,252,479 and No. 5,139,941; WO 94/13788; WO 93/24641.

[00463] Another viral vector is a pox virus such as a vaccinia virus, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox.

[00464] The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral

capsid proteins, as appropriate. For example, lentiviral vectors can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, Baculovirus, and the like. Mononegavirales, e.g., VSV or respiratory syncytial virus (RSV) can be pseudotyped with Baculovirus. U.S. Patent No. 7,041,489. AAV vectors can be made to target different cells by engineering the vectors to express different capsid protein serotypes. *See, e.g.*, Rabinowitz et al., 76 J. Virol. 791-801 (2002).

[00465] In one embodiment, the invention provides compositions containing a RNA effector molecule, as described herein, and an acceptable carrier. The composition containing the RNA effector molecule is useful for enhancing the production of an immunogenic agent by a cell by modulating the expression or activity of a target gene in the cell. Such compositions are formulated based on the mode of delivery. Provided herein are exemplary RNA effector molecules useful in improving the production of an immunogenic agent. In one embodiment, the RNA effector molecule in the composition is a siRNA. Alternatively, the RNA effector molecule in the composition is not a siRNA.

[00466] In another embodiment, a composition is provided herein comprising a plurality of RNA effector molecules that permit inhibition of expression of an immune response pathway and a cellular process; such as INFRA1 or IFNB genes, and PTEN, BAK, FN1 or LDHA genes. The composition can optionally be combined (or administered) with at least one additional RNA effector molecule targeting an additional cellular process including, but not limited to: carbon metabolism and transport, apoptosis, RNAi uptake and/or efficiency, reactive oxygen species production, cell cycle control, protein folding, pyroglutamation protein modification, deamidase, glycosylation, disulfide bond formation, protein secretion, gene amplification, viral replication, viral infection, viral particle release, control of pH, and protein production.

[00467] In one embodiment, the compositions described herein comprise a plurality of RNA effector molecules. In one embodiment of this aspect, each of the plurality of RNA effector molecules is provided at a different concentration. In another embodiment of this aspect, each of the plurality of RNA effector molecules is provided at the same concentration. In another embodiment of this aspect, at least two of the plurality of RNA effector molecules are provided at the same concentration, while at least one other RNA effector molecule in the plurality is provided at a different concentration. It is appreciated one of skill in the art that a variety of combinations of RNA effector molecules and concentrations can be provided to a cell in culture to produce the desired effects described herein.

[00468] In one embodiment, a first RNA effector molecule is administered to a cultured cell, and then a second RNA effector molecule is administered to the cell (or vice versa). In a

further embodiment, the first and second RNA effector molecules are administered to a cultured cell substantially simultaneously.

[00469] In another embodiment, a composition containing a RNA effector molecule described herein, e.g., a dsRNA directed against a host cell target gene, is administered to a cultured cell with a non-RNA agent useful for enhancing the production of an immunogenic by the cell.

[00470] The compositions featured herein are administered in amounts sufficient to inhibit expression of target genes. In general, a suitable dose of RNA effector molecule will be in the range of 0.001 to 200.0 milligrams per unit volume per day. In another embodiment, the RNA effector molecule is provided in the range of 0.001 nM to 200 mM per day, generally in the range of 0.1 nM to 500 nM, inclusive. For example, the dsRNA can be administered at 0.01 nM, 0.05 nM, 0.1 nM, 0.5 nM, 0.75 nM, 1 nM, 1.5 nM, 2 nM, 3 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 100 nM, 200 nM, 400 nM, or 500 nM per single dose.

[00471] The composition can be administered once daily, or the RNA effector molecule can be administered as two, three, or more sub-doses at appropriate intervals throughout the day or delivery through a controlled release formulation. In that case, the RNA effector molecule contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation, which provides sustained release of the RNA effector molecule over a several-day-period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents to a particular site, such as could be used with the agents of the present invention. It should be noted that when administering a plurality of RNA effector molecules, one should consider that the total dose of RNA effector molecules will be higher than when each is administered alone. For example, administration of three RNA effector molecules each at 1 nM (e.g., for effective inhibition of target gene expression) will necessarily result in a total dose of 3 nM to the cell. One of skill in the art can modify the necessary amount of each RNA effector molecule to produce effective inhibition of each target gene while preventing any unwanted toxic effects to the embryo resulting from high concentrations of either the RNA effector molecules or delivery agent.

[00472] The effect of a single dose on target gene transcript levels can be long-lasting, such that subsequent doses are administered at not more than 3-, 4-, or 5-day intervals, or at not more than 1-, 2-, 3-, or 4-week intervals.

[00473] In one embodiment, the administration of the RNA effector molecule is ceased at least 6 hr, at least 12 hr, at least 18 hr, at least 36 hr, at least 48 hr, at least 60 hr, at least 72 hr, at

least 96 hr, or at least 120 hr, or at least 1 week, before isolation of the immunogenic agent. Thus in one embodiment, contacting a host cell (e.g. in a large scale host cell culture) with a RNA effector molecule is complete at least 6 hr, at least 12 hr, at least 18 hr, at least 36 hr, at least 48 hr, at least 60 hr, at least 72 hr, at least 96 hr, or at least 120 hr, or at least 1 week, before isolation of the immunogenic agent.

[00474] It is also noted that, in certain embodiments, it can be beneficial to contact the cells in culture with a RNA effector molecule such that a constant number (or at least a minimum number) of RNA effector molecules per each cell is maintained. Maintaining the levels of the RNA effector molecule as such can ensure that modulation of target gene expression is maintained even at high cell densities.

[00475] Alternatively, the amount of a RNA effector molecule can be administered according to the cell density. In such embodiments, the RNA effector molecule(s) is added at a concentration of at least 0.01 fmol/10^6 cells, at least 0.1 fmol/10^6 cells, at least 0.5 fmol/10^6 cells, at least 0.75 fmol/10^6 cells, at least 1 fmol/10^6 cells, at least 2 fmol/10^6 cells, at least 5 fmol/10^6 cells, at least 10 fmol/10^6 cells, at least 20 fmol/10^6 cells, at least 30 fmol/10^6 cells, at least 40 fmol/10^6 cells, at least 50 fmol/10^6 cells, at least 60 fmol/10^6 cells, at least 100 fmol/10^6 cells, at least 200 fmol/10^6 cells, at least 300 fmol/10^6 cells, at least 400 fmol/10^6 cells, at least 500 fmol/10^6 cells, at least 700 fmol/10^6 cells, at least 800 fmol/10^6 cells, at least 900 fmol/10^6 cells, or at least 1 pmol/10^6 cells, or more.

[00476] In an alternate embodiment, the RNA effector molecule is administered at a dose of at least 10 molecules per cell, at least 20 molecules per cell (molecules/cell), at least 30 molecules/cell, at least 40 molecules/cell, at least 50 molecules/cell, at least 60 molecules/cell, at least 70 molecules/cell, at least 80 molecules/cell, at least 90 molecules/cell at least 100 molecules/cell, at least 200 molecules/cell, at least 300 molecules/cell, at least 400 molecules/cell, at least 500 molecules/cell, at least 600 molecules/cell, at least 700 molecules/cell, at least 800 molecules/cell, at least 900 molecules/cell, at least 1000 molecules/cell, at least 2000 molecules/cell, at least 5000 molecules/cell or more, inclusive.

[00477] In some embodiments, the RNA effector molecule is administered at a dose within the range of 10-100 molecules/cell, 10-90 molecules/cell, 10-80 molecules/cell, 10-70 molecules/cell, 10-60 molecules/cell, 10-50 molecules/cell, 10-40 molecules/cell, 10-30 molecules/cell, 10-20 molecules/cell, 90-100 molecules/cell, 80-100 molecules/cell, 70-100 molecules/cell, 60-100 molecules/cell, 50-100 molecules/cell, 40-100 molecules/cell, 30-100 molecules/cell, 20-100 molecules/cell, 30-60 molecules/cell, 30-50 molecules/cell, 40-50 molecules/cell, 40-60 molecules/cell, or any range there between.

[00478] In one embodiment of the methods described herein, the RNA effector molecule is provided to the cells in a continuous infusion. The continuous infusion can be initiated at day zero (e.g., the first day of cell culture or day of inoculation with a RNA effector molecule) or can be initiated at any time period during the immunogen production process. Similarly, the continuous infusion can be stopped at any time point during the immunogenic agent production process. Thus, the infusion of a RNA effector molecule or composition can be provided and/or removed at a particular phase of cell growth, a window of time in the production process, or at any other desired time point. The continuous infusion can also be provided to achieve a "desired average percent inhibition" for a target gene, as that term is used herein.

[00479] In one embodiment, a continuous infusion can be used following an initial bolus administration of a RNA effector molecule to a cell culture. In this embodiment, the continuous infusion maintains the concentration of RNA effector molecule above a minimum level over a desired period of time. The continuous infusion can be delivered at a rate of 0.03 pmol/L of culture/hour to 3 pmol/L of culture/hour, for example, at 0.03 pmol/L/hr, 0.05 pmol/L/hr, 0.08 pmol/L/hr, 0.1 pmol/L/hr, 0.2 pmol/L/hr, 0.3 pmol/L/hr, 0.5 pmol/L/hr, 1.0 pmol/L/hr, 2 pmol/L/hr, or 3 pmol/L/hr, or any value there between.

[00480] In one embodiment, the RNA effector molecule is administered as a sterile aqueous solution. In one embodiment, the the RNA effector molecule is formulated in a non-lipid formulation. In another embodiment, the RNA effector molecule is formulated in a cationic or non-cationic lipid formulation. In still another embodiment, the RNA effector molecule is formulated in a cell medium suitable for culturing a host cell (e.g., a serum-free medium). In one embodiment, an initial concentration of RNA effector molecule(s) is supplemented with a continuous infusion of the RNA effector molecule to maintain modulation of expression of a target gene. In another embodiment, the RNA effector molecule is applied to cells in culture at a particular stage of cell growth (e.g., early log phase) in a bolus dosage to achieve a certain concentration (e.g., 1 nM), and provided with a continuous infusion of the RNA effector molecule.

[00481] The RNA effector molecule(s) can be administered once daily, or the RNA effector molecule treatment can be repeated (e.g., two, three, or more doses) by adding the composition to the culture medium at appropriate intervals/frequencies throughout the production of the immunogenic agent. As used herein the term "frequency" refers to the interval at which transfection of the cell culture occurs and can be optimized by one of skill in the art to maintain the desired level of inhibition for each target gene. In one embodiment, RNA effector molecules are contacted with cells in culture at a frequency of every 48 hours. In other

embodiments, the RNA effector molecules are administered at a frequency of e.g., every 4 hr, every 6 hr, every 12 hr, every 18 hr, every 24 hr, every 36 hr, every 72 hr, every 84 hr, every 96 hr, every 5 days, every 7 days, every 10 days, every 14 days, every 3 weeks, or more during the production of the immunogenic agent. The frequency can also vary, such that the interval between each dose is different (e.g., first interval 36 hr; second interval 48 hr; third interval 72 hr, etc).

[00482] The term "***frequency***" can be similarly applied to nutrient feeding of a cell culture during the production of an immunogenic agent. The frequency of treatment with RNA effector molecule(s) and nutrient feeding need not be the same. To be clear, nutrients can be added at the time of RNA effector treatment or at an alternate time. The frequency of nutrient feeding can be a shorter interval or a longer interval tha RNA effector molecule treatment. For example, the dose of RNA effector molecule can be applied at a 48-hour-interval while nutrient feeding can be applied at a 24-hour-interval. During the entire length of the interval for producing the immunogenic product (e.g., 3 weeks) there can be more doses of nutrients tha RNA effector molecules or less doses of nutrients tha RNA effector molecules. Alternatively, the amount of treatments with RNA effector molecule(s) is equal to that of nutrient feedings.

[00483] The frequency of RNA effector molecule treatment can be optimized to maintain an "average percent inhibition" of a particular target gene. As used herein, the term "desired average percent inhibition" refers to the average degree of inhibition of target gene expression over time that is necessary to produce the desired effect and which is below the degree of inhibition that produces any unwanted or negative effects. For example, the desired inhibition of Bax/Bak is typically >80% inhibition to effect a decrease in apoptosis, while the desired average inhibition of LDH can be less (e.g., 70%) as high degrees of LDH average inhibition (e.g., 90%) decrease cell viability. In some embodiments, the desired average percent inhibition is at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., absent). One of skill in the art can use routine cell death assays to determine the upper limit for desired percent inhibition (e.g., level of inhibition that produces unwanted effects). One of skill in the art can also use methods to detect target gene expression (e.g., PERT) to determine an amount of a RNA effector molecule that produces gene modulation. *See* Zhang et al., 102 Biotech. Bioeng. 1438-47 (2009). The percent inhibition is described herein as an average value over time, since the amount of inhibition is dynamic and can fluctuate slightly between doses of the RNA effector molecule.

[00484] In one embodiment of the methods described herein, the RNA effector molecule is added to the culture medium of the cells in culture. The methods described herein can be applied to any size of cell culture flask and/or bioreactor. For example, the methods can be applied in bioreactors or cell cultures of 1 L, 3 L, 5 L, 10 L, 15 L, 40 L, 100 L, 500 L, 1000 L, 2000 L, 3000 L, 4000 L, 5000 L or larger. In some embodiments, the cell culture size can range from 0.01 L to 5000 L, from 0.1 L to 5000 L, from 1 L to 5000 L, from 5 L to 5000 L, from 40 L to 5000 L, from 100 L-5000 L, from 500 L to 5000 L, from 1000-5000 L, from 2000-5000 L, from 3000-5000 L, from 4000-5000 L, from 4500-5000 L, from 0.01 L to 1000 L, from 0.01-500 L, from 0.01-100 L, from 0.01-40 L, from 15-2000 L, from 40-1000 L, from 100-500 L, from 200-400 L, or any integer there between.

[00485] The RNA effector molecule(s) can be added during any phase of cell growth including, but not limited to, lag phase, stationary phase, early log phase, mid-log phase, late-log phase, exponential phase, or death phase. It is preferred that the cells are contacted with the RNA effector molecules prior to their entry into the death phase. In some embodiments, such as when targeting an apoptotic pathway, it may be desired to contact the cell in an earlier growth phase such as the lag phase, early log phase, mid-log phase or late-log phase (e.g., Bax/Bak inhibition). In other embodiments, it may be desired or acceptable to inhibit target gene expression at a later phase in the cell growth cycle (e.g., late-log phase or stationary phase), for example when growth-limiting products such as lactate are formed (e.g., LDH inhibition).

Compositions

[00486] Compositions for enhancing production of an immunogenic agent in cell culture by modulating the expression of a target gene in a host cell are also provided.

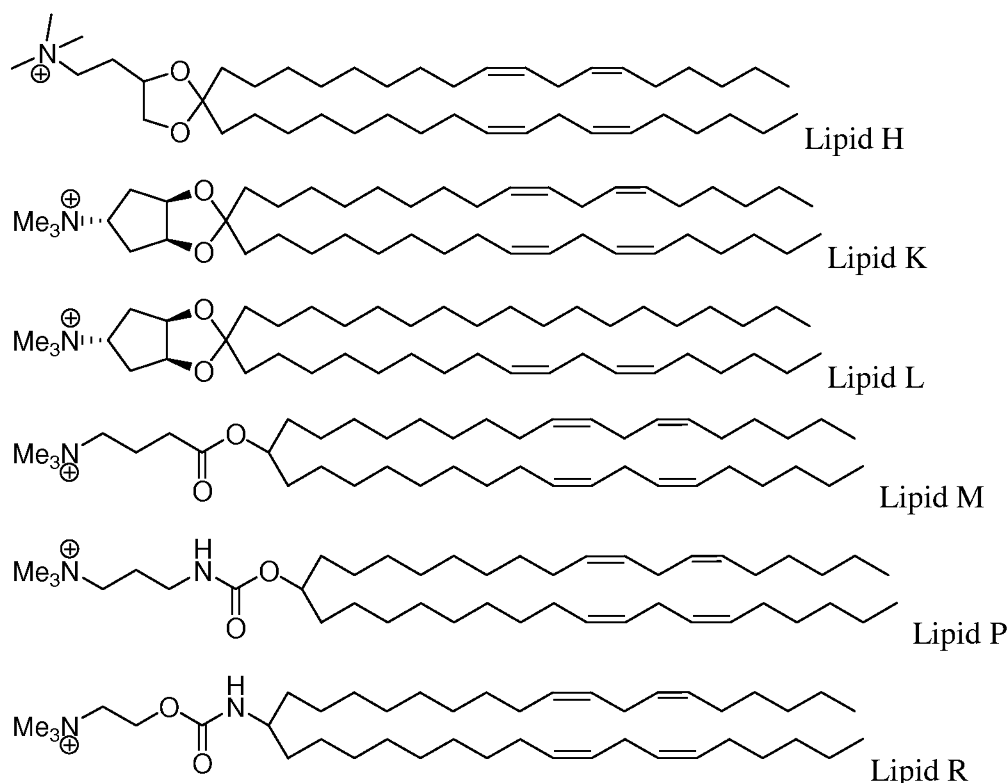
[00487] In one embodiment, the invention provides compositions containing a RNA effector molecule, as described herein, and an acceptable carrier. The composition containing the RNA effector molecule is useful for enhancing the production of an immunogenic agent by a cell by modulating the expression or activity of a target gene in the cell. Such compositions are formulated based on the mode of delivery. Provided herein are exemplary RNA effector molecules useful in improving the production of an immunogenic agent. In one embodiment, the RNA effector molecule in the composition is a siRNA. Alternatively, the RNA effector molecule in the composition is not a siRNA.

[00488] The RNA effector molecule compositions of the invention can be formulated as suspension in aqueous, non-aqueous, or mixed media and can be formulated in a lipid or non-lipid formulations, e.g., as described herein (*see, e.g.*, the instant specification under section

headings: ligand, lipid/oligonucleotide complexes, emulsions, surfactants, penetration enhancers, and additional carriers).

[00489] In one embodiment, the composition comprises at least one RNA effector molecule and a reagent that facilitates RNA effector molecule uptake, for example, an emulsion, a cationic lipid, a non-cationic lipid, a charged lipid, a liposome, an anionic lipid, a penetration enhancer, a transfection reagent or a modification to the RNA effector molecule for attachment, e.g., a ligand, a targeting moiety, a peptide, a lipophillic group, etc.

[00490] In some embodiments, the RNA effector molecule composition comprises a reagent that facilitates RNA effector molecule uptake which comprises "Lipid H" also known as lipid No. 200, "Lipid K" also known as lipid No. 201 or K8; "Lipid L" also known as lipid No. 202 or L8; "Lipid M" also known as lipid No. 203; "Lipid P" also known as lipid No. 204 or P8; or "Lipid R" also known as lipid No. 205, whose formulas are indicated as follows:



[00491] In another embodiment, the composition comprising a RNA effector molecule further comprises a growth medium, e.g. suitable for growth of the host cell. In one embodiment, the growth medium is a chemically defined media such as Biowhittaker® PowerCHO® (Lonza, Basel, Switzerland), Hyclone PF CHO™ (Thermo Scientific, Fisher Scientific), Gibco® CD DG44 (Invitrogen, Carlsbad, CA), Medium M199 (Sigma-Aldrich),

OPTIPRO™ SFM (Gibco), etc.). The RNA effector is ideally present in a concentration such that, when reconstituted, provides the optimal formulation.

[00492] In still another embodiment, the RNA effector molecule composition comprises a growth media supplement, e.g. an agent selected from the group consisting of essential amino acids (e.g., glutamine), 2-mercapto-ethanol, bovine serum albumin (BSA), lipid concentrate, cholesterol, catalase, insulin, human transferrin, superoxide dismutase, biotin, DL α-tocopherol acetate, DL α-tocopherol, vitamins (e.g., Vitamin A (acetate), choline chloride, D-calcium pantothenate, folic acid, Nicotinamide, pyridoxal hydrochloride, riboflavin, thiamine hydrochloride, i-Inositol), corticosterone, D-galactose, ethanolamine HCl, glutathione (reduced), L-carnitine HCl , linoleic acid, linolenic acid, progesterone, putrescine 2HCl, sodium selenite, T3 (triodo-I-thyronine), growth factors (e.g., EGF), iron, L-glutamine, L-alanyl-L-glutamine, sodium hypoxanthine, aminopterin and thymidine, arachidonic acid , ethyl Alcohol 100%, myristic acid, oleic acid, palmitic acid, almitoleic acid, pluronic F-68 ® (Invitrogen, Carlsbad, CA), stearic acid 10, TWEEN 80® nonionic surfactant (Invitrogen), sodium pyruvate, and glucose.

[00493] The RNA effector molecule composition can be provided in a sterile solution or lyophilized. In one embodiment the composition is packaged in discrete units by concentration and/or volume, e.g. to supply RNA effector molecule suitable for administration at various frequencies of administration and dosages, e.g. frequencies and dosages described herein.

[00494] In one embodiment, the composition is formulated for administration to cells according to a dosage regimen described herein, e.g., at a frequency of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 84 hr, 96 hr, 108 hr, or more. Alternatively the composition is formulated at a dosage for continuous infusion.

[00495] Compositions containing two or more RNA effector molecules directed against separate target genes are also provided. The compositions can be used to enhance production of an immunogenic agent in cell culture by modulating expression of a first target gene and at least a second target gene in the cultured cells. In another embodiment, compositions containing two or more RNA effector molecules directed against the same target gene are provided.

Lipid/oligonucleotide complexes

[00496] In some embodiments, a reagent that facilitates RNA effector molecule uptake comprises a charged lipid, an emulsion, a liposome, a cationic or non-cationic lipid, an anionic lipid, a transfection reagent or a penetration enhancer as described herein. In one embodiment, the reagent that facilitates RNA effector molecule uptake used herein comprises a charged lipid as described in U.S. Application Ser. No. 61/267,419, filed 7 December 2009.

[00497] The oligonucleotides of the present invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, RNA effector molecules can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C1-20 alkyl ester (e.g., isopropylmyristate IPM), monoglyceride, diglyceride, or acceptable salts thereof.

[00498] In one embodiment, the RNA effector molecules are fully encapsulated in the lipid formulation (e.g., to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle). The term "SNALP" refers to a stable nucleic acid-lipid particle: a vesicle of lipids coating a reduced aqueous interior comprising a nucleic acid such as a RNA effector molecule or a plasmid from which a RNA effector molecule is transcribed. SNALPs are described, e.g., in U.S. Patent Pubs. No. 2006/0240093, No. 2007/0135372; No. 2009/0291131; U.S. Patent Applications Ser. No. 12/343,342; No.12/424,367. The term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in WO 00/03683. The particles in this enbodiment typically have a mean diameter of about 50 nm to about 150 nm, or about 60 nm to about 130 nm, or about 70 nm to about 110 nm, or typically about 70 nm to about 90 nm, inclusive, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid- lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are reported in, e.g., U.S. Patents No. 5,976,567; No. 5,981,501; No. 6,534,484; No. 6,586,410; No. 6,815,432; and WO 96/40964.

[00499] The lipid to drug ratio (mass/mass ratio) (e.g., lipid to dsRNA ratio) can be in ranges of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1, inclusive.

[00500] A cationic lipid of the formulation can comprise at least one protonatable group having a pKa of from 4 to 15. The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I-(2,3- dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I-

(2,3- dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1 ,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane, or a mixture thereof. The cationic lipid can comprise from about 20 mol% to about 70 mol%, inclusive, or about 40 mol% to about 60 mol%, inclusive, of the total lipid present in the particle. In one embodiment, cationic lipid can be further conjugated to a ligand.

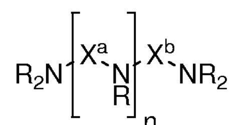
[00501] A non-cationic lipid can be an anionic lipid or a neutral lipid, such as distearoyl-phosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoyl-phosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoyl-phosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoyl-phosphatidylcholine (POPC), palmitoyloleoyl- phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1 -stearoyl-2-oleoyl- phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be from about 5 mol% to about 90 mol%, inclusive, of about 10 mol%, to about 58 mol%, inclusive, if cholesterol is included, of the total lipid present in the particle.

[00502] The lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA can be, for example, a PEG-dilauryloxypropyl (C12), a PEG-dimyristyloxypropyl (C14), a PEG-dipalmityloxypropyl (C16), or a PEG- distearyloxypropyl (C18). The lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol %

or about 2 mol % of the total lipid present in the particle. In one embodiment, PEG lipid can be further conjugated to a ligand.

[00503] In some embodiments, the nucleic acid-lipid particle further includes a steroid such as, cholesterol at, e.g., about 10 mol% to about 60 mol%, inclusive, or about 48 mol% of the total lipid present in the particle.

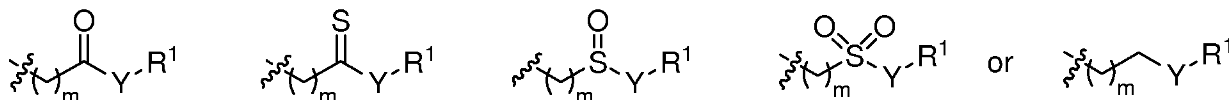
[00504] In one embodiment, the lipid particle comprises a steroid, a PEG lipid and a cationic lipid of formula (I):



formula (I)

wherein each Xa and Xb, for each occurrence, is independently C1-6 alkylene;

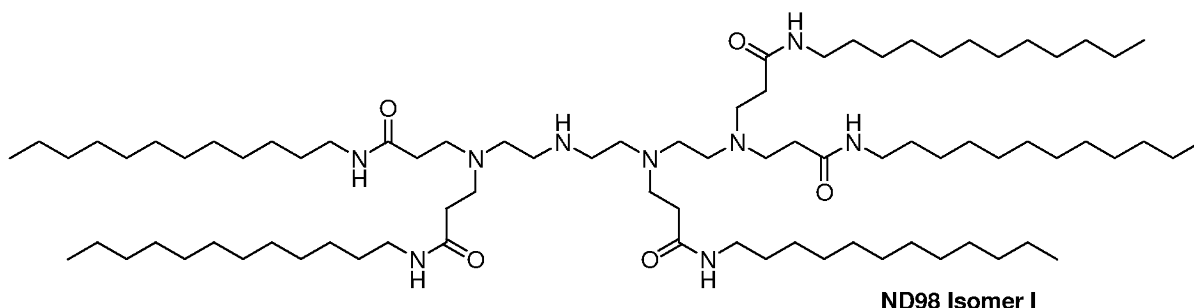
n is 0, 1, 2, 3, 4, or 5; each R is independently H,



m is 0, 1, 2, 3 or 4; Y is absent, O, NR^2, or S; R^1 is alkyl alkenyl or alkynyl; each of which is optionally substituted with one or more substituents; and R^2 is H, alkyl alkenyl or alkynyl; each of which is optionally substituted each of which is optionally substituted with one or more substituents.

[00505] In one example, the lipidoid ND98·4HCl (MW 1487) (Formula 2), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid RNA effector molecule nanoparticles (e.g., LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/mL; Cholesterol, 25 mg/mL; PEG-Ceramide C16, 100 mg/mL. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined, for example, in a 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous RNA effector molecule (e.g., in sodium acetate pH 5) such that the final ethanol concentration is about 35% to 45% and the final sodium acetate concentration is about 100 mM to 300 mM, inclusive. Lipid RNA effector molecule nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (e.g., 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged

with, for example, phosphate buffered saline (PBS) at about pH 7, e.g., about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula 2

LNP01 formulations are described elsewhere, e.g., WO 2008/042973.

[00506] In one embodiment, the reagent that facilitates RNA effector molecule uptake used herein comprises a charged lipid as described in U.S. Application Ser. No. 61/267,419, filed 7 December 2009, and U.S. Application Ser. No. 61/334,398, filed 13 May 2010. In various embodiments, the RNA effector molecule composition described herein comprises “Lipid H” also known as lipid No. 200, “Lipid K” also known as lipid No. 201 or K8; “Lipid L” also known as lipid No. 202 or L8; “Lipid M” also known as lipid No. 203; “Lipid P” also known as lipid No. 204 or P8; or “Lipid R” also known as lipid No. 205, whose formulas are indicated as follows:

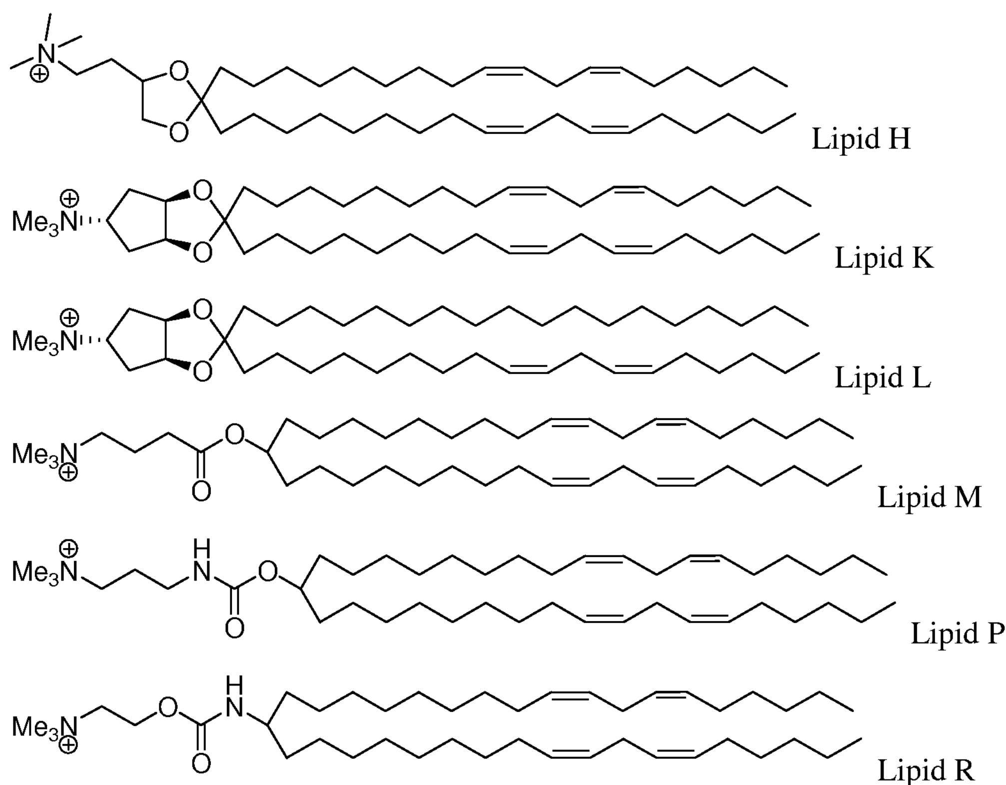


Table 19. Example lipid formulations				
Formulation Number	Cationic Lipid Number	Cationic Lipid Mol %	DOPE %	Cholesterol %
1	200 (Lipid H)	48.08	51.92	-
2	200 (Lipid H)	47.94	47.06	5
3	201 (Lipid K)	45.56	54.44	-
4 (K8)	201 (Lipid K)	47.94	47.06	5
5 (L8)	202 (Lipid L)	47.94	47.06	5
6	203 (Lipid M)	53.01	44.49	2.5
7	203 (Lipid M)	47.94	47.06	5
8 (P8)	204 (Lipid P)	47.94	47.06	5
9	205 (Lipid R)	47.94	47.06	5

[00507] In another embodiment, the RNA effector molecule composition described herein further comprises a lipid formulation comprising a lipid selected from the group consisting of Lipid H, Lipid K, Lipid L, Lipid M, Lipid P, and Lipid R, and further comprises a neutral lipid and a sterol. In particular embodiments, the lipid formulation comprises between approximately 25 mol % - 100 mol% of the lipid. In another embodiment, the lipid formulation comprises between 0 mol% - 50 mol% cholesterol. In still another embodiment, the lipid formulation comprises between 30 mol% - 65 mol% of a neutral lipid. In particular embodiments, the lipid formulation comprises the relative mol % of the components as listed in Table 20 as follows:

Table 20. Example lipid formulae			
Series	Lipid (Mol%)	DOPE	Chol
1	45.56	54.44	0
2	48.08	51.92	0
3	50.60	49.40	0
4	53.10	46.90	0
5	52.73	37.27	10
6	52.92	42.08	5
7	53.01	44.49	2.5
8	47.94	47.06	5

[00508] Additional exemplary lipid-siRNA formulations are as shown in Table 69, as follows:

Table 69. Lipid-siRNA formulations

	Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio	Process
SNALP	1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1	

SNALP-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1	
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1	Extrusion
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1	Extrusion
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1	In-line mixing
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1	In-line mixing
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing

[00509] LNP09 formulations and XTC comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/239,686, filed September 3, 2009. LNP11 formulations and MC3 comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/244,834, filed September 22, 2009. LNP12 formulations and TechG1 comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/175,770, filed May 5, 2009.

[00510] Formulations prepared by either the standard or extrusion-free method can be characterized in similar manners. For example, formulations are typically characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles can be measured by light scattering using, for example, a Malvern Zetasizer Nano ZS (Malvern, PA). Particles should be about 20 nm to 300 nm, such as 40 nm to 100 nm in size. The particle size distribution should be unimodal. The total RNA effector molecule concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated RNA effector molecule can be incubated with a RNA-binding dye, such as Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant,

e.g., 0.5% Triton-X100. The total RNA effector molecule in the formulation can be determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the "free" RNA effector molecule content (as measured by the signal in the absence of surfactant) from the total RNA effector molecule content. Percent entrapped RNA effector molecule is typically >85%. For lipid nanoparticle formulation, the particle size is at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm, at least 100 nm, at least 110 nm, or at least 120 nm. The suitable range is typically about at least 50 nm to about at least 110 nm, about at least 60 nm to about at least 100 nm, or about at least 80 nm to about at least 90 nm, inclusive.

[00511] Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo*. In order to cross intact cell membranes, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

[00512] Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; and liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation. *See, e.g.,* Wang et al., DRUG DELIV. PRINCIPLES & APPL. (John Wiley & Sons, Hoboken, NJ, 2005); Rosoff, 1988. Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

[00513] Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent can act. Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

[00514] Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged polynucleotide molecules to form a stable complex. The positively charged polynucleotide/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm. Wang et al., 147 Biochem. Biophys. Res. Commun., 980-85 (1987).

[00515] Liposomes which are pH-sensitive or negatively-charged, entrap polynucleotide rather than complex with it. Because both the polynucleotide and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some polynucleotide is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells. Zhou et al., 19 J. Controlled Rel. 269-74 (1992).

[00516] One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

[00517] Liposomes also include “sterically stabilized” liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside GM1, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES). Allen et al., 223 FEBS Lett. 42 (1987); Wu et al., 53 Cancer Res. 3765 (1993).

[00518] Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (507 Ann. N.Y. Acad. Sci. 64 (1987)), reported the ability of monosialoganglioside GM1, galactocerebroside sulfate and phosphatidylinositol to improve

blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (85 PNAS 6949 (1988)). U.S. Patent No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside GM1 or a galactocerebroside sulfate ester. U.S. Patent No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al.).

[00519] Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (53 Bull. Chem. Soc. Jpn. 2778 (1980)) described liposomes comprising a nonionic detergent, 2C1215G, that contains a PEG moiety. Illum et al. (167 FEBS Lett. 79 (1984)), noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (e.g., PEG) are described by Sears (U.S. Patents No. 4,426,330 and No. 4,534,899). In addition, antibodies can be conjugated to a polyakylene derivatized liposome (see e.g., U.S. Application Pub. No. 2008/0014255). Klibanov et al. (268 FEBS Lett. 235 (1990)), described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (1029 Biochim. Biophys. Acta 1029, (1990)), extended such observations to other PEG-derivatized phospholipids, e.g., DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. 0 445 131 B1 and WO 90/04384 to Fisher.

[00520] Liposome compositions containing 1 mol% to 20 mol% of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Patents No. 5,013,556; No. 5,356,633) and Martin et al. (U.S. Patent No. 5,213,804; European Patent No. 0 496813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Patent No. 5,225,212 and in WO 94/20073. Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391. U.S. Patents No. 5,540,935 and No. 5,556,948 describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces. Methods and compositions relating to liposomes comprising PEG can be found in, e.g., U.S. Patents No. 6,049,094; No. 6,224,903; No. 6,270,806; No. 6,471,326; No. 6,958,241.

[00521] As noted above, liposomes can, optionally, be prepared to contain surface groups, such as antibodies or antibody fragments, small effector molecules for interacting with cell-

surface receptors, antigens, and other like compounds, and these groups can facilitate delivery of liposomes and their contents to specific cell populations. Such ligands can be included in the liposomes by including in the liposomal lipids a lipid derivatized with the targeting molecule, or a lipid having a polar-head chemical group that can be derivatized with the targeting molecule in preformed liposomes. Alternatively, a targeting moiety can be inserted into preformed liposomes by incubating the preformed liposomes with a ligand-polymer-lipid conjugate.

[00522] Lipids can be derivatized using a variety of targeting moieties, such as ligands, cell surface receptors, glycoproteins, vitamins (e.g., riboflavin) and monoclonal antibodies by covalently attaching the ligand to the free distal end of a hydrophilic polymer chain, which is attached at its proximal end to a vesicle-forming lipid. There are a wide variety of techniques for attaching a selected hydrophilic polymer to a selected lipid and activating the free, unattached end of the polymer for reaction with a selected ligand, and as noted above, the hydrophilic polymer polyethyleneglycol (PEG) has been studied widely. Allen et al., 1237 Biochem. Biophys. Acta 99-108 (1995); Zalipsky, 4 Bioconj. Chem. 296-99 (1993); Zalipsky et al., 353 FEBS Lett. 1-74 (1994); Zalipsky et al., Bioconj. Chem. 705-08 (1995); Zalipsky, *in* STEALTH LIPOSOMES (Lasic & Martin, eds. CRC Press, Boca Raton, FL, 1995).

[00523] A number of liposomes comprising nucleic acids are known in the art, such as methods for encapsulating high molecular weight nucleic acids in liposomes. WO 96/40062. U.S. Patent No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes can include a dsRNA. U.S. Patent No. 5,665,710 describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 refers to liposomes comprising dsRNAs targeted to the *raf* gene. In addition, methods for preparing a liposome composition comprising a nucleic acid can be found in, e.g., U.S. Patents No. 6,011,020; No. 6,074,667; No. 6,110,490; No. 6,147,204; No. 6,271,206; No. 6,312,956; No. 6,465,188; No. 6,506,564; No. 6,750,016; No. 7,112,337.

[00524] Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g., they are self-optimizing, self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition.

[00525] Encapsulated nanoparticles can also be used for delivery of RNA effector molecules. Examples of such encapsulated nanoparticles include those created using yeast cell

wall particles (YCWP). For example, glucan-encapsulated siRNA particles (GeRPs) are payload delivery systems made up of a yeast cell wall particle (YCWP) exterior and a multilayered nanoparticle interior, wherein the multilayered nanoparticle interior has a core comprising a payload complexed with a trapping agent. Glucan-encapsulated delivery systems, such as those described in U.S. Patent Applications Ser. No. 12/260,998, filed October 29, 2008, can be used to deliver siRNA duplexes to achieve silencing *in vitro* and *in vivo.*

Emulsions

[00526] The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 µm in diameter. *See, e.g.,* Ansel's PHARM. DOSAGE FORMS & DRUG DELIV. SYS. (8th ed. Allen et al., eds., Lippincott Williams & Wilkins, NY, 2004); Idson, *in* 1 PHARM. DOSAGE FORMS 199 (Lieberman et al., eds., Marcel Dekker, Inc., NY, 1988); Rosoff, *in* 1 PHARM. DOSAGE FORMS 245 (Lieberman et al., eds., Marcel Dekker, Inc., NY, 1988); Block *in* 2 PHARM. DOSAGE FORMS 335 (Lieberman et al., eds., Marcel Dekker, Inc., NY, 1988); Higuchi et al., *in* REMINGTON'S PHARM. SCI. 301 (Mack Publishing Co., Easton, PA, 1985). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other.

[00527] In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

[00528] Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or

continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids. *See, e.g.,* ANSEL'S PHARM. DOSAGE FORMS & DRUG DELIV. SYS., 2004; Idson, *in* PHARM. DOSAGE FORMS,1988.

[00529] Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature. *See, e.g.,* ANSEL'S PHARM. DOSAGE FORMS & DRUG DELIV. SYS., 2004; Idson, *in* PHARM. DOSAGE FORMS,1988; Rieger, *in* PHARM. DOSAGE FORMS,1988. Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric. *See, e.g.,* ANSEL'S PHARM. DOSAGE FORMS & DRUG DELIV. SYS., 2004; Idson, *in* PHARM. DOSAGE FORMS,1988; Rieger, *in* PHARM. DOSAGE FORMS,1988.

[00530] Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

[00531] A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants. Block, *in* 1 PHARM. DOSAGE FORMS 335 (Lieberman et al., eds., Marcel Dekker, Inc., NY, 1988); Idson, *in* PHARM. DOSAGE FORMS (1988).

[00532] Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan,

guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

[00533] Because emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

[00534] In one embodiment, the compositions of RNA effector molecules and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution. *See, e.g.*, ANSEL'S PHARM. DOSAGE FORMS & DRUG DELIV.SYS. (8th ed., Allen et al, eds., Lippincott Williams & Wilkins, NY, 2004); Rosoff, *in* PHARM. DOSAGE FORMS, 1988. Typically, microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules. Leung & Shah, *in* CONTROLLED RELEASE DRUGS: POLYMERS & AGGREGATE SYS. 185-215 (Rosoff, ed., VCH Publishers, NY, 1989). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules. Schott, *in* REMINGTON'S PHARM. SCI. 271 (1985).

[00535] The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions. *See, e.g.*, ANSEL'S PHARM. DOSAGE FORMS & DRUG DELIV.SYS. (8th

ed., Allen et al, eds., Lippincott Williams & Wilkins, NY, 2004); Rosoff, 1988; Block, 1988. Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

[00536] Microemulsions can include surfactants, discussed further herein, not limited to ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

[00537] Microemulsions afford advantages of better drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, and decreased toxicity. *See, e.g.*, U.S. Patents No. 6,191,105; No. 7,063,860; No. 7,070,802; No. 7,157,099; Constantinides et al., 11 Pharm. Res. 1385 (1994); Ho et al., 85 J. Pharm. Sci. 138-43 (1996). Often, microemulsions can form spontaneously when their components are brought together at ambient temperature. This can be particularly advantageous when formulating thermolabile drugs, peptides or RNA effector molecules.

[00538] Microemulsions of the present invention can also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the RNA effector molecules and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention can be classified as belonging to one of five broad

categories--surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Lee et al., Crit. Rev. Therapeutic Drug Carrier Sys. 92 (1991).

[00539] There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

Surfactants

[00540] In some embodiments, RNA effector molecules featured in the invention are formulated in conjunction with one or more penetration enhancers, surfactants and/or chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxy-cholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (e.g., sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether.

[00541] Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations. *See e.g.*, Malmsten, SURFACTANTS & POLYMERS IN DRUG DELIV. (Informa Health Care, NY, 2002); Rieger, *in* PHARM. DOSAGE FORMS 285 (Marcel Dekker, Inc., NY, 1988).

[00542] If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18

depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

[00543] If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

[00544] If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class. If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

Penetration enhancers

[00545] In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly RNA effector molecules, to the cell. Most drugs are present in solution in both ionized and nonionized forms. Usually, only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

[00546] Penetration enhancers can be classified as belonging to one of five broad categories: surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. *See, e.g.,* Malmsten, 2002; Lee et al., Crit. Rev. Therapeutic Drug Carrier Sys. 92 (1991).

[00547] In connection with the present invention, penetration enhancers include surfactants (or “surface-active agents”), which are chemical entities that, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of RNA effector molecules

through cellular membranes and other biological barriers is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (*see, e.g.,* Malmsten, 2002; Lee et al., 1991); and perfluorochemical emulsions, such as FC-43 (Takahashi et al., 40 J. Pharm. Pharmacol. 252 (1988)).

[00548] Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacyclo-heptan-2-one, acylcarnitines, acylcholines, C1-20 alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.). *See, e.g.,* Touitou et al., ENHANCEMENT IN DRUG DELIV. (CRC Press, Danvers, MA, 2006); Lee et al., 1991; Muranishi, 7 Crit. Rev. Therapeutic Drug Carrier Sys. 1-33 (1990); El Hariri et al., 44 J. Pharm. Pharmacol. 651-54 (1992).

[00549] The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins. *See, e.g.,* Malmsten, 2002; Brunton, Chapt. 38 *in* GOODMAN & GILMAN'S PHARMACOLOGICAL BASIS THERAPEUTICS, 9TH ED. 934-35 (Hardman et al., eds., McGraw-Hill, NY, 1996). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (*see e.g.*, Malmsten, 2002; Lee et al., 1991; Swinyard, Chapt. 39 *in* REMINGTON'S PHARM. SCI., 18th Ed. 782-83 (Gennaro, ed., Mack Publishing Co., Easton, PA, 1990); Muranishi, 1990; Yamamoto et al., 263 J. Pharm. Exp. Ther. 25 (1992); Yamashita et al., 79 J. Pharm. Sci. 579-83 (1990).

[00550] Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of RNA effector molecules through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added

advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents. Jarrett, 618 J. Chromatogr. 315-39 (1993). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines). *See, e.g.*, Katdare et al., EXCIPIENT DEVEL. PHARM. BIOTECH. & DRUG DELIV. (CRC Press, Danvers, MA, 2006); Lee et al., 1991; Muranishi, 1990; Buur et al., 14 J. Control Rel. 43-51 (1990).

[00551] As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of RNA effector molecules through the alimentary mucosa. *See e.g.,* Muranishi, 1990. This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., 1991); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., 1987).

[00552] Agents that enhance uptake of RNA effector molecules at the cellular level can also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (U.S. Patent No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (WO 97/30731), are also known to enhance the cellular uptake of dsRNAs. Examples of commercially available transfection reagents include, for example LIPOFECTAMINE™, LIPOFECTAMINE 2000™, 293FECTIN™, CELLFECTIN™, DMRIE-C™, FREESTYLE™ MAX, LIPOFECTAMINE™ 2000 CD, LIPOFECTAMINE™, RNAiMAX, OLIGOFECTAMINE™, and OPTIFECT™ transfection reagents (each from Invitrogen); and X-tremeGENE Q2 Transfection Reagent (Roche Applied Science; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Avante Polar Lipids, Inc., Alabaster, AL), DOSPER Liposomal Transfection Reagent (Roche), or FuGENE® (Promega; Madison, WI) or TRANSFECTAM® Reagent (Promega), TRANSFAST™ Transfection Reagent (Promega), TFX™-20 Reagent (Promega), TFX™-50 Reagent (Promega); DREAMFECT™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences); TRANSPASS® D1 Transfection Reagent (New England Biolabs; Ipswich, MA); LYOVEC™/LIPOGEN™ (InvivoGen; San Diego, CA); PerFectin Transfection Reagent (Genlantis; San Diego, CA), NEUROPORTER Transfection Reagent (Genlantis), GENEPORTER Transfection reagent (Genlanti), GENEPORTER 2 Transfection reagent (Genlantis), CYTOFECTIN Transfection Reagent (Genlantis), BACULOPORTER Transfection Reagent (Genlantis),

TROGANPORTER™ transfection reagent (Genlantis); RIBOFECT (Bioline; Taunton, MA, U.S.), PLASFECT (Bioline), UNIFECTOR (B-Bridge International; Mountain View, CA), SUREFECTOR (B-Bridge International), or HIFECT™ (B-Bridge Int'l), among others.

Additional carriers

[00553] Other agents can be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

[00554] Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal.

[00555] The compositions of the present invention can additionally contain other adjunct components so long as such materials, when added, do not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents that do not deleteriously interact with the RNA effector molecules of the formulation.

[00556] Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

[00557] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or in cells, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit high therapeutic indices are particularly useful. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages for use in the instant methods. The dosage of compositions featured in the invention lies generally within a range of concentrations that includes the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized.

[00558] In yet another aspect, the invention provides a method for inhibiting the expression of a target gene in a host cell by administering a composition featured in the invention to the host cell such that expression of the target gene is decreased for an extended

duration, e.g., at least two, three, four days or more, e.g., one week, two weeks, three weeks, or four weeks or longer. The effect of the decreased expression of the target gene preferably results in a decrease in levels of the protein encoded by the target gene by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, or at least 60%, or more, as compared to pretreatment levels.

VII. Kits and Assays

[00559] In some embodiments, kits are provided for testing the effect of a RNA effector molecule or a series of RNA effector molecules on the production of an immunogenic agent by the cell, where the kits comprise a substrate having one or more assay surfaces suitable for culturing cells under conditions that allow production of an immunogenic agent. In some embodiments, the exterior of the substrate comprises wells, indentations, demarcations, or the like at positions corresponding to the assay surfaces. In some embodiments, the wells, indentations, demarcations, or the like retain fluid, such as cell culture media, over the assay surfaces.

[00560] In some embodiments, the assay surfaces on the substrate are sterile and are suitable for culturing host cells under conditions representative of the culture conditions during large-scale (e.g., industrial scale) production of the immunogenic agent. Advantageously, kits provided herein offer a rapid, cost-effective means for testing a wide-range of agents and/or conditions on the production of an immunogenic agent, allowing the cell culture conditions to be established prior to full-scale production of the immunogenic agent.

[00561] In some embodiments, one or more assay surfaces of the substrate comprise a concentrated test agent, such as a RNA effector molecule, such that the addition of suitable media to the assay surfaces results in a desired concentration of the RNA effector molecule surrounding the assay surface. In some embodiments, the RNA effector molecules can be printed or ingrained onto the assay surface, or provided in a lyophilized form, e.g., within wells, such that the effector molecules can be reconstituted upon addition of an appropriate amount of media. In some embodiments, the RNA effector molecules are reconstituted by plating cells onto assay surfaces of the substrate.

[00562] In some embodiments, kits provided herein further comprise cell culture media suitable for culturing a cell under conditions allowing for the production of an immunogenic agent of interest. The media can be in a ready to use form or can be concentrated (e.g., as a stock solution), lyophilized, or provided in another reconstitutable form.

[00563] In further embodiments, kits provided herein further comprise one or more reagents suitable for detecting production of the immunogenic agent by the cell, cell culture, or tissue culture. In further embodiments, the reagent(s) are suitable for detecting a property of the cell, such as maximum cell density, cell viability, or the like, which is indicative of production of the desired immunogenic agent. In some embodiments, the reagent(s) are suitable for detecting the immunogenic agent or a property thereof, such as the *in vitro* or *in vivo* biological activity, homogeneity, or structure of the immunogenic agent.

[00564] In some embodiments, one or more assay surfaces of the substrate further comprise a carrier for which facilitates uptake of RNA effector molecules by cells. Carriers for RNA effector molecules are known in the art and are described herein. For example, in some embodiments, the carrier is a lipid formulation such as LIPOFECTAMINE™ transfection reagent (Invitrogen; Carlsbad, CA) or a related formulation. Examples of such carrier formulations are described herein. In some embodiments, the reagent that facilitates RNA effector molecule uptake comprises a charged lipid, an emulsion, a liposome, a cationic or non-cationic lipid, an anionic lipid, a transfection reagent or a penetration enhancer as described throughout the application herein. In particular embodiments, the reagent that facilitates RNA effector molecule uptake comprises a charged lipid as described in U.S. Application Ser. No. 61/267,419, filed on December 7, 2009.

[00565] In some embodiments, one or more assay surfaces of the substrate comprise a RNA effector molecule or series of RNA effector molecules and a carrier, each in concentrated form, such that plating test cells onto the assay surface(s) results in a concentration the RNA effector molecule(s) and the carrier effective for facilitating uptake of the RNA effector molecule(s) by the cells and modulation of the expression of one or more genes targeted by the RNA effector molecules.

[00566] In some embodiments, the substrate further comprises a matrix which facilitates 3-dimensional (3-D) cell growth and/or production of the immunogenic agent by the cells. In further embodiments, the matrix facilitates anchorage-dependent growth of cells. Non-limiting examples of matrix materials suitable for use with various kits described herein include agar, agarose, methylcellulose, alginate hydrogel (e.g., 5% alginate + 5% collagen type I), chitosan, hydroactive hydrocolloid polymer gels, polyvinyl alcohol-hydrogel (PVA-H), polylactide-co-glycolide (PLGA), collagen vitrigel, PHEMA (poly(2-hydroxylmethacrylate)) hydrogels, PVP/PEO hydrogels, BD PURAMATRIX™ hydrogels, and copolymers of 2-methacryloyloxyethyl phophorylcholine (MPC).

[00567] In some embodiments, the substrate comprises a microarray plate, a biochip, or the like which allows for the high-throughput, automated testing of a range of test agents, conditions, and/or combinations thereof on the production of an immunogenic agent by cultured cells. For example, the substrate can comprise a 2-dimensional microarray plate or biochip having m columns and n rows of assay surfaces (e.g., residing within wells) which allow for the testing of m x n combinations of test agents and/or conditions (e.g., on a 24, 96 or 384-well microarray plate). The microarray substrates are preferably designed such that all necessary positive and negative controls can be carried out in parallel with testing of the agents and/or conditions.

[00568] In further embodiments, kits are provided comprising one or more microarray substrates seeded with a set of RNA effector molecules designed to modulate a particular pathway, function, or property of a cell which affects the production of the immunogenic agent. For example, in some embodiments, the RNA effector molecules are directed against target genes comprising a pathway involved in the expression, folding, secretion, or post-translational modification of a recombinant immunogenic agent by the cell.

[00569] In further embodiments, kits are provided herein comprising one or more microarray substrates seeded with a set of RNA effector molecules designed to address a particular problem or class of problems associated with the production of an immunogenic agent in cell-based systems. For example, in some embodiments, the RNA effector molecules are directed against target genes expressed by latent or endogenous viruses; or involved in cell processes, such as cell cycle progression, cell metabolism or apoptosis which inhibit or interfere production or purification of the immunogenic agent. In further embodiments, the RNA effector molecules are directed against target genes that mediate enzymatic degradation, aggregation, misfolding, or other processes that reduce the activity, homogeneity, stability, and/or other qualities of the immunogenic agent. In yet further embodiments, the effector molecules are directed against target genes that affect the infectivity of exogenous or adventitious contaminating microbes. In one embodiment, the immunogenic agent includes a glycoprotein, and the RNA effector molecules are directed against target genes involved in glycosylation (e.g., fucosylation) and/or proteolytic processing of glycoproteins by the host cell. In another embodiment, the immunogenic agent is a multi-subunit recombinant protein and the RNA effector molecules are directed against target genes involved in the folding and/or secretion of the protein by the host cell. In another embodiment, the RNA effector molecules are directed against target genes involved in post-translation modification of the immunogenic agent in the

cells, such as methionine oxidation, glycosylation, disulfide bond formation, pyroglutamation and/or protein deamidation.

[00570] In some embodiments, kits provided herein allow for the selection or optimization of a combination o two or more factors in production of the immunogenic agent. For example, the kits can allow for the selection of a suitable RNA effector molecule from among a series of candidate RNA effector molecules as well as a concentration of the RNA effector molecule. In further embodiments, kits provided herein allow for the selection of a first RNA effector molecule from a first series of candidate RNA effector molecules and a second RNA effector molecule from a second series of candidate RNA effector molecules. In some embodiments, the first and/or second series of candidate RNA effector molecules are directed against a common target gene. In further embodiments, the first and/or second series of RNA effector molecules are directed against two or more functionally related target genes or two or more target genes of a common host cell pathway.

[00571] In another embodiment, a kit for enhancing production of an immunogenic agent in a cell, comprising at least a first RNA effector molecule, a portion of which is complementary to at least a first target gene of a latent or endogenous virus; a second RNA effector molecule, a portion of which is complementary to at least a secon target gene of the cellular immune response; and, optionally, a third RNA effector molecule, a portion of which is complementary to at least a third target gene of a cellular process. For example, the first target gene is an ERV *env* gene, the second target gene is a IFNAR1 or IFNB gene, and the third target gene is a PTEN, BAK1, FN1, or LDHA gene. The kit can further comprise at least additional RNA effector molecule that targets a cellular process including, but not limited to, carbon metabolism and transport, apoptosis, RNAi uptake and/or efficiency, reactive oxygen species production, cell cycle control, protein folding, pyroglutamation protein modification, deamidase, glycosylation, disulfide bond formation, protein secretion, gene amplification, viral replication, viral infection, viral particle release, control of cellular pH, and protein production.

[00572] In yet another aspect, the invention provides a method for inhibiting the expression of a target gene in a cell. The method includes administering a composition featured in the invention to the cell such that expression of the target gene is decreased, such as for an extended duration, e.g., at least two, three, four days or more. The RNA effector molecules useful for the methods and compositions featured in the invention specifically target RNAs (primary or processed) of the target gene. Compositions and methods for inhibiting the expression of these target genes using RNA effector molecules can be prepared and performed as described herein.

[00573] The present invention may be as defined in any one of the following numbered paragraphs:

1. A method for producing an immunogenic agent in a large scale host cell culture, comprising: (a) contacting a host cell in a large scale host cell culture with at least a first RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell, (b) maintaining the host cell culture for a time sufficient to modulate expression of the at least one first target gene, wherein the modulation of expression improves production of an immunogenic agent in the host cell; (c) isolating the immunogenic agent from the host cell; wherein the large scale host cell culture is at least 1 Liter in size, and wherein the host cell is contacted with at least a first RNA effector molecule by addition of the RNA effector molecule to a culture medium of the large scale host cell culture such that the target gene expression is transiently inhibited.

2. A method for producing an immunogenic agent in a large scale host cell culture, comprising: (a) contacting a host cell in a large scale host cell culture with at least a first RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell; (b) maintaining the host cell culture for a time sufficient to modulate expression of the at least one first target gene, wherein the modulation of expression improves production of an immunogenic agent in the host cell; (c) isolating the immunogenic agent from the host cell; wherein the host cell is contacted with at least a first RNA effector molecule by addition of the RNA effector molecule to a culture medium of the large scale host cell culture multiple times throughout production of the immunogenic agent such that the target gene expression is transiently inhibited.

3. The method of paragraph 1 or 2, wherein the host cell in the large scale host cell culture is contacted with a plurality of RNA effector molecules, wherein the plurality of RNA effector molecules modulate expression of at least one target gene, at least two target genes, or a plurality of target genes.

4. A method for production of an immunogenic agent in a cell, the method comprising: (a) contacting a host cell with a plurality of RNA effector molecules, wherein the two or more RNA effector molecules modulate expression of a plurality of target genes; (b) maintaining the cell for a time sufficient to modulate expression of the plurality of target genes, wherein the modulation of expression improves production of the immunogenic agent in the cell; and (c) isolating the immunogenic agent from the cell, wherein the plurality of target genes comprises at least Bax, Bak, and LDH.

5. The method of paragraph 4, wherein the host cell is contacted with the plurality of RNA effector molecules by addition of the RNA effector molecule to a culture medium of the large scale host cell culture such that the target gene expression is transiently inhibited.

6. The method of paragraphs 1 to 5, wherein the RNA effector molecule, or plurality of RNA effector molecules, comprises a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least part of a target gene, and wherein said region of complementarity is 10-30 nucleotides in length.

7. The method of any of paragraphs 1 to 6, wherein the contacting step is performed by continuous infusion of the RNA effector molecule, or plurality of RNA effector molecules, into the culture medium used for maintaining the host cell culture to produce the immunogenic agent.

8. The method of any of paragraphs 1 to 7, wherein the modulation of expression is inhibition of expression, and wherein the inhibition is a partial inhibition.

9. The method of paragraph 7, wherein the partial inhibition is no greater than a percent inhibition selected from the group consisting of: 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, and 85%.

10. The method of any of paragraphs 1 to 6 or 8-9, wherein the contacting step is repeated at least once.

11. The method of any of paragraphs 1 to 6 or 8-9, wherein the contacting step is repeated multiple times at a frequency selected from the group consisting of: 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 84 hr, 96 hr, and 108 hr.

12. The method of any of paragraphs 1 to 11, wherein the modulation of expression is inhibition of expression and wherein the contacting step is repeated multiple times, or continuously infused, to maintain an average percent inhibition of at least 50% for the target gene(s) throughout the production of the immunogenic agent.

13. The method of paragraph 12, wherein the average percent inhibition is selected from the group consisting of at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100%.

14. The method of any of paragraphs 1 to 13, wherein the RNA effector molecule is contacted at a concentration of less than 100 nM.

15. The method of any of paragraphs 1 to 14, wherein the RNA effector molecule is contacted at a concentration of less than 20 nM.

16. The method of any of paragraphs 1 to 15, wherein said contacting a host cell in a large scale host cell culture with a RNA effector molecule is done at least 6 hr, at least 12 hr, at least 18 hr, at least 36 hr, at least 48 hr, at least 60 hr, at least 72 hr, at least 96 hr, or at least 120 hr, or at least 1 week, before isolation of the immunogenic agent or prior to harvesting the supernatant.

17. The method of any of paragraphs 1 to 16, wherein the RNA effector molecule is composition formulated in a lipid formulation.

18. The method of any of paragraphs 1 to 17, wherein the RNA effector molecule is a composition formulated in a non-lipid formulation.

19. The method of any of paragraphs 1 to 18, wherein the RNA effector molecule is not shRNA.

20. The method of any of paragraphs 1 to 19, wherein the RNA effector molecule is siRNA.

21. The method of any of paragraphs 1 to 20, wherein the RNA effector molecule is chemically modified.

22. The method of any of paragraphs 1 to 21, wherein the RNA effector molecule is not chemically modified.

23. The method of any of paragraphs 1 to 22, further comprising monitoring at least one measurable parameter selected from the group consisting of cell density, medium pH, oxygen levels, glucose levels, lactic acid levels, temperature, and protein production.

24. The method of any of paragraphs 2 to 23, wherein each of the plurality of different RNA effector molecules is added simultaneously or at different times.

25. The method of any of paragraphs 2 to 23, wherein each of the plurality of different RNA effector molecules is added at the same or different concentrations.

26. The method of any of paragraphs 2 to 6 or 8 to 25, wherein the plurality of different RNA effector molecules is added at the same or different frequencies.

27. The method of any of paragraphs 1 to 26, further comprising contacting the cell with a second agent.

28. The method of paragraph 27, wherein the second agent is selected from the group consisting of: an antibody, a growth factor, an apoptosis inhibitor, a kinase inhibitor, a phosphatase inhibitor, a protease inhibitor, and a histone demethylating agent.

29. The method of paragraph 28, wherein the kinase inhibitor is selected from the group consisting of: a MAP kinase inhibitor, a CDK inhibitor, and K252a.

30. The method of paragraph 28, wherein the phosphatase inhibitor is selected from the group consisting of: sodium vanadate and okadaic acid.

31. The method of paragraph 28, wherein the histone demethylating agent is 5-azacytidine.

32. The method of any of paragraphs 1 to 31, wherein the immunogenic agent is a polypeptide.

33. The method of any of paragraphs 1 to 31, wherein the immunogenic agent is a virus.

34. The method of paragraph 33, wherein the virus is PCV.

35. The method of any of paragraphs 1 to 34, wherein the cell is contacted with the RNA effector molecule at a phase of cell growth selected from the group consisting of: stationary phase, early log phase, mid-log phase, late-log phase, lag phase, and death phase.

36. The method of any of paragraphs 1 to 35, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, comprises a duplex region.

37. The method of any of paragraphs 1 to 36, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is 15 to 30 nucleotides in length.

38. The method of any of paragraphs 1 to 37, the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is 17 to 28 nucleotides in length.

39. The method of any one of paragraphs 1 to 38, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, comprises at least one modified nucleotide.

40. The method of any of paragraphs 1 to 39, wherein the cell is a plant cell, a fungal cell, or an animal cell.

41. The method of any of paragraphs 1 to 40, wherein the cell is a mammalian cell.

42. The method of paragraph 41, wherein the mammalian cell is a human cell.

43. The method of paragraph 42, wherein the human cell is an adherent cell selected from the group consisting of: SH-SY5Y cells, IMR32 cells, LAN5 cells, HeLa cells, MCFlOA cells, 293T cells, and SK-BR3 cells.

44. The method of paragraph 42, wherein the human cell is a primary cell selected from the group consisting of: HuVEC cells, HuASMC cells, HKB-Il cells, and hMSC cells.

45. The method of paragraph 42, wherein the human cell is selected from the group consisting of: U293 cells, HEK 293 cells, PERC6® cells, Jurkat cells, HT-29 cells, LNCap.FGC cells, A549 cells, MDA MB453 cells, HepG2 cells, THP-I cells, MCF7 cells, BxPC-3 cells, Capan-1 cells, DU145 cells, and PC-3 cells.

46. The method of paragraph 41, wherein the mammalian cell is a rodent cell selected from the group consisting of: BHK21 cells, BHK(TK–) cells, NS0 cells, Sp2/0 cells, EL4 cells, CHO

cells, CHO cell derivatives, NIH/3T3 cells, 3T3-Ll cells, ES-D3 cells, H9c2 cells, C2C12 cells, Madin Darby canine kidney (MDCK) cells and miMCD 3 cells.

47. The method of paragraph 46, wherein the CHO cell derivative is selected from the group consisting of: CHO-Kl cells, CHO-DUKX, CHO-DUKX B1, and CHO-DG44 cells.

48. The method of paragraph 42, wherein the cell is selected from the group consisting of: PERC6 cells, HT-29 cells, LNCaP-FGC cells A549 cells, MDA MB453 cells, HepG2 cells, THP-1 cells, miMCD-3 cells, HEK 293 cells, HeLaS3 cells, MCF7 cells, Cos-7 cells, BxPC-3 cells, DU145 cells, Jurkat cells, PC-3 cells, and Capan-1 cells,

49. The method of paragraph 41, wherein the cell is a rodent cell selected from the group consisting of: BHK21, BHK(TK–), NS0 cells, Sp2/0 cells, U293 cells, EL4 cells, CHO cells, and CHO cell derivatives.

50. The method of any of paragraphs 1 to 49, wherein the cell further comprises a genetic construct encoding the immunogenic agent.

51. The method of any of paragraphs 1 to 50, wherein the cell further comprises a genetic construct encoding a viral receptor.

52. The method of any of paragraphs 1 to 51, wherein the target gene encodes a protein that affects protein glycosylation.

53. The method of any of paragraphs 1 to 52, wherein the target gene encodes the immunogenic agent.

54. The method of any of paragraphs 1 to 53, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is added at a concentration selected from the group consisting of 0.1 nM, 0.5 nM, 0.75 nM, 1nM, 2 nM, 5 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 75 nM, and 100 nM.

55. The method of any of paragraphs 1 to 53, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is added at an amount of 50 molecules per cell, 100 molecules/cell, 200 molecules/cell, 300 molecules/cell, 400 molecules/cell, 500 molecules/ cell, 600 molecules/cell, 700 molecules/ cell, 800 molecules/cell, 900 molecules/cell, 1000 molecules/cell, 2000 molecules/cell, or 5000 molecules/cell.

56. The method of any of paragraphs 1 to 53, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is added at a concentration selected from the group consisting of: 0.01 fmol/106 cells, 0.1 fmol/106 cells, 0.5 fmol/106 cells, 0.75 fmol/106 cells, 1 fmol/106 cells, 2 fmol/106 cells, 5 fmol/106 cells, 10 fmol/106 cells, 20 fmol/106 cells, 30 fmol/106 cells, 40 fmol/106 cells, 50 fmol/106 cells, 60 fmol/106 cells, 100 fmol/106 cells, 200 fmol/106 cells, 300 fmol/106 cells, 400 fmol/106 cells, 500

fmol/106 cells, 700 fmol/106 cells, 800 fmol/106 cells, 900 fmol/106 cells, and 1 pmol/106 cells.

57. The method of any of paragraphs 1 to 56, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is selected from the group consisting of siRNA, miRNA, dsRNA, saRNA, shRNA, piRNA, tkRNAi, eiRNA, pdRNA, a gapmer, an antagomir, a ribozyme, and any combination thereof.

58. The method of any of paragraphs 1 to 57, wherein the method further comprises contacting the cell with at least one additional RNA effector molecule, or agent, that modulates a cellular process selected from the group consisting of: carbon metabolism and transport, apoptosis, RNAi uptake and/or efficiency, reactive oxygen species production, control of cell cycle, protein folding, protein pyroglutamation, protein deamidation, protein glycosylation, disulfide bond formation, protein secretion, gene amplification, viral replication, viral infection, viral particle release, control of cellular pH, and protein production.

59. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene, is selected from the group consisting of: GLUT1, GLUT2, GLUT3, GLUT4, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN), and lactate dehydrogenase (LDH), and wherein the modulation of expression improves production of a immunogenic agent in the cell by modulating carbon metabolism or transport in the cell.

60. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is lactate dehydrogenase (LDH) and the RNA effector molecule comprises a sequence selected from SEQ ID NOs:3152540-3152603.

61. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene selected from the group consisting of: Bcl-G, Bax, Bak, Bok, Bad, Bid, Bik, Blk, Hrk, BNIP3, PUMA, NOXA, BimL, Bcl-2, Bcl-xL, Bcl-B, Bcl-w, Boo, Mcl-1, CASP2, CASP3, CASP6, CASP7, CASP8, CASP9, and CASP10; and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating apoptosis of the cell.

62. The method of claim any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is Bak and the RNA effector molecule comprises a sequence selected from SEQ ID NOs:3152412-3152475.

63. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is Bax and the RNA effector molecule comprises a sequence selected from SEQ ID NOs:3152476-3152539.

64. The method of paragraph 16 or 17, wherein the RNA effector molecule significantly decreases the fraction of cells that enter early apoptosis.

65. The method of paragraph 3, wherein the plurality of target genes are at least Bax and Bak.

66. The method of paragraph 3, wherein the plurality of target genes are at least Bax, Bac, and LDH.

67. The method of any of paragraphs 4, 5, 65, or 66, wherein the RNA effector molecule, a portion of which is complementary to Bax comprises a sequence selected from SEQ ID NOs:3152476-3152539, wherein the RNA effector molecule, a portion of which is complementary to Bak, comprises a sequence selected fromSEQ ID NOs:3152412-3152475.

68. The method of paragraph 4 or 66, wherein the RNA effector molecule, a portion of which is complementary to LDH, comprises a sequence selected from SEQ ID NOs:3152540-3152603

69. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the expression of at least two target genes is modulated and the at least two target genes are selected from the group consisting of: Bcl-G, Bax, Bak, Bok, Bad, Bid, Bik, Blk, Hrk, BNIP3, PUMA, NOXA, and BimL .

70. The method of claim any of paragraphs 1 to 3, 6 to 58, further comprising contacting the cell with a RNA effector molecule comprising a sequence complementary to lactate dehydrogenase (LDH).

71. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene selected from the group consisting of: Ago1, Ago2, Ago3, Ago4, HIWI1, HIWI2, HIWI3, HILI, interferon receptor, ApoE, Eri1 and mannose/GalNAc-receptor, and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating RNAi uptake and/or efficacy in the cell.

72. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of NAD(p)H oxidase, peroxidase, constitutive neuronal nitric oxide synthase (cnNOS), myeloperoxidase (MPO), xanthine oxidase (XO), 15-lipoxygenase-1, NADPH cytochrome c2 reductase, NAPH cytochrome c reductase, NADH cytochrome b5 reductase, and cytochrome P4502E1, and wherein the modulation of expression improves production of the immunogenic agent in the cell by inhibiting production of reactive oxygen species in the cell.

73. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: MuLV protein, MVM protein, Reo-3 protein, PRV protein, and vesivirus protein; and wherein the modulation of expression improves production of the immunogenic agent in the cell by inhibiting viral infection of the cell.

74. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is xylose transferase.

75. The method of paragraph 73, wherein the at least one target gene is a vesivirus protein and the at least one RNA effector molecule comprises at least one strand that comprises at least 16 contiguous nucleotides of an oligonucleotide having a sequence selected from SEQ ID NOs:3152604-3152713.

76. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: CCNA1, CCNA2, CCNB1, CCNB2, CCNB3, CCND1, CCND2, CCND3, CCNE1, CCNE2, cyclin B, cyclin D, cyclin E, CDK2, CDK4, P10, P21, P27, p53, P57, p16INK4a, P14ARF, and CDK4, and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating the cell cycle of the cell.

77. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: IRE1, PERK, ATF4, ATF6, eIF2alpha, GRP78, GRP94, Bip, Hsp40, HSP47, HSP60, Hsp70, HSP90, HSP100, protein disulfide isomerase, peptidyl prolyl isomerase, calreticulin, calnexin, Erp57, and BAG-1; and wherein the modulation of expression improves production of the protein in the cell by enhancing folding of the protein.

78. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is a methionine sulfoxide reductase gene in the host cell, and wherein the modulation of expression improves production of the protein in the cell by inhibiting modification of the protein by methionine oxidation.

79. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the target gene is a glutaminyl cyclase gene in the host cell, and wherein the modulation of expression improves production of the protein in the cell by inhibiting modification of the
protein by pyroglutamation.

80. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: asparagine deamidase and glutamine deamidase; and wherein the modulation of expression improves production of the protein in the cell by inhibiting modification of the protein by deamidation.

81. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of dolichyl-diphosphooligosaccharide-protein glycosyltransferase, UDP glycosyltransferase, UDP-Gal:βGlcNAcβ1,4-galactosyltransferase, UDP-galactose-ceramide galactosyltransferase, fucosyltransferase, protein O-fucosyltransferase, N-acetylgalactosaminytransferase T-4, O-GlcNAc transferase, oligosaccharyl transferase, O-linked N-acetylglucosamine transferase, α-galactosidase, and β-galactosidase; and wherein the

modulation of expression improves production of the protein in the cell by modulating glycosylation of the protein.

82. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of protein disulfide isomerase and sulfhydryl oxidase; and wherein the modulation of expression improves production of the protein in the cell by modulating disulfide bond formation in the protein.

83. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of gamma-secretase, p115, a signal recognition particle (SRP) protein, secretin, and a kinase; and wherein the modulation of expression improves production of the protein in the cell by modulating secretion of the protein.

84. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is a dehydrofolate reductase gene in the host cell, wherein the modulation of expression improves production of the protein in the cell by enhancing gene amplification in the cell.

85. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is a gene of a virus or a target gene of a cell, thereby producing an immunogenic agent from a host cell having a reduced viral load.

86. The method of paragraph 85, wherein said virus is selected from the group consisting of: vesivirus, MMV, MuLV, PRV, and Reo-3.

87. The method of paragraph 85, wherein said at least one target gene encodes a viral protein.

88. The method of paragraph 85, wherein said at least one target gene encodes a non-viral protein.

89. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: pro-oxidant enzymes, BIK, BAD, BIM, HRK, BCLG, HR, NOXA, PUMA, BOK, BOO, BCLB, CASP2, CASP3, CASP6, CASP7, CASP8, CASP9, CASP10, BAX, BAK, BCL2, p53, APAFI, and HSP70; and wherein the modulation of expression improves production of the immunogenic agent in the cell by enhancing the viability of the cell.

90. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: CCNA1, CCNA2, CCNB1, CCNB2, CCNB3, CCND1, CCND2, CCND3, CCNE1, CCNE2, cyclin B, cyclin D, cyclin E, CDK2, CDK4, P10, P21, P27, p53, P57, p16INK4a, P14ARF, CDK4, Bcl-G, Bax, Bak, Bok, Bad, Bid, Bik, Blk, Hrk, BNIP3, PUMA, NOXA, BimL, Bcl-2, Bcl-xL, Bcl-B, Bcl-w, Boo, Mcl-1, A1, CASP2, CASP3, CASP6, CASP7, CASP8, CASP9, CASP10, GLUT1, GLUT2, GLUT3, GLUT4, phosphatidylinositol-

3,4,5-trisphosphate 3-phosphatase (PTEN), and lactate dehydrogenase (LDH); and wherein the modulation of expression improves production of the immunogenic agent in the cell by enhancing the specific productivity of the cell.

91. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: GLUT1, GLUT2, GLUT3, GLUT4, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN), lactate dehydrogenase (LDH), CCNA1, CCNA2, CCNB1, CCNB2, CCNB3, CCND1, CCND2, CCND3, CCNE1, CCNE2, cyclin B, cyclin D, cyclin E, CDK2, CDK4, P10, P21, P27, p53, P57, p16INK4a, P14ARF, and CDK4; wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating nutrient requirements of the cell.

92. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: lactate dehydrogenase and lysosomal V-type ATPase; and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating the pH of the cell.

93. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: cytoplasmic actin capping protein (CapZ), Ezrin (VIL2), Laminin A, and Cofilin (CFL1); and wherein the modulation of gene expression improves production of the immunogenic agent in the cell by modulating actin dynamics of the cell

94. The method of paragraph 93, wherein at least one RNA effector molecule inhibits expression of the target gene Cofilin.

95. The method of paragraph 93, wherein at least one RNA effector molecule increases expression of a target gene selected from the group consisting of: cytoplasmic actin capping protein (CapZ), Ezrin (VIL2), and Laminin A.

96. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is a gene of a host cell latent virus, an adventitious virus, a host cell endogenous retrovirus, or a host cell binding-ligand of such virus.

97. The method of paragraph 96, wherein the target gene is a gene of an endogenous retrovirus (ERV) selected from HERV-K, pt01-Chr10r-17119458, pt01-Chr5-53871501, BaEV, GaLV, HERV-T, ERV-3, HERV-E, HERV-ADP, HERV-I, MER4like, HERV-FRD, HERV-W, HERVH-RTVLH2, HERVH-RGH2, HERV-Hconsensus, HERV-Fc1, hg15-chr3-152465283, HERVL66, HSRV, HFV, HERV-S, HERV-L, HERVL40, HERVL74, HTLV-1, HTLV-2, HIV-1, HIV-2, MPMV, MMTV, HML1, HML2, HML3, HML4, HML7, HML8, HML5, HML10, HML6, HML9, MMTV, FLV, PERV, BLV, EIAV, JSRV, gg01-chr7-7163462, gg01-chrU-52190725, gg01-Chr4-48130894, ALV, gg01-chr1-15168845, gg01-chr4-77338201,

gg01-ChrU-163504869, gg01-chr7-5733782, Python-molurus, WDSV, SnRV, Xen1, Gypsy, and Ty1.

98. The method of paragraph 96, wherein the target gene is a gene of a latent virus selected from the group consisting of C serotype adenovirus, avian adenovirus, avian adenovirus-associated virus, human herpesvirus-4 (EBV), and circovirus.

99. The method of paragraph 98, wherein the latent virus is a circovirus, and the target gene is the rep gene of porcine circovirus type 1 (PCV1) or circovirus type 2 (PCV2).

100. The method of paragraph 98, wherein the latent virus is EBV and the target gene is latent membrane protein (LMP)-2A.

101. The method of paragraph 96, wherein the target gene is a gene of an adventitious virus selected from the group consisting of: exogenous retrovirus, human immunodeficiency virus type 1 (HIV-1), HIV-2, human T-cell lymphotropic virus type I (HTLV-I), HTLV-II, human hepatitis A (HHA), HHB, HHC, human cytomegalovirus, EBV, herpesvirus, human herpesvirus 6 (HHV6), HHV7, HHV8, human parvovirus B19, reovirus, polyoma (JC/BK) virus, SV40, human coronavirus, papillomavirus, human papillomavirus, influenza A, B, and C viruses, human enterovirus, human parainfluenza virus, human respiratory syncytial virus, vesivirus, porcine circovirus, lymphocytic choriomeningitis virus (LCMV), lactate dehydrogenase virus, porcine parvovirus, adeno-associated virus, reovirus, rabies virus, leporipoxviruse, avian leukosis virus (ALV), hantaan virus, Marburg virus, SV20, Semliki Forest virus, feline sarcoma virus, porcine parvovirus, mouse hepatitis virus (MHV), murine leukemia virus (MuLV), pneumonia virus of mice (PVM), Theiler's encephalomyelitis virus, murine minute virus, mouse adenovirus (MAV); mouse cytomegalovirus, mouse rotavirus (EDIM), Kilham rat virus, Toolan's H-1 virus, Sendai virus, rat coronavirus, pseudorabies virus, Cache Valley virus, bovine viral diarrhoea virus, bovine parainfluenza virus type 3, bovine respiratory syncytial virus, bovine adenovirus, bovine parvovirus, infectious bovine rhinotracheitis virus, bovine herpesvirus, bovine reovirus, bluetongue virus, bovine polyoma virus, bovine circovirus, vaccinia, orthopoxviruses other than vaccinia, pseudocowpox virus, and leporipoxvirus.

102. The method of paragraph 96, wherein target gene is a host cell binding ligand for an endogenous virus, a latent virus, or an adventitious virus.

103. The method of paragraph 102, wherein the target gene is SLC35A1, Gne, Cmas, B4GalT1, or B4GalT6.

104. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of FUT8, TSTA3, and GMDS; and wherein the

modulation of expression improves production of the immunogenic agent in the cell by modulating fucosylation.

105. The method of paragraph 104, further comprising contacting a host cell with at least one RNA effector molecule that targets a gene that encodes a sialytransferase.

106. The method of paragraph 105, wherein the sialytransferase is selected from the group consisting of ST3 β-galactoside α-2,3-sialyltransferase 1, ST3 β-galactoside α-2,3-sialyltransferase 4, ST3 β-galactoside α-2,3-sialyltransferase 3, ST3 β-galactoside α-2,3-sialyltransferase 5, ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6, and ST3 β-galactoside α-2,3-sialyltransferase 2.

107. The method of any of paragraphs 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of glutaminase and glutamine dehydrogenase; and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating ammonia buildup.

108. The method of any of paragraphs 1 to 108, further comprising contacting the host cell with at least one RNA effector molecule that modulates expression of glutaminase.

109. The method of any of paragraphs 1 to 108, further comprising contacting the host cell with at least one RNA effector molecule that modulates expression of glutamine synthetase.

110. A composition comprising: at least one RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell, and a cell medium suitable for culturing the host cell, wherein the RNA effector molecule is capable of modulating expression of the target gene and the modulation of expression enhances production of an immunogenic agent, wherein the at least one RNA effector molecule is an siRNA that comprises an antisense strand comprising at least 16 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs:9772-3152339 and SEQ ID NOs:3161121-3176783.

111. The composition of paragraph 110, comprising two or more RNA effector molecules, wherein the two or more RNA effector molecules are each complementary to different target genes.

112. A composition comprising: a plurality of RNA effector molecules, wherein a portion of each RNA effector molecule is complementary to at least one target gene of a host cell, and wherein the composition is capable of modulating expression of Bax, Bak, and LDH, and the modulation of expression enhances production of an immunogenic agent.

113. The composition of paragraph 110 or 112, further comprising at least one additional RNA effector molecule or agent

114. The composition of paragraph 110 or 112, wherein the at least one RNA effector molecule is siRNA.

115. The composition of paragraph 110 or 112, wherein the at least one RNA effector molecule comprises a duplex region.

116. The composition of paragraph 110 or 112, wherein the at least one RNA effector molecule is 15-30 nucleotides in length.

117. The composition of paragraph 110 or 112, wherein the at least one RNA effector molecule is 17-28 nucleotides in length.

118. The composition of paragraph 110 or 112, wherein the at least one RNA effector molecule comprises a modified nucleotide.

119. The composition of paragraph 110, wherein the cell medium is a serum-free medium.

120. The composition of any of paragraphs 110 to 119, wherein the composition is formulated in a non-lipd formulation.

121. The composition of any of paragraphs 110 to 119, wherein the composition is formulated in a lipid formulation.

122. The composition of paragraph 121, wherein the lipid in the formulation comprises a cationic or non-ionic lipid.

123. The composition of any of paragraphs 110 to 122, wherein the composition further comprises one or more cell culture media supplements.

124. The composition of any of paragraphs 110 to 123, wherein the at least one RNA effector molecule comprises a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least part of a target gene, and wherein said region of complementarity is 10 to 30 nucleotides in length.

125. A kit for enhancing production of an immunogenic agent by a cultured cell, comprising: (a) a substrate comprising one or more assay surfaces suitable for culturing the cell under conditions in which the immunogenic agent is produced; (b) one or more RNA effector molecules, wherein at least a portion of each RNA effector molecule is complementary to a target gene; and (c) a reagent for detecting the immunogenic agent or production thereof by the cell, wherein the one or more RNA effector molecules is an siRNA comprising an antisense strand that comprises at least 16 contiguous nucleotides of the nucleotide sequence selected from the group consisting of: SEQ ID NOs:9772-3152339 and SEQ ID NOs:3161121-3176783.

126. The kit of paragraph 125, wherein the one or more assay surfaces further comprises a matrix for supporting the growth and maintenance of host cells.

127. The kit of paragraph 125, wherein the one or more RNA effector molecules are deposited on the substrate.

128. The kit of paragraph 125, further comprising a carrier for promoting uptake of the RNA effector molecules by the host cell.

129. The kit of paragraph 128, wherein the carrier comprises a cationic lipid composition.

130. The kit of paragraph 128, wherein the carrier is deposited on the substrate.

131. The kit of paragraph 125, further comprising cell culture media suitable for culturing the host cell.

132. The kit of paragraph 125, further comprising instructions for culturing a host cell in the presence of one or more RNA effector molecules and assaying the cell for production of the immunogenic agent.

133. A kit for optimizing production of an immunogenic agent by cultured cells, comprising: (a) a microarray substrate comprising a plurality of assay surfaces, the assay surfaces being suitable for culturing the cells under conditions in which the immunogenic agent is produced; (b) one or more RNA effector molecules, wherein at least a portion of each RNA effector molecule is complementary to a target gene; and (c) a reagent for detecting the effect of the one or more RNA effector molecules on production of the immunogenic agent, wherein the one or more RNA effector molecules is an siRNA comprising an antisense strand that comprises at least 16 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs:9772-3152339 and SEQ ID NOs:3161121-3176783.

134. The kit of paragraph 133, wherein the substrate is a multi-well plate or biochip.

135. The kit of paragraph 133, wherein the substrate is a two-dimensional microarray plate or biochip.

136. The kit of paragraph 133, wherein the one or more RNA effector molecules are deposited on the assay surfaces of the substrate.

137. The kit of paragraph 135, wherein a plurality of different RNA effector molecules are deposited on assay surfaces across a first dimension of the microarray.

138. The kit of paragraph 137, wherein the plurality of RNA effector molecules are each complementary to a different target gene.

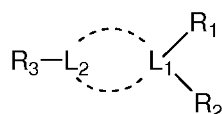
139. The kit of paragraph wherein the different target genes are Bax, Bak, and LDH.

140. The kit of paragraph 137, wherein a plurality of RNA effector molecules are each complementary to a different region of the same target gene.

141. The kit of paragraph 137, wherein each of the RNA effector molecules comprising the plurality is deposited at varying concentrations on assay surfaces along the second dimension of the microarray.

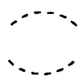
142. The method of any of paragraphs 1-109, wherein the RNA effector molecule, a portion of which is complementary to the target gene, is a corresponding siRNA that comprises an antisense strand comprising at least 16 contiguous nucleotides of a nucleotide sequence, wherein the nucleotide sequence is set forth in the tables herein.

143. The method of paragraph 121, wherein the lipid formulation comprises a lipid having the following formula:



wherein:

R_1 and R_2 are each independently for each occurrence optionally substituted C_{10}-C_{30} alkyl, optionally substituted C_{10}-C_{30} alkoxy, optionally substituted C_{10}-C_{30} alkenyl, optionally substituted C_{10}-C_{30} alkenyloxy, optionally substituted C_{10}-C_{30} alkynyl, optionally substituted C_{10}-C_{30} alkynyloxy, or optionally substituted C_{10}-C_{30} acyl;

 represents a connection between L_2 and L_1 which is:

(1) a single bond between one atom of L_2 and one atom of L_1, wherein

L_1 is $C(R_x)$, O, S or N(Q);

L_2 is $-CR_5R_6-$, -O-, -S-, -N(Q)-, $=C(R_5)-$, -C(O)N(Q)-, -C(O)O-, -N(Q)C(O)-, -OC(O)-, or -C(O)-;

(2) a double bond between one atom of L_2 and one atom of L_1; wherein

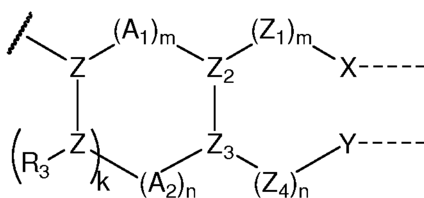
L_1 is C;

L_2 is $-CR_5=$, -N(Q)=, -N-, -O-N=, -N(Q)-N=, or -C(O)N(Q)-N=;

(3) a single bond between a first atom of L_2 and a first atom of L_1, and a single bond between a second atom of L_2 and the first atom of L_1, wherein

L_1 is C;

L_2 has the formula



wherein

X is the first atom of L_2, Y is the second atom of L_2, - - - - - represents a single bond to the first atom of L_1, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, -OS(O)(Q_2)O-, and -OP(O)(Q_2)O-;

Z_1 and Z_4 are each, independently, -O-, -S-, -CH_2-, -CHR^5-, or -CR^5R^5-;

Z_2 is CH or N;

Z_3 is CH or N;

or Z_2 and Z_3, taken together, are a single C atom;

A_1 and A_2 are each, independently, -O-, -S-, -CH_2-, -CHR^5-, or -CR^5R^5-;

each Z is N, C(R_5), or C(R_3);

k is 0, 1, or 2;

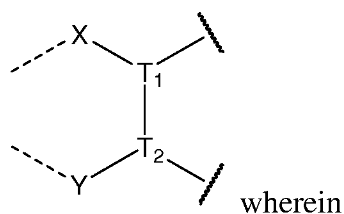
each m, independently, is 0 to 5;

each n, independently, is 0 to 5;

where m and n taken together result in a 3, 4, 5, 6, 7 or 8 member ring;

(4) a single bond between a first atom of L_2 and a first atom of L_1, and a single bond between the first atom of L_2 and a second atom of L_1, wherein

(A) L_1 has the formula:



wherein

X is the first atom of L_1, Y is the second atom of L_1, - - - - - represents a single bond to the first atom of L_2, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, -OS(O)(Q_2)O-, and -OP(O)(Q_2)O-;

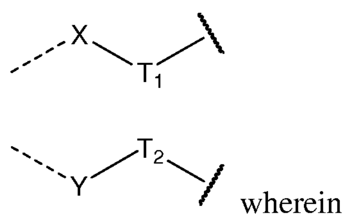
T_1 is CH or N;

T_2 is CH or N;

or T_1 and T_2 taken together are C=C;

L_2 is CR_5; or

(B) L_1 has the formula:



wherein

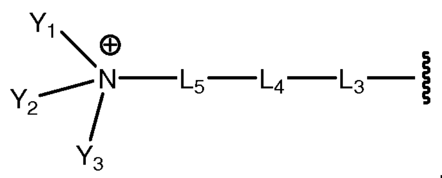
X is the first atom of L_1, Y is the second atom of L_1, - - - - - represents a single bond to the first atom of L_2, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, -OS(O)(Q_2)O-, and -OP(O)(Q_2)O-;

T_1 is -CR_5R_5-, -N(Q)-, -O-, or -S-;

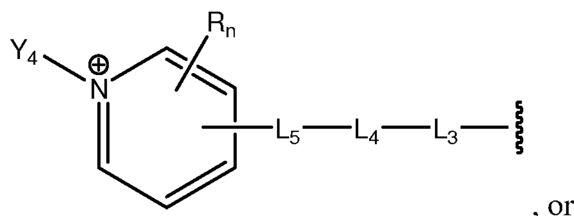
T_2 is -CR_5R_5-, -N(Q)-, -O-, or -S-;

L_2 is CR_5 or N;

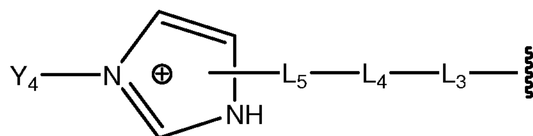
R_3 has the formula:



,



, or



wherein

each of Y_1, Y_2, Y_3, and Y_4, independently, is alkyl, cycloalkyl, aryl, aralkyl, or alkynyl; or

any two of Y_1, Y_2, and Y_3 are taken together with the N atom to which they are attached to form a 3- to 8- member heterocycle; or

Y_1, Y_2, and Y_3 are all be taken together with the N atom to which they are attached to form a bicyclic 5- to 12- member heterocycle;

each R_n, independently, is H, halo, cyano, hydroxy, amino, alkyl, alkoxy, cycloalkyl, aryl, heteroaryl, or heterocyclyl;

L_3 is a bond, -N(Q)-, -O-, -S-, -$(CR_5R_6)_a$-, -C(O)-, or a combination of any two of these;

L_4 is a bond, -N(Q)-, -O-, -S-, -$(CR_5R_6)_a$-, -C(O)-, or a combination of any two of these;

L_5 is a bond, -N(Q)-, -O-, -S-, -$(CR_5R_6)_a$-, -C(O)-, or a combination of any two of these;

each occurrence of R_5 and R_6 is, independently, H, halo, cyano, hydroxy, amino, alkyl, alkoxy, cycloalkyl, aryl, heteroaryl, or heterocyclyl; or two R_5 groups on adjacent carbon atoms are taken together to form a double bond between their respective carbon atoms; or two R_5 groups on adjacent carbon atoms and two R_6 groups on the same adjacent carbon atoms are taken together to form a triple bond between their respective carbon atoms;

each a, independently, is 0, 1, 2, or 3;

wherein

an R_5 or R_6 substituent from any of L_3, L_4, or L_5 is optionally taken with an R_5 or R_6 substituent from any of L_3, L_4, or L_5 to form a 3- to 8- member cycloalkyl, heterocyclyl, aryl, or heteroaryl group; and

any one of Y_1, Y_2, or Y_3, is optionally taken together with an R_5 or R_6 group from any of L_3, L_4, and L_5, and atoms to which they are attached, to form a 3- to 8- member heterocyclyl group;

each Q, independently, is H, alkyl, acyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl or heterocyclyl; and

each Q_2, independently, is O, S, N(Q)(Q), alkyl or alkoxy.

EXAMPLES

Example 1. RNA effector molecule synthesis

[00574] Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

[00575] *Oligonucleotide Synthesis*: All oligonucleotides are synthesized on an AKTAoligopilot synthesizer. Commercially available controlled pore glass solid support (dT-CPG, 500Å, Prime Synthesis) and RNA phosphoramidites with standard protecting groups, 5'-*O*-dimethoxytrityl N6-benzoyl-2'-*t*-butyldimethylsilyl-adenosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-*t*-butyldimethylsilyl-cytidine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N2--isobutryl-2'-*t*-butyldimethylsilyl-guanosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, and 5'-*O*-dimethoxytrityl-2'-*t*-butyldimethylsilyl-uridine-3'-O-N,N'-diisopropyl-2-cyanoethylphosphoramidite (Pierce Nucleic Acids Technologies) were used for the oligonucleotide synthesis. The 2'-F phosphoramidites, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-fluro-cytidine-3'-*O*-N,N'-diisopropyl-2-cyanoethyl-phosphoramidite and 5'-*O*-dimethoxytrityl-2'-

fluro-uridine-3'-O-N,N'-diisopropyl-2-cyanoethyl-phosphoramidite are purchased from (Promega). All phosphoramidites are used at a concentration of 0.2M in acetonitrile (CH_3CN) except for guanosine which is used at 0.2M concentration in 10% THF/ANC (v/v). Coupling/recycling time of 16 min is used. The activator is 5-ethyl thiotetrazole (0.75M, American International Chemicals); for the PO-oxidation iodine/water/pyridine is used and for the PS-oxidation PADS (2%) in 2,6-lutidine/ACN (1:1 v/v) is used.

[00576] The 3'-ligand conjugated strands are synthesized using solid support containing the corresponding ligand. For example, the introduction of cholesterol unit in the sequence is performed from a hydroxyprolinol-cholesterol phosphoramidite. Cholesterol is tethered to *trans*-4-hydroxyprolinol via a 6-aminohexanoate linkage to obtain a hydroxyprolinol-cholesterol moiety. The 5'-end Cy-3 and Cy-5.5 (fluorophore) labeled RNA effector molecules are synthesized from the corresponding Quasar®570 indocarbocyanine Cy™3 phosphoramidite are purchased from Biosearch Technologies (Novato, CA). Conjugation of ligands to 5'-end and or internal position is achieved by using appropriately protected ligand-phosphoramidite building block. An extended 15 min coupling of 0.1 M solution of phosphoramidite in anhydrous CH_3CN in the presence of 5-(ethylthio)-1*H*-tetrazole activator to a solid-support-bound oligonucleotide. Oxidation of the internucleotide phosphite to the phosphate is carried out using standard iodine-water, as reported in the literature, or by treatment with *tert*-butyl hydroperoxide/ acetonitrile/water (10:87:3) with 10 min oxidation wait time conjugated oligonucleotide. Phosphorothioate is introduced by the oxidation of phosphite to phosphorothioate by using a sulfur transfer reagent such as DDTT (purchased from AM Chemicals), PADS and or Beaucage reagent. The cholesterol phosphoramidite is synthesized in house and used at a concentration of 0.1 M in dichloromethane. Coupling time for the cholesterol phosphoramidite is 16 min.

[00577] *Deprotection I (Nucleobase Deprotection)*: After completion of synthesis, the support is transferred to a 100 mL glass bottle (VWR). The oligonucleotide is cleaved from the support with simultaneous deprotection of base and phosphate groups with 80 mL of a mixture of ethanolic ammonia [ammonia:ethanol (3:1)] for 6.5 h at 55°C. The bottle is cooled briefly on ice and then the ethanolic ammonia mixture is filtered into a new 250-mL bottle. The CPG is washed with 2 x 40 mL portions of ethanol/water (1:1 v/v). The volume of the mixture is then reduced to ~ 30 mL by roto-vap. The mixture is then frozen on dry ice and dried under vacuum on a speed vac.

[00578] *Deprotection II (Removal of 2'-TBDMS group)*: The dried residue is resuspended in 26 mL of triethylamine, triethylamine trihydrofluoride (TEA•3HF) or pyridine-HF and DMSO (3:4:6) and heated at 60°C for 90 minutes to remove the *tert*-butyldimethylsilyl

(TBDMS) groups at the 2' position. The reaction is then quenched with 50 mL of 20 mM sodium acetate and the pH is adjusted to 6.5. Oligonucleotide is stored in a freezer until purification.

[00579] *Analysis*: The oligonucleotides are analyzed by high-performance liquid chromatography (HPLC) prior to purification and selection of buffer and column depends on nature of the sequence and or conjugated ligand.

[00580] *HPLC Purification*: The ligand-conjugated oligonucleotides are purified by reverse-phase preparative HPLC. The unconjugated oligonucleotides are purified by anion-exchange HPLC on a TSK gel column packed in house. The buffers are 20 mM sodium phosphate (pH 8.5) in 10% CH_3CN (buffer A); and 20 mM sodium phosphate (pH 8.5) in 10% CH_3CN, 1 M NaBr (buffer B). Fractions containing full-length oligonucleotides are pooled, desalted, and lyophilized. Approximately 0.15 OD of desalted oligonucleotides are diluted in water to 150 μL and then pipetted into special vials for CGE and LC/MS analysis. Compounds are then analyzed by LC-ESMS and CGE.

[00581] *RNA effector molecule preparation*: For the general preparation of RNA effector molecules, equimolar amounts of sense and antisense strand are heated in 1 x PBS at 95°C for 5 min and slowly cooled to room temperature. Integrity of the duplex is confirmed by HPLC analysis.

[00582] siRNAs designed to degrade hamster Bax, Bak, and LDH mRNA were synthesized based on publicly available sequence data. A set of approximately 32 siRNAs was designed and synthesized for each target. Each siRNA was added to cell media at 10 nM for 3 days to screen for effect. In a 96 well plate, 29.5 μL of CD CHO media (Gibco) was added to test wells and 47 μL to control wells. To this, 17.5 μL of 100 nM siRNAs in CD CHO media was added to the test wells. To all wells, 3 μL of Lipofectamine™ RNAiMAX transfection reagent (Invitrogen) diluted 1:10 in CD CHO media was added. The mixture was allowed to incubate at room temperature for 15 min and then 125 μL of CD CHO media containing 20,000-30,000 cells was added to all wells. The plates were then placed in a 37°C CO_2 incubator for 3 days.

[00583] After three days, cells were visually inspected for toxicity and then RNA was extracted using a MagMAX™ 96-well RNA extraction kit (Applied Biosys./Ambion®, Austin, TX) following manufacturer's instructions. cDNA was made from the RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosys.) according to manufacturer's instructions. Finally, qPCR was used to quantify a 25-fold dilution of the target cDNA with a Roche Lightcycler 480 PCR instrument and Roche PCR Probes master mix. Relative

knockdown of target genes was calculated using the ΔΔCt method using GAPDH as the internal standard.

[00584] For qPCR the following primers and probes were used:

Bax

Forward primer 5'-GGAGCAGCTCGGAGGCG-3' (SEQ ID NO: 3152400)

Reverse primer 5'-AAAAGGCCCCTGTCTTCATGA-3' (SEQ ID NO:3152401)

Probe 5'-6FAM-CGGGCCCACCAGCTCTGAGCA-TAMRA-3' (SEQ ID NO:3152402)

Bak

Forward primer 5'-CCTCCTAGGCAGGACTGTGA-3' (SEQ ID NO:3152403)

Reverse primer 5'-CCAAGATGCTGTTGGGTTCT-3' (SEQ ID NO:3152404)

Probe 5'-6FAM-TCAGGAACAAGAGACCCAGG-TAMRA-3' (SEQ ID NO:3152405)

LDH

Forward primer 5'-TCTGTCTGTGGCTGACTTGG-3' (SEQ ID NO:3152406)

Reverse primer 5'-TCACAACATCGGAGATTCCA-3' (SEQ ID NO:3152407)

Probe 5'-6FAM-TGAAGAATCTTAGGCGGGTG-TAMRA-3' (SEQ ID NO:3152408)

GAPDH

Forward primer 5'-TGGCTACAGCAACAGAGTGG–3' (SEQ ID NO:3152409)

Reverse primer 5'-GTGAGGGAGATGATCGGTGT–3' (SEQ ID NO:3152410)

Probe 5' – VIC-AGTCCCTGTCCAATAACCCC– TAMRA–3' (SEQ ID NO:3152411)

[00585] Following the initial screen at 10 nM, the most potent siRNAs were further tested at concentrations ranging from 100 nM to 1 pM under identical conditions as described above except that the concentrations of siRNAs in the 17.5 μL CD CHO media was modified as needed to obtain the desired final concentration.

[00586] An LDH activity assay kit (Cayman Chemical, Ann Arbor, MI) was used to test for reduced levels of LDH after 3 to 4 days of treatment with LDH siRNAs. To lyse cells in the 175 μL of media in the 96-well plate wells, 20 μL of 1% TritonX-100 was added and the plates shaken for 10 min at room temperature. The assay was carried out according to manufacturer's protocol.

Table 22. dsRNA against hamster Bak1				
start pos.	SEQ ID NO	sense (5'-3')	antisense (5'-3')	SEQ ID NO
89		AGGAGGUCUUUCGAAGCUA	UAGCUUCGAAAGACCUCCU	2260032
90		GGAGGUCUUUCGAAGCUAU	AUAGCUUCGAAAGACCUCC	2259864

Table 22. dsRNA against hamster Bak1				
93		GGUCUUUCGAAGCUAUGUU	AACAUAGCUUCGAAAGACC	2259871
95		UCUUUCGAAGCUAUGUUUU	AAAACAUAGCUUCGAAAGA	
99		UCGAAGCUAUGUUUUCCAU	AUGGAAAACAUAGCUUCGA	2259966
163		AACCCCGAGAUGGACAAUU	AAUUGUCCAUCUCGGGGUU	
185		UCCUAGAACCCAACAGCAU	AUGCUGUUGGGUUCUAGGA	
188		UAGAACCCAACAGCAUCUU	AAGAUGCUGUUGGGUUCUA	
232		AUCAUUGGAGAUGACAUUA	UAAUGUCAUCUCCAAUGAU	
241		GAUGACAUUAACCGGAGAU	AUCUCCGGUUAAUGUCAUC	2260016
262		GACACAGAGUUCCAGAAUU	AAUUCUGGAACUCUGUGUC	
318		CGAACUCUUCACCAAGAUU	AAUCUUGGUGAAGAGUUCG	2259868
331		AAGAUUGCCUCCAGCCUAU	AUAGGCUGGAGGCAAUCUU	2259985
333		GAUUGCCUCCAGCCUAUUU	AAAUAGGCUGGAGGCAAUC	2259918
334		AUUGCCUCCAGCCUAUUUA	UAAAUAGGCUGGAGGCAAU	2259976
335		UUGCCUCCAGCCUAUUUAA	UUAAAUAGGCUGGAGGCAA	2259895
415		UAUGUCUACCAACGUGGUU	AACCACGUUGGUAGACAUA	2260083
476		UCAUACUGCACCAUUGCAU	AUGCAAUGGUGCAGUAUGA	
546		CAGAGACCCAAUCCUGAUU	AAUCAGGAUUGGGUCUCUG	
551		ACCCAAUCCUGAUUGUGAU	AUCACAAUCAGGAUUGGGU	2259907
561		GAUUGUGAUGACAAUUCUU	AAGAAUUGUCAUCACAAUC	2259857
599		AGUACGUGGUACACAGAUU	AAUCUGUGUACCACGUACU	2259943
607		GUACACAGAUUCUUCAGAU	AUCUGAAGAAUCUGUGUAC	

[00587] Exemplary dsRNA sequences against hamster (*Cricetulus griseus*) Bax are disclosed herein as SEQ ID NOs:3152476-3152539, wherein the even numbered SEQ ID NOs (e.g., NO:3152476) represent the sense strand and the odd numbered SEQ ID NOs (e.g., NO:3152477) represent the complementary antisense strand; in embodiments described herein, the RNA effector molecule can comprise at least 16 contiguous nucleotides of these sequences.

[00588] Exemplary dsRNA sequences against hamster (*Cricetulus griseus*) LDH-A are disclosed herein as SEQ ID NOs:3152540-3152603, wherein the even numbered SEQ ID NOs (e.g., NO:3152540) represent the sense strand and the odd numbered SEQ ID NOs (e.g., NO:3152541) represent the complementary antisense strand; in embodiments described herein, the RNA effector molecule can comprise at least 16 contiguous nucleotides of these sequences.

Table 25. Sense and antisense exemplary dsRNA against hamster Bax, Bak, and LDH-A.					
Target	SEQ ID NO	Sense (3' to 5')	AntiSense (5' to 3')	SEQ ID NO	IC_{50} (nM)
Bax Duplex					
A7	3152794	CCGUCUACCAAGAAGUU GAdTdT	UCAACUUCUUGGUAGAC GGdTdT	3152795	0.38
B2	3152796	CAGCUGACAUGUUUGCU GAdTdT	UCAGCAAACAUGUCAGC UGdTdT	3152797	1.46
B4	3152798	GUUGUUGCCCUUUUCUA CUdTdT	AGUAGAAAAGGGCAAC AACdTdT	3152799	0.08
B11	3152800	GACAGUGACUAUCUUUG UGdTdT	CACAAAGAUAGUCACUG UCdTdT	3152801	0.22

Table 25. Sense and antisense exemplary dsRNA against hamster Bax, Bak, and LDH-A.					
Target	SEQ ID NO	Sense (3' to 5')	AntiSense (5' to 3')	SEQ ID NO	IC_{50} (nM)
C6	3152802	AGCUCUGAGCAGAUCAUGAdTdT	UCAUGAUCUGCUCAGAGCUdTdT	3152803	0.17
Bak Duplex					
A2	3152804	GUCUUUCGAAGCUAUGUUUdTdT	AAACAUAGCUUCGAAAGACdTdT	3152805	0.07
A10	3152806	GCAGCUUGCUAUCAUUGGAdTdT	UCCAAUGAUAGCAAGCUGCdTdT	3152807	0.38
A11	3152808	GCUAUCAUUGGAGAUGACAdTdT	UGUCAUCUCCAAUGAUAGCdTdT	3152809	0.14
B9	3152810	GCCUAUUUAAGAGCGGCAUdTdT	AUGCCGCUCUUAAAUAGGCdTdT	3152811	0.08
C7	3152812	CGUGGUACACAGAUUCUUCdTdT	GAAGAAUCUGUGUACCACGdTdT	3152813	0.04
LDH Duplex					
C10	3152814	CUACUUAAGGAAGAACAGAdTdT	UCUGUUCUUCCUUAAGUAGdTdT	3152815	0.06
D5	3152816	CAAGCUGGUCAUUGUCACAdTdT	UGUGACAAUGACCAGCUUGdTdT	3152817	0.06
D7	3152818	UCAUCAUUCCCAACGUUGUdTdT	ACAACGUUGGGAAUGAUGAdTdT	3152819	0.13
E2	3152820	GAGUGGAGUGAAUGUAGCUdTdT	AGCUACAUUCACUCCACUCdTdT	3152821	0.40
E4	3152822	ACAAGGAGCAGUGGAAUGAdTdT	UCAUUCCACUGCUCCUUGUdTdT	3152823	0.15

Example 2. Enhanced production of glucocerebrosidase in human HT-1080 cells

[00589] The production of human glucocerebrosidase is enhanced in human HT-1080 cells in which the glucocerebrosidase gene has been activated as described in U.S. Patent No. 5,641,670 (Gene-Activated® GCB (GA-GCB)) by contacting the cells with one or more RNA effector molecules, wherein at least a portion of each RNA effector molecule is complementary to a target gene encoding a host cell mannosidase. The RNA effector molecules inhibits expression of target genes encoding class 1 processing and/or class 2 processing mannosidases, such as Golgi mannosidase IA, Golgi mannosidase IB, Golgi mannosidase IC, and/or Golgi mannosidase II. The coding strand sequences of various mannosidases have been disclosed. *See, e.g.*, Bause, 217 Eur. J. Biochem. 535-40 (1993); Gonzalez et al., 274 J. Biol. Chem. 21375-86 (1999); Misago et al., 92 PNAS 11766-70 (1995); Tremblay et al., 8 Glycobio. 585-95 (1998); Tremblay et al., 275 J. Biol. Chem. 31655-60 (2000). RNA effector molecules targeting the mannosidases can be designed according to the rules of Watson and Crick base pairing and other considerations as disclosed herein, or otherwise known in the art.

[00590] *Effect of RNA Effector Molecules on GA-GCB Glycoforms*: HT-1080 cells producing GA-GCB are plated and the Production Medium is adjusted to provide RNA effector

molecule concentrations ranging from 0 (no drug) to 10 μg/mL. The medium is harvested and the cells are re-fed every 24 hr for 3 days. Samples from the third day are subjected to isoelectric focusing (IEF) analysis to assay the effect of the RNA effector molecules on the expressed glucocerebrosidase. The apparent isoelectric point (pI) of the protein increases in a concentration dependent manner with the concentration of the RNA effector molecules. The RNA effector molecule(s) showing the steepest increase in pI are identified as preferred RNA effector molecules for enhancing production of glucocerebrosidase.

[00591] *Effect of RNA Effector Molecules on GA-GCB Production*: Ten roller bottles (surface area, 1700 cm2 each) are seeded in Growth Medium (DMEM with 10% calf serum) with HT-1080 cells producing GA-GCB. Following 2 weeks of growth, the medium is aspirated and 200 mL of fresh Production Medium (DMEM/F12, 0% calf serum) is added to three sets of roller bottles. Two sets of four roller bottles are treated with ~1 μg/mL of the RNA effector molecules. The third group of two roller bottles receives no drug treatment. After about 24 hr, the medium from each roller bottle is harvested and pooled, and a sample is taken for GA-GCB enzymatic activity analysis. The enzyme activity analysis is performed as follows: test article is mixed with the enzyme substrate (4-methylumbelliferyl-β-D-glucopyranoside) and incubated for 1 hr at 37°C. The reaction is stopped by the addition of NaOH/Glycine buffer and fluorescence is quantified by the use of a fluorescence spectrophotometer. Specific activities are expressed as 2,500 Units/mg, where one unit is defined as the conversion of 1 μMole of substrate in 1 hr at 37°C. The entire procedure is repeated for 7 days. Stable production of GA-GCB isobserved for all roller bottles throughout the seven daily harvests. Absolute levels of the enzyme, however, may vary according to RNA effector molecule treatment group.

[00592] *Purification and Characterization of hmGCB*: HmGCB is purified from the culture medium of human fibroblasts grown in the presence of RNA effector molecules. The four N-linked glycans present on hmGCB are released by peptide N-glycosidase F and purified using a Sep-pak C18 cartridge. Oligosaccharides eluting in the 5% acetic acid fraction are permethylated using sodium hydroxide and methyl iodide, dissolved in methanol:water (80:20), and portions of the permethylated glycan mixture are analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS). The sample is analyzed on a VOYAGER™ STR BIOSPECTROMETRY™ Research Station laser-desorption mass spectrometer (Applied Biosys.) coupled with Delayed Extraction using a matrix of 2,5-dihydroxybenzoic acid. A pattern of pseudomolecular ions is seen in the range m/z 1500-2500, indicating the presence of high-mannose glycans ranging from $Man_5GlcNAc_2$ to $Man_9GlcNAc_2$.

[00593] The most abundant high mannose glycans present are $Man_9GlcNAc_2$ and $Man_8GlcNAc_2$, with decreasing abundances of $Man_7GlcNAc_2$, $Man_6GlcNAc_2$, and $Man_5GlcNAc_2$. A trace amount of a fucosylated biantennary complex glycan containing two sialic acid residues is observed. An approximate indication of the relative abundancy of each glycan is obtained by measuring the peak heights. A more accurate assessment of the average chain length of the high mannose glycans is obtained by MALDI-TOF-MS analysis of the intact glycoprotein. A sharp peak is obtained at about m/z 62,483.1 due to the homogeneity of the glycan chains. The mass of the mature peptide calculated from the amino acid sequence is about 55,577.6, indicating the four N-linked glycan chains contribute 6905.5 to the total mass of hmGCB. From this number, it can be calculated that the average glycan length is 8.15 mannose residues.

[00594] *Effect of RNA Effector Molecules on GA-GCB Uptake into Macrophages*: GA-GCB produced in HT-1080 cells is used in an *in vitro* assay to determine uptake efficiency in a mouse macrophage cell line. The specific objective of the experiment is to determine the absolute and mannose receptor-specific uptake of GA-GCB in mouse J774E cells. One day prior to assay, J774E cells are plated at 50,000 cells/cm^2 in 12-well plates in Growth Medium. For the assay, 0.5 mL of Production Medium (DMEM/F12, 0% calf serum) containing 50 nM vitamin D3 (1,2-5, Dihydroxy vitamin D3) is added to the cells. Unpurified GA-GCB is added to the test wells at a final concentration of 10 μg/mL in the presence or absence of 2 μg/mL mannan (a competitor for the mannose receptor).

[00595] The following forms of GA-GCB are used: GA-GCB from cells treated with a RNA effector molecule (1 μg/mL) and GA-GCB (1 μg/mL) from untreated cells. Control wells receive no GA-GCB. The wells are incubated for 4 hr at 37°C., and then are washed extensively in buffered saline, scraped into GA-GCB enzyme reaction buffer, passed through two freeze/thaw cycles, and clarified by centrifugation. The supernatant is then quantitatively tested for enzyme activity and total protein. Enzyme activity is determined as follows: sample is mixed with the enzyme substrate (4-methylumbelliferyl-β-D-glucopyranoside) and incubated for 1 hr at 37°C. The reaction is stopped by the addition of NaOH/Glycine buffer. Fluorescence is quantified by the use of a fluorescence spectrophotometer. Total protein is determined in freeze/thaw cell lysates by bicinchoninic acid (BCA). Activity is reported as units/mg total protein, where one Unit is defined as the conversion of 1 μMole of substrate in 1 hr at 37°C. Cells treated with a RNA effector molecule will receive the RNA effector molecule in the presence or absence of mannan (2 μg/mL). Internalization of GA-GCB into mouse J744E cells is reported as Units/mg of cell lysates.

[00596] The results demonstrate that uptake of GA-GCB from RNA effector molecule treated cells is about 7-fold to 14-fold over background and about 67%-73% inhibitable by mannan. In addition, they also demonstrate that uptake of GA-GCB from untreated cells is about 3-fold over background and 53% inhibitable by mannan. Thus, the inhibition of intracellular mannosidases by RNA effector molecules results in GA-GCB that can be transported into cells efficiently via the mannose receptor. Improvement in targeting of GA-GCB to cells via mannose receptors can therefore be optimized by production of GA-GCB in the presence of one or more RNA effector molecules.

Example 3. Growth curves of suspended CHO-S cells treated with different siRNAs

[00597] Flasks were set up with approximately 400,000 cells/mL in 50 mL of total volume. First, 2.5 μL of 20 μM Invitrogen Stealth FITC-siRNA or 50 μL of 1 μM Bax siRNA and 50 μL of 1 μM Bak siRNA or 50 μL of 1 μM LDH siRNA were added to three different 14.3 mL volumes of CD CHO media (GIBCO). The solutions were gently mixed and then 85.5 μL of LIPOFECTAMINE™ RNAiMAX transfection reagent (Invitrogen) was added to each and the solutions gently mixed again. The solutions were allowed to incubate at room temperature for 15 min. After 15 min, 32.8 mL of warmed media was added to each solution. Finally, 2.9 mL of media with 7,000,000 cells/mL was added and the flasks put on a shaker plate set at 160 rpm in a 37°C CO_2 incubator. Each following day an aliquot was taken from the media to count cells and determine their viability in a Beckman-Coulter cell counter.

[00598] On days 2 and 4, additional siRNAs were added. To do this, 25 mL was removed from each flask and spun at ~400 x g for 5 minutes to pellet the cells. Then, 14.3 mL of the cell-free media was removed to a separate tube and siRNAs and LIPOFECTAMINE™ RNAiMAX reagent were added as above. The solutions were gently mixed and allowed to incubate at room temperature for 15 min. The solutions were added back to their respective cell pellet, mixed with a pipette to break up cell clumps and then introduced back to their original flasks.

Example 4: Inhibition of Bax, Bak and LDH enhances viability of cells in culture

[00599] Bax and Bak are members of the mitochondrial-regulating BCL-2 protein family that play pivotal pro-apoptotic (capable of inducing programmed cell death) roles. As described herein, potent siRNAs directed against Bax and Bak with IC_{50}s in the low pico molar range were added at periodic intervals to CHO cells grown in a 1 L bioreactor. In addition, an siRNA directed against lactate dehydrogenase (LDH) was also included in the siRNA formulation. LDH catalyzes the conversion of pyruvate to lactate during times of anaerobic stress. Lactate is a

major metabolic waste product produced in cells grown in culture and has been shown to inhibit both cell growth and metabolic pathways. Because the activation of the Bax/Bak and LDH pathways is thought to limit the growth potential of cells in culture, the effect of adding potent siRNAs directed against these genes to CHO cells grown in suspension under 1 L bioprocessing-like conditions was evaluated. When compared to CHO cells treated with a non-specific FITC-labeled siRNA, the Bax/Bak/LDH siRNA-treated cells grew to a cell density that was 90% greater than the control with a corresponding 2-fold decreased apoptotic death rate.

[00600] *Materials and methods*: Suspension-adapted CHO cells were obtained from Invitrogen and were grown (0.2 x 10^6 cells / mL seed density) in a 1 L disposable bioreactor (Sartorius, Bohemia, NY) at 37°C and 5.5% CO_2 using DG44 chemically defined media (Invitrogen; #12610-010) with constant stirring at a rate suggested by the manufacturer. Starting on day-4 following seeding, the cell cultures were supplemented with 5% culture volume (30 mL) using CHO CD Efficient Feed media (Invitrogen; 10234, 10240). The cultures were then fed every 48 hr using the same feed media and volume.

[00601] Bax, Bak, and LDH siRNA sequences are provided in Table 10 and synthesized initially at small scale without modification (except for 3' dTdT) by RLD small scale synthesis followed by medium scale synthesis. Control siRNA was purchased from Invitrogen (FITC-labeled oligo; #44-2926). Each siRNA was added to the 1 L bioreactor at a final concentration of 1 nM and formulated for transfection using Lipofectamine RNAiMax transfection reagent (Invitrogen). Bax, Bak, and LDH siRNAs were formulated together for a final combined siRNA concentration of 3 nM. The control siRNA formulation contained 6 mL DG44 media, 240 μL LIPOFECTAMINE™ RNAiMax reagent, and 30 μL FITC-labeled oligo (20 μM stock concentration). The experimental siRNA formulation contained 6 mL DG44 media, 240 μL LIPOFECTAMINE RNAiMax reagent, and 6 uL of each Bax, Bak, and LDH siRNA (100 μM stock concentrations). Both control and experimental siRNAs were incubated at room temperature for 15 min prior to addition to the culture media starting on day 0 and dosed again at similar concentrations every 48 hr for a total of six doses. Each day, 5 mL culture samples were removed, the cells counted and viability determined using Trypan blue dye (Sigma Aldrich) exclusion with a hemocytometer. All cell samples were taken before any further addition of siRNA or nutrient feeds. The remaining cells were aliquoted, spun down to form a cell pellet and frozen at -70°C until needed for the following assays: qPCR, lactate, glucose, LDH, and caspase 3.

Table 26. Oligo sense strand sequences of siRNAs

Target	SEQ ID NO	Strand	IC_{50}	Sequence 5'to 3'

LDH C10	3152814	sense	16 pM	CUACUUAAGGAAGAACAGAdTdT
Bak A2 A-54123.1	3152804	sense	70 pM	GUCUUUCGAAGCUAUGUUUdTdT
Bax B4 A-54091.1	3152798	sense	80 pM	GUUGUUGCCCUUUUCUACUdTdT

[00602] *Results*: The addition of Bax/Bak/LDH siRNAs to CHO cell cultures improves viable cell density by approximately 2-fold (Figure 6) when compared to a control transfection using a non-specific FITC-labeled siRNA. The control cell population reached a maximum cell density of ~1.5 x 10^6 cells per mL on day 6; whereas, the Bax/Bak/LDH siRNA-treated cells achieved a maximum cell density of ~1.8 x 10^6 cells per mL on day 7. The integral cell area (IGA) for the Bax/Bak/LDH-treated cells increased ~90% over the control siRNA-treated cells (Figure 6, inset).

[00603] Fifty percent viability of the control cells was observed on day 10 and on day 16 for the Bax/Bak/LDH-treated cells (Figure 7). Both samples exhibited comparably high viability starting on day-0 until day-5. Cell viability started to decay below 90% starting on day 6 for the control-treated sample and on day 7 for the experimental. Cell death rates are directly proportional to the slope of the percent viability response curve. Sharper slopes indicate faster apoptotic death rates compared to shallower slopes. The rate of apoptotic cell death was 2.8-fold faster for the control compared to the Bax/Bak/LDH siRNA-treated culture (Figure 7, inset).

[00604] These data strongly support the concept that soluble siRNAs when added to CHO cells grown in suspension in a 1 L bioreactor can have a positive effect on both cell density and viability when compared to a non-specific control siRNA.

[00605] Both lactate dehydrogenase enzyme activity and lactate levels are decreased in CHO cells following Bax/Bak/LDH siRNA treatment.

[00606] Lactate dehydrogenase enzyme activity was followed during the course of the cell growth curve (Figure 8). Area under the curve (AUC) analysis indicated a 67% decrease in enzymatic activity in the Bax/Bak/LDH siRNA-treated cells compared to the control siRNA-treated cells. A corresponding decrease in lactate levels was observed (Figure 9). The observed lactate level decrease in the Bax/Bak/LDH siRNA-treated culture as determined by AUC analysis was approximately 33%, about one-half that observed for the enzyme activity decrease, suggesting the LDH pyruvate to lactate conversion rate increased to compensate for decreased enzyme concentrations.

[00607] Glucose consumption in control siRNA-treated cells decreases following day 7 of the growth curve. Glucose was used as part of the culture feeding strategy and monitored throughout the growth curve. Prior to day 7, both the control and experimental cultures utilized glucose to the same extent (Figure 10). After day 7, the Bax/Bak/LDH siRNA-treated cells

continued to use glucose as they did prior to day 7 but the control cell population appeared to decrease their glucose consumption.

[00608] These data demonstrate that Bax/Bax/LDH siRNAs, when added to 1 L CHO bioprocessing cultures, promote glucose utilization post log phase growth compared to the control siRNA-treated culture that does not suggesting the control cells are dead or incapable of glucose metabolism.

[00609] Bax/Bak/LDH siRNAs when added to 1 L CHO bioprocessing cultures significantly decrease Caspase 3 activity compared to the control siRNA. Caspase 3 activation is the penultimate step that leads to DNA degradation in cells undergoing apoptotic death. Since both Bax and Bak proteins are upstream of this process, it is expected that a Bax/Bak knockdown would decrease Caspase 3 activity as well. A biphasic Caspase 3 activity response was observed (Figure 11) for both the control and experimental conditions. During log phase growth, both the Bax/Bak/LDH-siRNA-treated and control siRNA-treated cell cultures had similar Caspase 3 levels. The reason for active Caspase 3 in non-apoptotic cells is uncertain; but during post log phase, the Bax/Bak/LDH siRNA-treated cell culture had markedly less Caspase 3 activity compared to the control cell population with no Caspase 3 activity observed on day 9 and <10% activity present the experimental cell population on day 12 compared to control.

[00610] These data demonstrate the Bax/Bak/LDH siRNAs block the ability of Bax and Bak to activate mitochondrial-induced apoptosis, confirming the appropriate target pathway has been affected.

[00611] Bax/Bak/LDH siRNAs, when dosed multiple times over a 2-week time course, can maintain >80% mRNA knockdown. A recent publication has reported that both Bax and Bak mRNA should be comparably knocked down to maintain a maximum block of apoptosis (Lim et al., 8 Metabolic Eng. 509–22 (2006)), although another group suggested >80% mRNA knockdown was sufficient for LDH (Kim & Lee, 74 Appl. Microbiol. Biotech. 152-59 (2007)) to reduce LDH activity. Therefore, the aim of multiple siRNA doses was to keep the percent knockdown for all three genes to be >80%. Bax and LDH message knockdown through most of the time course was in fact >80% (Figure 12). The Bak mRNA knockdown hovered above and below the 80% mark through the time course. This siRNA appeared to benefit most from the multiple doses as suggested by the zigzag response pattern that seems to correlate with each new dose. A zigzag effect is also observed with the other siRNAs, but not as dramatic as the Bak siRNA.

[00612] These data demonstrate that all three siRNAs used in this study maintained target mRNA knockdown throughout the two week time course. Even though the message knockdown IC_{50} for the Bak siRNA was similar to Bax, the mRNA knockdown maintenance during the time course was not comparable. The reason for this is uncertain but suggests that other Bak siRNAs should be evaluated.

[00613] *Summary*: Silencing RNAs, directed against the apoptotic regulators Bax and Bak, in combination with an siRNA directed against a key metabolic enzyme, lactate dehydrogenase, were evaluated for knockdown activity in Chinese Hamster Ovary cells during a two week time course using a 1 L bioreactor. The results presented herein clearly support the concept that silencing RNAs can be appropriately formulated for efficient uptake into CHO cells grown in suspension under bioprocessing-like conditions. Bax/Bak/LDH siRNAs when dosed multiple times over the two week time course maintained >80% mRNA knockdown which was sufficient to lower both Caspase 3 and LDH activities resulting in increased cell density and viability compared to a non-specific siRNA control. Furthermore, these data demonstrate that multiple siRNAs (at least three) can be added simultaneously with multiple doses in suspension cell cultures with each having its desired knockdown effect and that transfection reagents can be identified that are well tolerated by CHO cells with minimal effect on viability.

Example 5. Improved ADCC of antibodies by use of RNA effectors

[00614] Many therapeutic antibodies, particularly anticancer therapeutic antibodies, require antibody-dependent cellular cytotoxicity (ADCC) for efficacy. In order to achieve high ADCC, it is believed that proper glycosylation of the antibody is necessary. For example, antibodies lacking the core fucose of the Fc oligosaccharides have been found to exhibit much higher ADCC in humans than their fucosylated counterparts. In addition, extensive α 2,6-sialation of N-linked oligosaccharides in antibodies is also thought to reduce ADCC.

[00615] Therefore, it is desirable to produce antibodies with substantially reduced amounts of fucosylation, as well as reduced α 2,6-sialation.

[00616] Fucosylation, particularly α 1,6-fucosylation of antibodies is achieved through a number of enzymatic steps, including:

(i) GDP-mannose 4,6 dehydratase (encoded by GMDS), catalyzing the conversion of GDP-mannose to GDP-4-keto-6-deoxymannose;

(ii) GDP-4-keto-6-deoxy-D-mannose epimerase reductase (encoded by TSTA3), which catalyzes the two step epimerase and the reductase reactions in GDP-D-mannose

metabolism, converting GDP-4-keto-6-D-deoxymannose to GDP-L-fucose, GDP-L-fucose is the substrate of several fucosyltransferases; and

(iii) Fucosyltransferase 8 (α 1,6 fucosyltransferase) (encoded by FUT8), which catalyzes the transfer of fucose from GDP-fucose to N-linked type complex glycopeptides.

[00617] Cells which are deleted or deficient in the α 1,6, fucosyltransferases have been isolated, and are currently used to produce antibodies with reduced fucosylation. However, the cells have a slow doubling time, and require special conditions to grow. Furthermore, the cells are not available in many genetic backgrounds.

[00618] High sialation of antibodies has also been suggested to result in reduced ADCC. Sialation occurs through the action of sialyltransferases such as those described herein.

[00619] Therefore, increased ADCC of antibodies is achieved by producing the antibody in host cells using the methods described herein. For example, host cells expressing antibodies are contacted with siRNAs directed against any one of:

FUT8: Antisense sequence containing at least 16 contiguous nucleotides from SEQ ID NOs:209841-210227; or siRNAs comprising at least one strand selected from SEQ ID NOs:3152714-3152753, or those described herein;

GMDS: dsRNA comprising an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of the oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs:1688202-1688519; and SEQ ID NOs:3152754-3152793;

TSTA3: a dsRNA molecule targeting TSTA3 can comprise an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide molecule selected from the group consisting of SEQ ID NOs:1839578-1839937.

[00620] Twelve separate cultures CHO cells expressing a human anti-CD20 antibody are grown in culture flasks, initially seeded on day 1 at a density of ~200,000 cells/ml, and on day 2 are given the following treatments:

Flask A: Transfection agent only;

Flask B: Transfection agent containing 1 nM (final concentration after addition) Luciferase dsRNA as negative control;

Flask C: 1 nM FUT8 dsRNA in transfection reagent;

Flask D: 1 nM TSTA3 dsRNA in transfection reagent;

Flask E: 1 nM GMDS dsRNA in transfection reagent;

Flask F: 1 nM TSTA3 dsRNA + 1 nM FUT8 dsRNAs in transfection reagent;

Flask G: 1 nM GMDS dsRNA + 1 nM FUT8 dsRNAs in transfection reagent;

Flask H: 1 nM TSTA3 dsRNA + 1 nM GMDS dsRNAs in transfection reagent;

Flask I: 1 nM St6GalNac6 dsRNA + 1 nM FUT8 dsRNAs in transfection reagent;

Flask J: 1 nM St6GalNac6 dsRNA + 1 nM GMDS dsRNAs in transfection reagent;

Flask K: 1 nM St6GalNac6 dsRNA + 1 nM TSTA3 dsRNAs in transfection reagent;

Flask L: 1 nM St6GalNac6 dsRNA + 1 nM FUT8 dsRNAs + 1 nM GMDS dsRNA in transfection reagent;

[00621] Cells are grown for an additional 4 days, and supernatant of each flask is collected. Antibodies are isolated from the supernatant using protein A-sepharose chromatography. The partially purified antibodies are characterized for overall yield (by ELISA using anti-human Ab), antigen binding (e.g., CD20 binding), and for ADCC (using, for example, the lactate dehydrogenase release assay). The oligosaccharide structure of the antibodies isolated from the different cells are characterized MALDI-TOF mass spectrometry in positive-ion mode.

[00622] Exemplary dsRNA sequences against hamster (*Cricetulus griseus*) fucosyltransferase (FUT8) are disclosed herein as SEQ ID NOs:3152714-3152753, wherein the even numbered SEQ ID NOs (e.g., NO:3152714) represent the sense strand and the odd numbered SEQ ID NOs (e.g., NO:3152715) represent the complementary antisense strand; in embodiments described herein, the RNA effector molecule can comprise at least 16 contiguous nucleotides.

Table 43. Screen of FUT8 siRNAs at 1 nM with 1 day incubation on adherent DG44 cells

SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (1nM)
3157117	AD-25348	AUGCCCGCAUUUUCAGAGUdTdT	94.8
209866	AD-25349	UAAUCCAACGCCAGGAACCdTdT	96.6
3157118	AD-25350	AAAAGAAUGAGCAUAAUCCdTdT	93.0
3157119	AD-25351	AAAUGACCACCUAUAUAAAdTdT	28.9
3157120	AD-25352	AACCAAAUGACCACCUAUAdTdT	83.8
3157121	AD-25353	UAUCUCGAACCAAAUGACCdTdT	94.3
209898	AD-25354	UUAUCUCGAACCAAAUGACdTdT	87.9
3157122	AD-25357	AACCAGAGCUCUUUAGCUCdTdT	93.2
3157123	AD-25358	AUCUGUCAUGAUAGACCUUdTdT	66.0
210049	AD-25359	UUAUUCUCCGCUGGACCAGdTdT	80.3
3157124	AD-25360	AUGAGUGUUCGCUGGGUGCdTdT	72.7
3157125	AD-25361	UUACAGGUCUAAACACAGUdTdT	74.3
3157126	AD-25362	AACUGGAUGUUUGAAGCCAdTdT	84.3
3157127	AD-25363	UUGGAGUACUUUGUCUUUGdTdT	86.6
3157128	AD-25364	AAUUGGAGUACUUUGUCUUdTdT	77.3
3125129	AD-25365	UAAUUGGAGUACUUUGUCUdTdT	81.7
209878	AD-25366	AUAAUUGGAGUACUUUGUCdTdT	77.9
209904	AD-25367	AAGUGUAUAUCCAGGAUCAdTdT	81.7
3125130	AD-25355	UUGCAAGAAUCUUGGAGAGdTdT	92.0
209885	AD-25356	AAAACACGGACUCUUCCUGdTdT	93.3

Table 44. Dose-response of FUT8 siRNAs in DG44 cells					
	%mRNA knockdown				
	100 nM	10 nM	1 nM	100 pM	10 picom
AD-25348	97.4	93.7	83.0	47.6	24.6
AD-25349	99.2	97.4	87.0	74.3	22.8
AD-25353	96.5	97.0	89.9	57.2	67.2
AD-25357	94.0	91.5	55.1	51.9	14.9
AD-25356	96.1	95.6	92.7	75.0	26.7

Example 6. Use of Bax/Bak in high-glucose culture

[00623] In general, inclusion of high concentrations of glucose (e.g., at least 15 mM) during growth of cells in bioprocessing results in accumulation of lactic acid in the growth media which can be deleterious to cell growth. Lactic acid accumulation results in premature apoptosis. Since providing high levels of a carbon source such as glucose would be otherwise highly advantageous, a method of growing cells in high glucose without triggering lactic acid accumulation and subsequent apoptosis would be highly desirable.

[00624] In this example, a RNA effector molecule targeting pro-apoptotic genes are used to allow cells to grow at higher glucose concentrations of at least 10 mM (for example, at least 15 mM, at least 20 mM, at least 25 mM, at least 30 mM or more) in the growth medium without undergoing apoptosis.

[00625] On day 0, host cells capable of expressing the immunogenic agent are contacted with 1 nM each of RNA effectors targeting Bax and Bak (optionally also with 1 nM dsRNA targeting LDH) in growth medium containing normal levels (~4-6 mM) of glucose. Approximately 24 hr afterwards, cells are switched to media containing 15 mM glucose. Subsequently, RNA effectors targeting Bax and Bak are further provided at 1 nM every 3 – 5 days. Protein production in these cells is compared with those from cells not transfected with RNA effector molecules (or transfected with an unspecific control RNA effector).

[00626] Other RNA effectors useful to permit growth in high glucose can include those targeting any pro-apoptotic genes, including those described herein. Other examples include RNA effector molecules comprising an an antisense strand comprising at least 16 contiguous nucleotides (e.g., at least 17, at least 18, at least 19 nucleotides) of an oligonucleotide nucleotide having a sequence selected from the group consisting of the following:

Table 30. Caspase				
SEQ ID NO:	consL	Descrption	Avg Coverage	siRNA SEQ ID NOs:
5854	1136	caspase 1	2.306	1964106-1964500
8056	612	caspase 2	1.166	2718675-2719039

5746	1157	caspase 3	11.813	1924836-1925195
7120	855	caspase 6	4.965	2408466-2408843
6798	926	caspase 7	0.436	2301618-2301960
8917	414	caspase 8	0.2	2995593-2995870
4250	1492	caspase 9	1.769	1412589-1412860
5608	1188	caspase 12	0.856	1875252-1875646

Example 7. Efficacy of siRNA's in PK15 cells and in DG44 cells

[00627] *siRNA screening in DG44 cells*: siRNAs against CHO targets of interest are designed and synthesized. Sets of siRNAs (duplex) to be screened are added to cell media at between 100 pM and 10 nM for between 1 and 4 days for effect. In a 96 well plate, 29.5 µL of CD DG44 media (GIBCO™ Invitrogen) supplemented with 8 mM L-glutamine and 0.18% PLURONIC F68® is added to test wells and 47 µL to control wells. To this, 17.5 µL of siRNA at 10 times the final desired concentration in CD DG44 media is added to the test wells. To all wells, 3 µL of LIPOFECTAMINE® transfection reagent RNAiMAx (Invitrogen) diluted 1:10 in CD DG44 media is added. The mixture is allowed to incubate at room temperature for 15 min and then 125 µL of CD DG44 media containing approximately 20,000 DG44 cells is added to all wells. The plates are then placed in a 37°C CO_2 incubator for between 1 and 4 days.

[00628] After incubation, cells are visually inspected for toxicity and RNA extracted using a MagMax 96-well RNA extraction kit (Ambion, Life Technologies Corp., Carlsbad, California) following the manufacturer's instructions. cDNA is made from the RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies Corp.) according to the manufacturer's instructions. Finally, qPCR is used to quantify an appropriate dilution of the target cDNA with a Roche Lightcycler 480 PCR instrument and Roche PCR Probes master mix. Relative knockdown of target genes was calculated using the ΔΔCt method using GAPDH as the internal standard. The % mRNA knockdown for target genes cofilin1, LDLR, GNE, SLC35A1,GALE, FUT8, GMDS, and XYLAT are shown elsewhere herein.

[00629] The most potent siRNAs are tested further in a range of concentrations. The method for this testing was the same as above except that a range of siRNA concentrations were tested simultaneously.

[00630] *siRNA screening in PK15 cells*: siRNAs against PCV1 targets of interest are designed and synthesized. Sets of siRNAs to be screened are added to cell media at 10 nM for 1 day for effect. In a 96-well plate, 29.5 µL of Minimum Essential Medium, Eagle's, with Earle's Balanced Salt (EMEM) media (ATCC) are added to test wells and 47 µL to control wells. To this, 17.5 µL of siRNA at 100 nM in CD DG44 media is added to the test wells. To all

wells, 4 μL of LIPOFECTAMINE® RNAiMAx reagent (Invitrogen) diluted 1:10 in EMEM media is added. The mixture is allowed to incubate at room temperature for 15 min and then 125 μL of EMEM media containing approximately 20,000 PK15 cells is added to all wells. The plates were then placed in a 37°C CO_2 incubator for 1 day.

[00631] After incubation, cells are visually inspected for toxicity and then RNA is extracted using a MagMax 96-well RNA extraction kit (Ambion) following the manufacturer's instructions. cDNA was made from the RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. Finally, qPCR is used to quantify an appropriate dilution of the target cDNA with a Roche Lightcycler 480 PCR instrument and Roche PCR Probes master mix. Relative knockdown of target genes is calculated using the ΔΔCt method using GAPDH as the internal standard.

Table 31. Screen of cofilin1 siRNAs in DG44 cells

SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (1nM)	%mRNA knockdown (100pM)
1914036	AD-30721	UUCAUUUACACACAGAACUdTdT	95.2	89.3
1914037	AD-30722	GAAUCAGAGGAUCAGAAGCdTdT	96.4	74.2
1914038	AD-30723	AAGAGCACUGCCUUCUUGCdTdT	76.7	42.6
1914039	AD-30724	UGUAAUUCGUGCUUGAUUCdTdT	96.8	82.2
1914040	AD-30725	UUAGAAGUUGGCAGCAUGGdTdT	87.5	66.3
1914041	AD-30726	UCCGCUUCAACCCAAGAGGdTdT	95.7	71.1
1914042	AD-30727	AAGGGGUGCACUCUCAGGGdTdT	92.6	60.5
1914043	AD-30728	UGUGGUUAGAAGUUGGCAGdTdT	88.8	70.0
1914044	AD-30729	UUCCGUUUAAGUAGGAGCCdTdT	17.4	16.5
1914045	AD-30730	GACACCAUCAGAGACAGCCdTdT	90.8	48.6
1914046	AD-30731	UAUUGUGGUUAGAAGUUGGdTdT	92.4	75.4
1914047	AD-30732	ACUUGGUCCGCUUCAACCCdTdT	91.8	75.2
1914048	AD-30733	AAAGGCUUGCCCUCCAGAGdTdT	40.2	9.0
1914050	AD-30734	CUUCCGUUUAAGUAGGAGCdTdT	-18.8	-5.6
1914051	AD-30735	AGCACAGUCACUAUUGUGGdTdT	93.8	71.2
1914052	AD-30736	UUGAACACUUUGAUGACACdTdT	88.2	73.9
1914053	AD-30737	UUUGCUUGUAAUUCGUGCUdTdT	91.6	76.1
1914054	AD-30738	UUGACAAAAGUGGUGUAGGdTdT	83.9	49.9
1914055	AD-30739	AUAGCGGCAGUCCUUGUCGdTdT	77.9	45.1
1914056	AD-30740	UAAAUUGAAGGUCCCUUACdTdT	94.3	84.5
1914057	AD-30741	UAUCAUUGAACACUUUGAUdTdT	83.9	51.6
1914058	AD-30742	UCGUAGAGAGCAUAGCGGCdTdT	77.3	14.8
1914060	AD-30743	UGACAAAAGUGGUGUAGGGdTdT	85.6	58.6
1914061	AD-30744	UGCAACACCCAUGAGCAGGdTdT	81.4	39.5

Table 32. Dose-response of cofilin1 siRNAs in DG44 cells

	%mRNA knockdown

	10 nM	1 nM	100 pM	10 picom	1 picom	0.1 picom
AD-30721	99.2	98.8	97.4	92.9	63.4	7.7
AD-30724	99.3	99.0	99.0	95.5	62.1	9.0
AD-30731	97.1	98.7	97.5	92.7	58.7	-3.1
AD-30737	99.3	99.4	98.8	87.7	36.3	-1.7
AD-30740	97.9	97.6	96.2	94.6	76.8	33.1

Table 33. Screen of LDLR siRNAs in DG44 cells at 1nM

SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (1nM)
1522123	AD-29793	AAAUAUAUAAAACAGAGCCdTdT	57.7
1522125	AD-29794	AACAGUGCAAUCUAAGAGCdTdT	64.9
1522126	AD-29795	AUAUCUACACCAUUCAGGCdTdT	33.5
1522127	AD-29796	GAAUCAACCCAAUAGAGGCdTdT	40.8
1522128	AD-29797	CUUGCUUUGAGCAACACAGdTdT	26.9
1522129	AD-29798	ACCAAAUUAACAUCUGAGCdTdT	23.0
1522130	AD-29799	UCAUUUAUGACAUCCGUCCdTdT	71.7
1522131	AD-29800	UCAUCUUCCAAAAUGGUCUdTdT	71.3
1522132	AD-29801	ACUGGAAGCCGACUGAUCCdTdT	57.7
1522133	AD-29802	AGUGUGAUGCCAUUUGGCCdTdT	64.9
1522134	AD-29803	AAUUGUGGCAAUUCUAAGUdTdT	33.5
1522135	AD-29804	AAAAUGGUCUUCCGAUUGCdTdT	40.8
1522136	AD-29805	UCUUAUCUUGCUUUGAGCAdTdT	78.1
1522137	AD-29806	AGGGUGAUGGACAAGACCCdTdT	6.9
1522138	AD-29807	UGUUCAUGCCACAUCAUCCdTdT	67.5
1522139	AD-29808	UAAAUUGUGGCAAUUCUAAdTdT	3.2
1522140	AD-29809	AAAUUAACAUCUGAGCCUGdTdT	16.3
1522141	AD-29810	AAGGAGGAUGACCAGUGCGdTdT	23.0
1522142	AD-29811	UCUAAGAGCACAGAUGCAGdTdT	81.3
1522143	AD-29812	AAGAGAAGGUUCUCACAGCdTdT	48.0
1522145	AD-29813	UUGGAAUCAACCCAAUAGAdTdT	36.6
1522146	AD-29814	GUGAUCUCAGGCCUGAUGGdTdT	39.8
1522148	AD-29815	AAAGUGCGAAGAGAGAAUCdTdT	66.7

Table 34. Dose response of LDLR siRNAs in DG44 cells

	%mRNA knockdown					
	10 nM	1 nM	100 pM	10 picom	1 picom	0.1 picom
AD-29799	91.9	91.3	83.0	43.5	17.6	-8.7
AD-29800	87.9	84.4	66.5	9.8	-10.3	-18.6
AD-29805	89.1	87.4	83.6	55.2	15.6	-11.4
AD-29807	95.8	93.2	83.1	45.3	4.4	-15.3
AD-29811	93.8	89.1	85.7	69.1	33.3	3.4

Table 35. Screen of GNE siRNAs in DG44 cells at 1nM

SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (1nM)
420095	AD-29121	UACUGACUCUACCAUGGCUdTdT	89.2
419972	AD-29122	UCCAUGAACAAUCAUGAUGdTdT	94.7
420052	AD-29123	AGUUUGUCAUAGGAAGGGCdTdT	85.7

419975	AD-29124	AGCAGUUUGUCAUAGGAAGdTdT	97.5
420033	AD-29125	AUCCGAAUGAUGCUCAUAUdTdT	80.0
420029	AD-29126	AGUGCAACAAUGUAAUCUUdTdT	38.4
420005	AD-29127	AAUGAGAUAAGUGCAUCCAdTdT	66.8
420224	AD-29128	ACAUGCUUGACUGCACGAAdTdT	28.3
420003	AD-29129	AAAUGGGACAUGCUUGACUdTdT	95.8
420090	AD-29130	AUAAACUGGUCAAAUGGGAdTdT	88.3
419965	AD-29131	UAUAAACUGGUCAAAUGGGdTdT	70.1
420219	AD-29132	AUCAUACAACCAGCAUGGGdTdT	51.7
420140	AD-29134	UUAUCUUGGGUGUCAGCAUdTdT	90.7
419984	AD-29135	UUUAUCUUGGGUGUCAGCAdTdT	91.9
420214	AD-29136	AGGUGGAGCGCUUGUAAUAdTdT	-55.8
419970	AD-29137	UUUACCGAACUGAAGGUGGdTdT	81.6
420077	AD-29138	UACUGUUUACCGAACUGAAdTdT	29.3
420096	AD-29139	UCUUAACUAUUUCACCCUUdTdT	45.3
420025	AD-29140	UUCUUAACUAUUUCACCCUdTdT	79.1
420098	AD-29141	UCUGCAGGAUUAAACUAAUdTdT	26.8
420186	AD-29142	AAAUGCCUACUCCCAGAAUdTdT	44.1
420121	AD-29143	UUGGCCAAACUUCCUUUCUdTdT	83.5
419964	AD-29144	UGUAAUGAGUGUCACAAAGdTdT	87.5
420243	AD-29145	UGCCUGUAAUGAGUGUCACdTdT	79.1
420011	AD-29146	AUAUUCUGGGCCUUCACGUdTdT	83.8
419974	AD-29147	UGUUCGUAGGAUAUUCUGGdTdT	79.6
420084	AD-29148	AGCUGUUCGUAGGAUAUUCdTdT	77.3
420143	AD-29149	ACGUCCUUGACAAUGUGGAdTdT	87.3
420275	AD-29150	AGGGUCAACCAAGUCUGAAdTdT	10.5
420017	AD-29151	UGAAGAAGUACUGAUCUAAdTdT	86.1
420297	AD-29133	UCUUCCUAUCUGGCGUGUUdTdT	74.5
420064	AD-29152	AGAUCCACCUUUAAUCUAGdTdT	-167.6

Table 36: Dose-response of GNE siRNAs in DG44 cells						
	%mRNA knockdown					
	10 nM	1 nM	100 pM	10 picom	1 picom	0.1 pM
AD-29122	95.5	93.6	75.9	28.8	47.7	17.9
AD-29124	94.4	89.6	63.3	7.3	-3.2	-13.3
AD-29129	97.7	90.7	87.0	36.9	-18.5	-20.2
AD-29134	94.6	89.3	66.1	-5.0	-43.4	-3.5
AD-29135	89.9	90.6	80.5	12.3	-4.6	-38.0

Table 37. Screen of SLC35A1 siRNAs at 1 nM on DG44 cells for 3 days			
SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (1nM)
1368055	AD-29063	AAGCUACGGUAUAAGCUGCdTdT	92.0
1367961	AD-29064	AAAGCUACGGUAUAAGCUGdTdT	83.6
1368040	AD-29065	UGUGUAUCUUAAAGCUACGdTdT	79.3
1367983	AD-29066	UUGUGUAUCUUAAAGCUACdTdT	90.5
1367981	AD-29067	ACUUUAUAACUUCUGUGACdTdT	94.4
1368010	AD-29068	AAUCUACCCAAACUUCCAGdTdT	99.5
1367999	AD-29069	UUAAAUCUACCCAAACUUCdTdT	99.5

Table 37. Screen of SLC35A1 siRNAs at 1 nM on DG44 cells for 3 days			
1368000	AD-29070	AGAUGCCUUAAAUCUACCCdTdT	99.3
1368085	AD-29071	AAGAUGCCUUAAAUCUACCdTdT	97.9
1367995	AD-29072	ACUAAGAGCUAGGAAAGCCdTdT	99.5
1368045	AD-29073	AUACUAAGAGCUAGGAAAGdTdT	99.4
1368017	AD-29074	UAGGUAACCUGGUAUACUGdTdT	99.4
1368068	AD-29075	ACCCAUGUAAUUUGCUGAGdTdT	75.3
1367960	AD-29076	AUAGCUAUUGCACCAAAGCdTdT	99.6
1367978	AD-29077	AAUACAGCAAUAGCUAUUGdTdT	98.4
1368053	AD-29078	AUCCAGAGCACAAUACAGCdTdT	99.3
1367953	AD-29079	AAUAAACUCCUGCAAAUCCdTdT	97.9
1367957	AD-29080	AAGUGAAUGUUUCUCACCCdTdT	96.2
1368159	AD-29081	UAGUCGUUAUAGGAGUAUCdTdT	99.2
1368033	AD-29082	AGUUUAGUCGUUAUAGGAGdTdT	99.5
1367956	AD-29083	AUUAUUGACAGUUUAGUCGdTdT	99.1
1368012	AD-29084	UAUUAUUGACAGUUUAGUCdTdT	99.7
1367967	AD-29085	UGUUUAAGCUACCAUCUGGdTdT	98.1
1367977	AD-29086	UAUUGUUUAAGCUACCAUCdTdT	98.7
1368065	AD-29087	UGAUAUUGUUUAAGCUACCdTdT	98.0
1367973	AD-29088	UUGAUAUUGUUUAAGCUACdTdT	99.3
1368038	AD-29089	UUGAAUAUUGUAGUUUCACdTdT	98.3
1368089	AD-29090	ACUUGAACCUUCAGAUACCdTdT	98.0
1368077	AD-29091	UACCUGAACGAGAGAACAGdTdT	98.7
1368067	AD-29092	UCUCUUAUUCAUUCUUCACdTdT	98.6
1368039	AD-29093	UUACCCAGACAGAAGUCAGdTdT	99.1
1367952	AD-29094	AAGUAUAUCAGCUAACAGCdTdT	99.7
1367965	AD-29095	AGUUCACAAAUUGAGAGCCdTdT	98.6
1367963	AD-29096	AAGUUCACAAAUUGAGAGCdTdT	97.2

Table 38. Dose-response of SLC35A1 siRNAs in DG44 cells						
	% mRNA knockdown					
	10 nM	1 nM	100 pM	10 picom	1 picom	0.1 pM
AD-29068	99.7	99.3	95.3	51.8	34.9	39.5
AD-29069	94.8	94.1	88.8	56.9	59.8	60.1
AD-29076	99.9	99.4	96.1	76.7	55.8	30.2
AD-29084	99.9	99.7	99.0	95.4	54.9	1.6
AD-29094	99.8	99.6	99.5	97.4	54.1	-9.1

Table 39. Screen of SLC35A2 siRNAs at 1 nM on DG44 cells for 3 days			
SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (1nM)
464723	AD-29097	UAUCGGAUGCUAAGGAUGAdTdT	86.9
464762	AD-29098	AUAUCGGAUGCUAAGGAUGdTdT	89.9
464953	AD-29099	UACGAGCAUAUCGGAUGCUdTdT	80.2
464679	AD-29100	AAAUAAUGGGUCCAGGUGAdTdT	90.8
464814	AD-29101	UUAUGGCUUUGACUGCACUdTdT	92.9
464729	AD-29102	UAUCCCUCUAGAAGUGUGGdTdT	92.2
464852	AD-29103	AUAUCCCUCUAGAAGUGUGdTdT	87.8
464750	AD-29104	UUCCCAAAGAGGUUAGCCUdTdT	81.4

464833	AD-29105	AUUAGUCGUUACUGAAGAAdTdT	75.6
464844	AD-29106	UUACAACAGGCCGAUCUUCdTdT	83.0
464676	AD-29107	AAGUAAAUGGUGCUUAUUGdTdT	88.3
464859	AD-29108	AUCACAAAUGCCCGACAUAdTdT	85.4
464748	AD-29109	UAUCACAAAUGCCCGACAUdTdT	92.4
464820	AD-29110	AUAUCACAAAUGCCCGACAdTdT	90.4
464675	AD-29111	AACCUGAUAUCACAAAUGCdTdT	92.2
464701	AD-29112	AAUUCUGACACCGCCAUGAdTdT	49.8
464847	AD-29113	AUCAAUUCUGACACCGCCAdTdT	83.4
464702	AD-29114	UAAGGAGUUAGUAAGCUUUdTdT	86.4
464778	AD-29115	UACAGUUAAGGAGUUAGUAdTdT	79.5
464881	AD-29116	AUACAGUUAAGGAGUUAGUdTdT	90.1
464961	AD-29117	AUCCUGACAUAUGUUCAUUdTdT	66.0
464804	AD-29118	UAUCCUGACAUAUGUUCAUdTdT	83.7
464726	AD-29119	UUGGCAUUGGGUAUCCUGAdTdT	83.2
464799	AD-29120	UUAUUUGGCAUUGGGUAUCdTdT	89.2

Table 40. Dose-response of SLC35A2 siRNAs in DG44 cells						
	%mRNA knockdown					
	10 nM	1 nM	100 pM	10 picom	1 picom	0.1 pM
AD-29101	66.4	60.3	16.4	24.1	-0.1	19.5
AD-29102	92.6	87.4	81.8	-4.3	66.5	53.3
AD-29109	85.7	84.2	79.5	37.5	-2.2	-31.6
AD-29110	74.4	84.4	77.3	-20.3	-63.2	-51.2
AD-29111	97.3	86.1	80.9	27.2	-15.8	-2.9

Table 41. Screen of GALE siRNAs at 1 nM with 3 days incubation on adherant DG44 cells			
SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (1nM)
1888656	AD-28691	UCUAUAAUAAUCCAGAGGCdTdT	92.1
1888657	AD-28692	AACACGAGAUUCUUCACCCdTdT	88.8
1888660	AD-28693	AAAGCUGUGCUUCUUAAAGdTdT	70.3
1888659	AD-28694	AUUGAAGUAGCGUAGCAGCdTdT	87.4
1888663	AD-28695	UGUAUCAUAGUCACCACCAdTdT	92.6
1888662	AD-28696	UCCACGAAUGGCAUUAUGGdTdT	86.4
1888665	AD-28697	ACCACAUGAAUGUAAUCCCdTdT	90.1
1888674	AD-28698	AACUCUAUAAUAAUCCAGAdTdT	66.3
1888673	AD-28699	ACUUGGACUUUCCAUAGGGdTdT	78.1
1888689	AD-28700	UAUGGGAUCUUCUUCCCUGdTdT	73.7
1888690	AD-28701	UAUAAUAAUCCAGAGGCUUdTdT	89.3
1888698	AD-28702	UCCUCUGUAUCAUAGUCACdTdT	90.5
1888695	AD-28703	AUGAAUGUAAUCCCUUACAdTdT	86.5
1888703	AD-28704	UGUAAUCCCUUACACCUGUdTdT	89.8
1888702	AD-28705	AGAACUUGGACUUUCCAUAdTdT	93.3
1888706	AD-28706	AACCACAUUGCUCCUUCAGdTdT	65.7
1888707	AD-28707	AUAAUAAUCCAGAGGCUUCdTdT	73.0
1888705	AD-28708	ACAGCCUUAAAGCUGUGCUdTdT	73.7
1888719	AD-28709	UAAUCCCUUACACCUGUGCdTdT	91.3
1888708	AD-28710	UUCGUAAGGAGGUCUUUAGdTdT	95.2

1888701	AD-28711	UCGUAAGGAGGUCUUUAGGdTdT	94.0
1888710	AD-28712	UCUUAAAGAGGUGCUGUAGdTdT	91.3
1888716	AD-28713	UAAGGAGGUCUUUAGGCCUdTdT	82.4
1888738	AD-28714	UCCCUGUUAGGUUAACUCUdTdT	92.2
1888735	AD-28715	UUUUGGUCCUUCGUAAGGAdTdT	89.7
1888723	AD-28716	UUGAAGUAGCGUAGCAGCAdTdT	66.8
1888769	AD-28717	UAAAGCUGUGCUUCUUAAAdTdT	25.5
1888781	AD-28718	UGAACACGAGAUUCUUCACdTdT	93.6
1888774	AD-28719	AAGUGGAUGACAGCCUUAAdTdT	60.9

Table 42. Dose-response of GALE siRNAs in DG44 cells					
	%mRNA knockdown				
	10 nM	1 nM	100 pM	10 picoM	1 picoM
AD-28695	99.1	97.8	92.1	75.5	32.8
AD-28705	96.4	94.9	94.2	76.7	45.4
AD-28710	97.1	97.8	94.9	88.3	42.3
AD-28711	98.7	97.8	95.2	75.6	24.9
AD-28718	98.6	98.3	93.6	61.4	3.6

Table 45. Screen of GMDS siRNAs at 10 nM with 1 day incubation on adherent DG44 cells			
SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (10nM)
1688259	AD-25328	UUCGACCUGUAUUAAAUGAdTdT	93.1
1688271	AD-25329	AAUUCGACCUGUAUUAAAUdTdT	93.9
1688246	AD-25331	UAUAAAUGUUCAAUUCGACdTdT	91.3
3157131	AD-25332	UUCAUGUUUCCUUCAAUAUdTdT	78.9
1688245	AD-25333	AGUGCAACUUCAUGUUUCCdTdT	89.8
3157132	AD-25334	UCCAAGGUAAAUCUUAGCUdTdT	90.6
3157133	AD-25338	AUAGUCCUUGGCAUGGCCCdTdT	87.9
3157134	AD-25340	UUCCUUCCCACACAAUGGUdTdT	89.9
3157135	AD-25342	UUGCCGGUCUCUUUACAUCdTdT	85.1
1688220	AD-25344	AAGUAAAAUGAGUAUGUGAdTdT	92.7
1688283	AD-25345	UAGUGACAUAAUUUCAAGUdTdT	92.7
3157136	AD-25346	UUGUCUAGUGACAUAAUUUdTdT	87.1
3157137	AD-25347	AAAAACAAUCUCAAGACUCdTdT	91.2
1688483	AD-25330	AUGUUCAAUUCGACCUGUAdTdT	81.4
3157138	AD-25335	UGUCCAAGGUAAAUCUUAGdTdT	77.3
3157139	AD-25336	UUGUCCAAGGUAAAUCUUAdTdT	83.3
3157140	AD-25337	UUGGCAUGGCCCCAGUCUCdTdT	63.0
1688295	AD-25339	UUCCCCAGUAGCUAUGACAdTdT	80.8
1688307	AD-25341	UCUCUUUACAUCUGCCCACdTdT	90.8
1688317	AD-25343	AAAGGCAACGCGGGGCUUCdTdT	80.9

Table 46. Dose-response of GMDS siRNAs in DG44 cells					
	%mRNA knockdown				
	100 nM	10 nM	1 nM	100 pM	10 picom
AD-25328	94.9	89.4	75.9	49.5	-3.3
AD-25329	92.1	89.1	80.1	49.9	12.6

AD-25331	94.2	88.9	87.6	73.8	27.5
AD-25344	96.4	91.2	85.8	63.8	21.4
AD-25345	92.9	86.3	78.0	45.1	26.7

Table 47. Screen of XYLT2 siRNAs at 100 pM with 4 days incubation on adherant DG44 cells

SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (10nM)
1554777	AD-28123	AUUAGCAGUAAGUAGUGAGdTdT	50.5
1554779	AD-28124	AAGUAGUGAGCACUACACCdTdT	-18.5
3157141	AD-28125	UUUCCUGAGAGGUAGUUUGdTdT	84.1
1554785	AD-28126	UCUUAGGUCUGCUUGGAGCdTdT	65.3
3157142	AD-28127	UCAGUGUCCUCAUCUACCGdTdT	52.9
3157143	AD-28128	AGGUUGGAUCAAUAGGGCCdTdT	-77.2
3157144	AD-28129	AGAACUGAAGCAAUCGAACdTdT	76.1
3157145	AD-28130	UGCGGUUGAAGGUCAAUGGdTdT	43.0
1554792	AD-28131	AGACAAAACCUCUCCAGAGdTdT	34.5
1554793	AD-28132	ACUUCUUAGGUCUGCUUGGdTdT	62.6
1554795	AD-28133	UGUCAUAUGAUGUGGCCACdTdT	1.7
1554806	AD-28134	ACCGUGAUGUCAUAUGAUGdTdT	67.5
1554808	AD-28135	AAAGAAGGUGGGUCUGGAGdTdT	62.9
1554809	AD-28136	UCUACCGUGAUGUCAUAUGdTdT	72.2
1554815	AD-28137	UAGGUUGGAUCAAUAGGGCdTdT	-109.9
1554821	AD-28138	ACGAUGUGUUUGUACUGGCdTdT	70.0
3157146	AD-28139	AGCAGUAAGUAGUGAGCACdTdT	72.4
3157147	AD-28140	UACGGUUCCAGUUGGUGACdTdT	81.1
1554825	AD-28141	ACAAGGAAGCGAAUCUCGCdTdT	76.0
3157148	AD-28142	AGCACGAACCAGUCAGAACdTdT	61.6
1554838	AD-28143	AUGUAUUCAUUGUGGGGUGdTdT	39.3
1554860	AD-28144	ACAGCCCACUUCUUAGGUCdTdT	54.0
1554886	AD-28145	UGACACGCAAGUUGUUGUCdTdT	2.4

Table 48. Dose-response of XYLT2 siRNAs in DG44 cells

	%mRNA knockdown				
	10 nM	1 nM	100 pM	10 picom	1 picom
AD-28125	89.0	91.3	77.2	42.0	29.3
AD-28129	93.3	88.1	81.6	50.9	24.5
AD-28139	92.0	92.6	68.5	34.7	15.9
AD-28140	96.0	96.4	75.7	53.7	13.8
AD-28141	94.9	92.5	81.6	60.8	34.7

Table 49. siRNAs against PCV1 Rep screened at 10 nM overnight in PCV1 infected PK15 cells

SEQ ID NO:	Name	antisense sequence	%mRNA knockdown (10nM)
3290845	AD-36165.2	AAcACCcACCUCUuAUGGGdTsdT	-3.5
3290846	AD-36171.2	uAAGGGUGAAcACCcACCUdTsdT	11.4
3290847	AD-36177.2	UuAAGGGUGAAcACCcACCdTsdT	17.6
3290848	AD-36183.2	AUuAAGGGUGAAcACCcACdTsdT	23.4

Table 49. siRNAs against PCV1 Rep screened at 10 nM overnight in PCV1 infected PK15 cells			
3290849	AD-36189.2	uAUuAAGGGUGAAcACCcAdTsdT	-35.7
3290850	AD-36195.2	UuAUuAAGGGUGAAcACCCdTsdT	3.4
3290851	AD-36201.2	AAGCUCCCGuAUUUUGUUUdTsdT	-5.7
3290852	AD-36207.2	AAGGGAGAUUGGAAGCUCCdTsdT	-17.3
3290853	AD-36166.2	UUCCUCUCCGcAAAcAAAAdTsdT	29.3
3290854	AD-36172.2	AAACCUUCCUCUCCGcAAAdTsdT	63.0
3290855	AD-36178.2	UUCcAAACCUUCCUCUCCGdTsdT	49.8
3290856	AD-36184.2	uACCCUCUUCcAAACCUUCdTsdT	-36.1
3290857	AD-36190.2	UUCuACCCUCUUCcAAACCdTsdT	-11.3
3290858	AD-36196.2	AAUUCGcAAACCCCUGGAGdTsdT	22.6
3290859	AD-36202.2	AAAUUCGcAAACCCCUGGAdTsdT	28.3
3290860	AD-36208.2	uAGcAAAAUUCGcAAACCCdTsdT	48.2
3290861	AD-36167.2	UUCUuAGcAAAAUUCGcAAdTsdT	16.5
3290862	AD-36173.2	AAGUCUGCUUCUuAGcAAAdTsdT	63.5
3290863	AD-36179.2	AAAGUCUGCUUCUuAGcAAdTsdT	42.0
3290864	AD-36185.2	AAAAGUCUGCUUCUuAGcAdTsdT	57.8
3290865	AD-36191.2	uAAAAGUCUGCUUCUuAGCdTsdT	61.2
3290866	AD-36197.2	UuAAAAGUCUGCUUCUuAGdTsdT	55.4
3290867	AD-36203.2	UUcACCUUGUuAAAAGUCUdTsdT	64.8
3290868	AD-36209.2	uACcACUUcACCUUGUuAAdTsdT	66.2
3290869	AD-36168.2	AuACcACUUcACCUUGUuAdTsdT	21.8
3290870	AD-36174.2	AAuACcACUUcACCUUGUUdTsdT	30.3
3290871	AD-36180.2	AAAuACcACUUcACCUUGUdTsdT	48.9
3290872	AD-36186.2	UUCGCUUUCUCGAUGUGGCdTsdT	51.0
3290873	AD-36192.2	UUCCUUUCGCUUUCUCGAUdTsdT	53.3
3290874	AD-36198.2	UuAUUCUGCUGGUCGGUUCdTsdT	17.1
3290875	AD-36204.2	UUCUUuAUUCUGCUGGUCGdTsdT	13.2
3290876	AD-36210.2	uACUGcAGuAUUCUUuAUUdTsdT	61.8
3290877	AD-36169.2	UuACUGcAGuAUUCUUuAUdTsdT	35.4
3290878	AD-36175.2	UUuACUGcAGuAUUCUUuAdTsdT	0.3
3290879	AD-36181.2	AUGUGGCCUUCUUuACUGCdTsdT	-20.2
3290880	AD-36187.2	uAUGUGGCCUUCUUuACUGdTsdT	18.2
3290881	AD-36193.2	AAGuAUGUGGCCUUCUUuAdTsdT	-218.2
3290882	AD-36199.2	uAAGuAUGUGGCCUUCUUUdTsdT	47.1
3290883	AD-36205.2	AuAAGuAUGUGGCCUUCUUdTsdT	41.0
3290884	AD-36211.2	uACUcAcAGcAGuAGAcAGdTsdT	-30.6
3290885	AD-36170.2	AAAGGGuACUcAcAGcAGUdTsdT	23.7
3290886	AD-36176.2	AAAAGGGuACUcAcAGcAGdTsdT	27.6
3290887	AD-36182.2	AACUGCUCGGCuAcAGUcAdTsdT	31.7
3290888	AD-36188.2	uACGUuAcAGGGAACUGCUdTsdT	40.3
3290889	AD-36194.2	UUCUcAcAuACGUuAcAGGdTsdT	53.3
3290890	AD-36200.2	AAUUUCUcAcAuACGUuACdTsdT	58.2
3290891	AD-36206.2	AAAUUUCUcAcAuACGUuAdTsdT	63.5
3290892	AD-36212.2	UUCCCGCUcACUUUcAAAAdTsdT	58.5
3290893	AD-36213.1	AUCUUCCCGCUcACUUUcAdTsdT	-100.7
3290894	AD-36219.1	AUcACGCUGCUGcAUCUUCdTsdT	16.5
3290895	AD-36225.1	uAcAGCUGUCUUCcAAUcAdTsdT	36.7
3290896	AD-36231.1	AAAAUuACGGGCCcACUGGdTsdT	20.2
3290897	AD-36237.1	uAGGCUcAGcAAAAUuACGdTsdT	24.0

Table 49. siRNAs against PCV1 Rep screened at 10 nM overnight in PCV1 infected PK15 cells			
3290898	AD-36243.1	UUCcAGuAGGUGUCGCuAGdTsdT	41.0
3290899	AD-36249.1	UUCuACuAGGCUUCcAGuAdTsdT	64.0
3290900	AD-36255.1	UUUCuACuAGGCUUCcAGUdTsdT	63.7
3290901	AD-36214.1	AUUUCuACuAGGCUUCcAGdTsdT	43.4
3290902	AD-36220.1	AUCCcACcACUuAUUUCuAdTsdT	11.7
3290903	AD-36226.1	AUCcAUCCcACcACUuAUUdTsdT	18.5
3290904	AD-36232.1	uAUCcAUCCcACcACUuAUdTsdT	24.5
3290905	AD-36238.1	AuAUCcAUCCcACcACUuAdTsdT	40.1
3290906	AD-36244.1	AUGAuAUCcAUCCcACcACdTsdT	29.3
3290907	AD-36250.2	UUCUCcAUGAuAUCcAUCCdTsdT	50.7
3290908	AD-36256.1	UUCUUCUCcAUGAuAUCcAdTsdT	22.1
3290909	AD-36215.1	AACUUCUUCUCcAUGAuAUdTsdT	27.3
3290910	AD-36221.1	AAcAACUUCUUCUCcAUGAdTsdT	46.0
3290911	AD-36227.2	AAcAAcAACUUCUUCUCcAdTsdT	52.4
3290912	AD-36233.1	AAAcAAcAACUUCUUCUCCdTsdT	55.1
3290913	AD-36239.2	AAAAcAAcAACUUCUUCUCdTsdT	46.6
3290914	AD-36245.1	AUCcAAAAcAAcAACUUCUdTsdT	56.9
3290915	AD-36251.2	AUcAUCcAAAAcAAcAACUdTsdT	25.8
3290916	AD-36257.1	AAUcAUCcAAAAcAAcAACdTsdT	76.0
3290917	AD-36222.1	AAGGuAACcAGCcAuAAAAdTsdT	53.0
3290918	AD-36228.2	AUCCcAAGGuAACcAGCcAdTsdT	-5.0
3290919	AD-36234.2	AUcAUCCcAAGGuAACcAGdTsdT	30.3
3290920	AD-36240.1	AuACCGGUcAcAcAGUCUCdTsdT	16.8
3290921	AD-36246.1	AUGGAuACCGGUcAcAcAGdTsdT	39.5
3290922	AD-36252.1	AAUGGAuACCGGUcAcAcAdTsdT	29.0
3290923	AD-36258.1	uAcAGUcAAUGGAuACCGGdTsdT	14.4
3290924	AD-36217.1	uAGUCUCuAcAGUcAAUGGdTsdT	-5.7
3290925	AD-36223.1	UUGCUGGuAAUcAAAAuACdTsdT	33.3
3290926	AD-36229.1	AUUGCUGGuAAUcAAAAuAdTsdT	38.2
3290927	AD-36235.1	UUGAGGAGuACcAUUCCUGdTsdT	18.5
3290928	AD-36241.1	uAGAGAGCUUCuAcAGCUGdTsdT	16.5
3290929	AD-36247.2	AuAGAGAGCUUCuAcAGCUdTsdT	58.5
3290930	AD-36253.1	AAGuAGuAAUCCUCCGAuAdTsdT	-20.6
3290931	AD-36259.1	AAAGuAGuAAUCCUCCGAUdTsdT	10.2
3290932	AD-36218.1	UUGcAAAGuAGuAAUCCUCdTsdT	22.1
3290933	AD-36224.2	AUUGcAAAGuAGuAAUCCUdTsdT	36.3
3290934	AD-36230.2	UUCcAAAAUUGcAAAGuAGdTsdT	-16.0
3290935	AD-36236.1	UUCUCcAGcAGUCUUCcAAdTsdT	-1.0
3290936	AD-36242.1	AUUGUUCUCcAGcAGUCUUdTsdT	22.3
3290937	AD-36248.1	uACCUCCGUGGAUUGUUCUdTsdT	14.5
3290938	AD-36254.1	UUcAAAUCGGCCUUCGGGUdTsdT	58.1
3290939	AD-36260.1	UUuAuAUGGGAAAAGGGcAdTsdT	27.4

Table 50. siRNAs against PCV1 Cap screened at 10 nM overnight in PCV1 infected PK15 cells			
SEQ ID NO.	Name	antisense sequence	%mRNA knockdown (10nM)
	AD-35779.1	AUGUUUCcAAGAUGGCUGCdTsdT	8.2

Table 50. siRNAs against PCV1 Cap screened at 10 nM overnight in PCV1 infected PK15 cells			
	AD-35785.1	UUCUCCGGAGGAUGUUUCCdTsdT	61.9
	AD-35791.1	uAUGGUCUUCUCCGGAGGAdTsdT	-1.8
	AD-35797.1	AuAUGGUCUUCUCCGGAGGdTsdT	43.5
	AD-35803.1	AAuAUGGUCUUCUCCGGAGdTsdT	52.8
	AD-35809.1	AAAuAUGGUCUUCUCCGGAdTsdT	27.0
	AD-35815.1	uAACGGUUUCUGAAGGCGGdTsdT	1.6
	AD-35821.1	AUCUGuAACGGUUUCUGAAdTsdT	-226.3
	AD-35780.1	AAGAuACCCGUCUUUCGGCdTsdT	5.6
	AD-35786.1	UUGAAGAuACCCGUCUUUCdTsdT	42.3
	AD-35792.1	AUUGAAGAuACCCGUCUUUdTsdT	-15.4
	AD-35798.1	AAUUGAAGAuACCCGUCUUdTsdT	6.3
	AD-35810.1	UUCUCuAGAAAGGCGGGAAdTsdT	-761.2
	AD-35816.1	AAUUCUCuAGAAAGGCGGGdTsdT	52.5
	AD-35822.1	AAAUUCUCuAGAAAGGCGGdTsdT	66.4
	AD-35781.1	uAcAAAUUCUCuAGAAAGGdTsdT	36.0
	AD-35787.1	AUGGUGAGuAcAAAUUCUCdTsdT	43.9
	AD-35793.1	uAUGGUGAGuAcAAAUUCUdTsdT	-15.4
	AD-35799.1	UUCcAAGAUGGCUGCGAGUdTsdT	13.2
	AD-35805.1	AUUCcAAGAUGGCUGCGAGdTsdT	15.0
	AD-35811.1	AAcAUUCcAAGAUGGCUGCdTsdT	-16.2
	AD-35817.1	uAAcAUUCcAAGAUGGCUGdTsdT	12.0
	AD-35823.1	UuAAcAUUCcAAGAUGGCUdTsdT	-135.6
	AD-35782.1	uAUUGGAAAGGuAGGGGuAdTsdT	30.4
	AD-35788.1	AuACGGuAGuAUUGGAAAGdTsdT	41.9
	AD-35794.1	AAuACGGuAGuAUUGGAAAdTsdT	19.5
	AD-35800.1	uAAuACGGuAGuAUUGGAAdTsdT	22.8
	AD-35806.1	UUCuAAuACGGuAGuAUUGdTsdT	-9.9
	AD-35812.1	UUUCuAAuACGGuAGuAUUdTsdT	49.4
	AD-35818.1	uAGCCUUUCuAAuACGGuAdTsdT	29.5
	AD-35824.1	UuAGCCUUUCuAAuACGGUdTsdT	38.2
	AD-35783.1	UUuAGCCUUUCuAAuACGGdTsdT	52.8
	AD-35789.1	AUUuAGCCUUUCuAAuACGdTsdT	63.7
	AD-35795.1	uAUUuAGCCUUUCuAAuACdTsdT	30.4
	AD-35801.1	UUcAuAUUuAGCCUUUCuAdTsdT	52.8
	AD-35807.1	AUUcAuAUUuAGCCUUUCUdTsdT	-35.3
	AD-35813.1	AAUUcAuAUUuAGCCUUUCdTsdT	30.9
	AD-35819.1	UUGAUuAGAGGUGAUGGGGdTsdT	-17.0
	AD-35825.1	UUUGAUuAGAGGUGAUGGGdTsdT	54.7
	AD-35784.1	AAcACCUCUUUGAUuAGAGdTsdT	40.3
	AD-35790.1	AAcAGUGGACCcAAcACCUdTsdT	20.7
	AD-35796.1	AAcAAcAGUGGACCcAAcAdTsdT	27.5
	AD-35802.1	uAAcAAcAGUGGACCcAACdTsdT	6.3
	AD-35808.1	AuAAcAAcAGUGGACCcAAdTsdT	-71.3
	AD-35814.1	AAGAuAAcAAcAGUGGACCdTsdT	9.5
	AD-35820.1	AUCcAAGAuAAcAAcAGUGdTsdT	24.9
	AD-35826.1	UUGGcAUCcAAGAuAAcAAdTsdT	7.6
	AD-35833.1	AAAGUUGGcAUCcAAGAuAdTsdT	46.2
	AD-35839.1	uAcAAAGUUGGcAUCcAAGdTsdT	37.3
	AD-35845.1	UuAcAAAGUUGGcAUCcAAdTsdT	-31.6

Table 50. siRNAs against PCV1 Cap screened at 10 nM overnight in PCV1 infected PK15 cells			
	AD-35851.1	uAGGGGUcAuAGGcAAGUdTsdT	-29.8
	AD-35857.1	uAuAGGGGUcAuAGGcAAdTsdT	10.1
	AD-35863.1	AuAuAGGGGUcAuAGGcAdTsdT	35.5
	AD-35869.1	AAuAuAGGGGUcAuAGGCdTsdT	54.1
	AD-35828.1	uAAuAuAGGGGUcAuAGGCdTsdT	36.0
	AD-35834.1	UuAAuAuAGGGGUcAuAGGdTsdT	28.0
	AD-35840.1	AAAGGGCUGCCUuAUGGUGdTsdT	28.0
	AD-35846.1	uAAAGGGCUGCCUuAUGGUdTsdT	-20.3
	AD-35852.1	uAGGuAAAGGGCUGCCUuAdTsdT	-21.1
	AD-35858.1	uACCUGGAGUGGuAGGuAAdTsdT	-28.0
	AD-35864.1	AAGuACCUGGAGUGGuAGGdTsdT	0.3
	AD-35870.1	UUGGUCcAGCUcAGGUUUGdTsdT	30.0
	AD-35829.1	UUUGGUCcAGCUcAGGUUUdTsdT	49.8
	AD-35835.1	UUGUUUGGUCcAGCUcAGGdTsdT	-4.7
	AD-35841.1	AUGGAGCcAcAGCUGGUUUdTsdT	33.7
	AD-35847.1	AAAUGGAGCcAcAGCUGGUdTsdT	-456.5
	AD-35853.1	uAAAUGGAGCcAcAGCUGGdTsdT	15.5
	AD-35859.1	UuAAAUGGAGCcAcAGCUGdTsdT	-18.6
	AD-35865.1	UUuAAAUGGAGCcAcAGCUdTsdT	62.7
	AD-35871.1	AUUuAAAUGGAGCcAcAGCdTsdT	41.9
	AD-35830.1	uAUUuAAAUGGAGCcAcAGdTsdT	56.3
	AD-35836.1	UUGGUGUGGGuAUUuAAAUdTsdT	29.0
	AD-35842.1	AUUGGUGUGGGuAUUuAAAdTsdT	36.4
	AD-35848.1	UUUUGGAGCGcAuAGCCGAdTsdT	-41.1
	AD-35854.1	AUUUUGGAGCGcAuAGCCGdTsdT	-6.2
	AD-35860.1	UUGGGCUGUGGCUGcAUUUdTsdT	-6.2
	AD-35866.1	UUUGGGCUGUGGCUGcAUUdTsdT	29.0
	AD-35872.1	AAUUUUGGGCUGUGGCUGCdTsdT	41.5
	AD-35831.1	uAAUUUUGGGCUGUGGCUGdTsdT	44.3
	AD-35837.1	AuAAUUUUGGGCUGUGGCUdTsdT	23.9
	AD-35843.1	uACcAcAuAAUUUUGGGCUdTsdT	37.3
	AD-35849.1	UuACcAcAuAAUUUUGGGCdTsdT	12.6
	AD-35855.1	uAGUcAGCCUuACcAcAuAdTsdT	21.2
	AD-35861.1	AuAGUcAGCCUuACcAcAUdTsdT	-23.7
	AD-35867.1	AAuAGUcAGCCUuACcAcAdTsdT	50.5
	AD-35873.1	AAAuAGUcAGCCUuACcACdTsdT	34.2
	AD-35832.1	uAAAuAGUcAGCCUuACcAdTsdT	78.3
	AD-35838.1	uAcAuAAAuAGUcAGCCUUdTsdT	41.6
	AD-35844.1	UUGuAcAuAAAuAGUcAGCdTsdT	26.8
	AD-35850.1	AUUGuAcAuAAAuAGUcAGdTsdT	66.3
	AD-35856.1	UUCUCUGAAUUGuAcAuAAdTsdT	80.4
	AD-35862.1	AUUCUCUGAAUUGuAcAuAdTsdT	43.0
	AD-35868.1	AAAUUCUCUGAAUUGuAcAdTsdT	75.6
	AD-35874.1	uAAAUUCUCUGAAUUGuACdTsdT	67.5

Example 8. Transiently transfected siRNAs in DG44 suspension cultures show significant and long term interference for up to 18 days at concentrations as low as 0.1 nM.

[00632] *RNA interference of suspension cultures grown at different temperatures*: GFP expressing CHO DG44 cells that are stably transfected with a CMV-GFP construct (Stratagene, Santa Clara, CA) were seeded at day 0 in wells of 96 well microtiter plates (at 2 x 10^4 cells per well for 37°C cells, and 10^5 cells per well for 28°C cells), and were transiently transfected with siRNAs against GFP at 0.1, 1, and 10 nM (formulated with LIPOFECTAMINE® RNAiMax reagent), also at day 0. GFP expression was measured fluorometrically; inhibition of expression (expressed as % of expression compared to RNAiMax only controls at the respective temperatures and times). Inhibition of expression was monitored for up to 18 days after the initial siRNA transfection.

[00633] Control *experiments*: Expression of GFP in the CHO DG44 cells that were either untreated or RNAiMax only treated were monitored over time. The results are shown if Figure 20 (untreated) and Figure 21 (treated with lipid (RnaiMax only, no siRNAs). GFP is expressed over the course of the entire time period; however, expression of GFP in the 28°C cells eventually became much higher, indicating continued protein expression, even in the absence of cell division (Figs. 20 and 21).

[00634] The lipid treated controls (Fig. 20) were used as controls for measuring efficacy of RNA interference. The graphs in Figure 22A-22C show significant inhibition of expression of GFP at siRNA concentrations as low as 0.1 nM (Fig. 22A). Furthermore, inhibition of expression was maintained as long as the measurements were taken (i.e., in some cases, up to 18 days after initial expression)

Example 13: Scalable siRNA uptake protocol for CHO cells grown in a 40 L Bioreactor

[00635] As known to those of skill in the art liposome mediated delivery of siRNA using lipid polynucleotide carriers is commonly used in research applications, however, as described in PCT publication WO 2009/012173 (filed July 11, 2008), the use of lipid polynucleotide carriers, e.g., common liposome transfection reagents, has been found to be detrimental when used in bioprocessing of protein. Polynucleotide carriers have been reported to be deleterious to the growth of host cells at the concentrations typically used presumably due to toxicity such that they impair the ability of host cells to produce the desired biological material on an industrial level. In addition polynucleotide carriers have been observed to cause adverse and unwanted changes in the phenotype of host cells, e.g., CHO cells, compromising the ability of the host cells to produce the biological product of interest. Accordingly, the artisan would expect that

the use of such polynucleotide carriers would hinder a cells ability to produce a desired protein. Surprisingly, we have found, as described throughout herein, that RNA effector molecules (e.g., targeting BAX, BAC and/or LDH) can be delivered transiently to host cells in culture by using polynucleotide carriers (e.g., liposome mediated delivery) during the bioprocessing procedure in large scale cultures (e.g., 1 L and, e.g., 40 L) without detrimental effects on the cells under conditions tested on the cells, e.g., cell viability and density is maintained. Thus, large scale production of biological products can be done on an industrial scale using lipid reagents to facilitate RNA effector uptake in cells when they are in culture (e.g., suspension culture), for example, to result in effective transient modulation of gene expression that improve production of biological products (e.g.,polypeptides).

[00636] Furthermore, we have studied various lipid compositions to identify efficient uptake enhancing reagents that promote efficient siRNA uptake into production cell lines with minimal impact on cell growth and viability. We had earlier demonstrated greater than 90% reduction in LDH activity (using siRNA directed against LDH) in 96-well plate cultures while screening a panel of quaternary cationic lipid formulations (data not shown). In this example, we show that siRNA formulated with P8 as an uptake inducer (*see, e.g.*, Table 19) is better tolerated than commercial RNAiMax with respect to the respective formulations effect on cell density and cell viability in 50 ml cultures. We scaled up our cultures to a large scale bioreactor and found that using P8 formulated siRNA directed against LDH achieved 80%-90% reduction in LDH activity for 6 days with a single 1 nM dose. We then scaled up our cultures to 3 L and 40 L. We found that formulation P8 promoted efficient uptake of an siRNA directed against lactate dehydrogenase (LDH-A) and resulted in >90% of LDH reduction of LDH activity in CHO cells grown in either a 3 L or 40 L bioreactor. Surprisingly, in scale-up experiments comparing 3 L to 40 L cultures, there is perfect linearity of silencing efficiency. The results are shown herein.

Materials/Methods

[00637] *Formulation of transfection reagents*: Cationic lipid and colipids (e.g.,cholesterol and DOPE) in chloroform were dried by a N_2 stream followed by vacuum-desiccation to remove residual organic solvent. The dried lipid film was hydrated using 10mM HEPES buffer, pH 7.4 at 37°C. The formed liposomes were extruded to yield an average particle size of ~200 nm.

[00638] *Testing of transfection reagents on plated GFP-CHO cells*: Nine different proprietary transfection formulations (*see e.g.*, Table 19) and Lipofectamine RNAiMax (Invitrogen) were used to deliver 1 nM of a potent siRNA against GFP to a GFP-CHO cell line. RNAiMax was tested at 0.4 μL/mL and the nine formulations were used at 0.5, 1, and 2.5 μg/mL. Mixtures of transfection reagents and siRNA were made in black optical bottom 96 well

plates and then cells were added. After 2 days, the relative GFP intensities were measured using a fluorescent plate reader.

[00639] *Testing of transfection reagents on suspended DG44 CHO cells*: The three most active transfection agents (K8, L8 and P8) from the GFP-CHO testing were used to transfect suspended CHO cells. Aliquots of 5 μL of 10 μM LDH-A siRNA were added to a tube and 500 μL CD DG44 media added to it. Transfection reagent was added to the mixture, the tube mixed by pipette aspiration and incubated at room temperature for 15 min. Then the mixture was added to 49.5 mL of media containing 200,000 cells/mL. The flask was incubated and shaken at 120 rpm for several days. LDH activity was measured by VetTest 8008 slide analyzer.

[00640] *40L transfection*: DG44 cells were grown in Invitrogen CD DG44 media. To seed the 40 L bioreactor, cells were taken from four 1 L disposable bioreactors. The starting cell density in the 40 L of culture was 120,000 cells/mL. The bioreactor was allowed to equilibrate with the cells added for 1 hr prior to transfection. For transfection, 400 μL of LDH-A siRNA (pair of SEQ ID NO:3152560 and NO:3152561) (100 uM stock solution) was added to 400 mL of media and mixed. Then 32 mL of 1 mg/mL P8 reagent was added and again mixed. This was allowed to incubate for 15 min at room temperature and then added to the 40 L bioreactor. Cell density and viability were measured using a Vi-Cell cell counter, and to determine the efficiency of transfection, LDH activity was measured using a VetTest 8008 slide analyzer.

Results and Discussion

[00641] *Evaluation of nine cationic lipid formulations for uptake efficiency in CHO cells in shake flasks*: To gauge the effectiveness of the lipid formulations, they were used with a potent GFP siRNA in GFP-CHO cells. Compared with an effective concentration of LIPOFECTAMINE® RNAiMAx reagent, three compounds were active (Fig. 23). These formulations were designated K8, L8, and P8. No obvious cytotoxicity was observed at the concentrations tested of any formulation.

[00642] Because K8 was the most active formulation in the GFP-CHO cells, it was tested using DG44 CHO cells in 50 mL of culture in a 250 mL shake flask and a potent LDH siRNA. A range of K8 concentrations was tested along with an effective concentration of LIPOFECTAMINE® RNAiMAx transfection reagent. After 3 days, LDH activity was lower in cultures where K8 was used (Fig. 24). There was also a higher cell density in flasks that had 0.6 μg/mL or 1.2 μg/mL of K8 compared to RNAiMAx reagent. It appears that RNAiMAx reagent inhibited growth of CHO cell in suspension when compared to K8-treated cells. The highest concentrations of K8 reduced the cell density, even though the LDH activity was still reduced.

[00643] Because some transfection reagents didn not seem to have the same activity in shake flasks as in a 96-well plate, the three most active formulations were tested similarly in 50 mL of DG44 culture in 250 mL shake flasks. Surprisingly, formulation P8, which was only marginally active against GFP-CHO cells, performed the best using suspended DG44 cell culture (Fig. 25). After 5 days, 0.8 μg/mL of P8 resulted in the most LDH activity knockdown. Also, it is significant that the cell density in the presence of P8 was greater than or equal to control cells without transfection reagent added. P8 at a final concentration of 0.8 μg/mL has been used numerous times in smaller bioreactors and (data not shown) and was tested in a 40 L system.

[00644] Figure 26 shows cell density (Fig. 26A) or % cell viability (Fig. 26B) over time in suspension CHO cell 50 mL shake flasks using P8 formulation or commercial formulation RNAiMax at the recommended concentration. Lipid formulations were dosed onto cells at day 0. P8 was found to be better tolerated than commercial RNAiMax. Figure 30 is a graph that shows that when using the P8 formulated siRNA directed against Lactate Dehydrogenase (LDH) achieves 80%-90% knockdown of LDH activity for 6 days with a single 1 nM dose in a 1 L bioreactor. Knockdown of LDH activity was found to be durable, with effects lasting over 6 days.

[00645] *Evaluation of cationic lipid formulation P8 for uptake efficiency in a 3L vs 40L bioreactor*: Figure 28 shows the results of a single dose of an 1nM LDH siRNA formulated with P8 lipid on viable cell density and % LDH activity over an elapsed time of 6 days in 3 L and 40 L cultures. Surprisingly, in scale-up experiments comparing 3 L to 40 L cultures, there is perfect linearity of silencing efficiency indicating success at even larger scales. Multiple dose protocols can be used to extend the duration of effect.

[00646] *Evaluation of cationic lipid formulation P8 for uptake efficiency in a 40L bioreactor*: After seeding the 40 L bioreactor, the cells generally grew with a doubling time of approximately 24 hr and the cell viability was over 98% (Figure 26B). The cells reached a peak concentration of 3.1×10^6 cells/mL at day 5 and then began to decline. As expected in this unfed batch culture, by day 6 the cells were in decline.

[00647] The LDH activity of the siRNA treated cells was reduced as the cells were growing following seeding and transfection. The LDH activity was reduced ~80% even as the cells had doubled over 3 times (Figure 30). There was diminished LDH activity through the entire experiment. Based on the significantly diminished LDH activity, the transfection was successful with no detectable toxicity in the CHO cells.

[00648] These experiments show that transfection of cells in culture with siRNAs can work in the large volumes necessary for biological production.

Example 14: Use of RNA effectors to titrate expression of target genes

[00649] Unlike cells with stably transfected shRNA, use of dsRNA molecules allows modulation of expression of practically any target gene within a host cell without the need for cell engineering. In addition, as mentioned previously, cells with constitutively inhibited target genes may not grow well and may display unwanted characteristics (e.g., need for special growth media or other growth conditions, increased rate of mutation, etc). Having the ability to modulate expression of a target gene at the desired point during growth of a cell or production of a biologic is therefore highly desirable.

[00650] Yet another advantage of using RNA effector molecules such as dsRNA agents that do not rely on stable transfection is the potential ability to fine-tune expression of a given target gene. In some cases it may be important to regulate expression of a target gene such that its expression level is only moderately altered (e.g., decreased by ~50% from the untreated state) so as to avoid unwanted phenotypes or to improve the quality of biologic production. As such, we performed experiments to find conditions in which expression of a given target gene could be titrated.

[00651] On day 0, CHO DG-44 cells grown in CD DG44 media (Invitrogen), were transfected with dsRNA targeting the LDHA gene (as described herein; see e.g., Table 62) at 0 nM, 10 pM, 50 pM, 100 pM, 500 pM, 1 nM and 5 nM (final concentrations in 25mL of culture), in a formulation containing the Lipid P, in formulation 8 (i.e., formulation "P8"; see Table 19) in a 500 µL volume. The dsRNA duplex used has an apparent EC_{50} of ~ 50pM under similar conditions. After transfection, cells were added to a flask containing 24.5 mL of media (at a cell density of 200,000 cells/mL) and grown at 37°C. After 3 days, LDH activity was measured and normalized to cell density.

The LDH activity is shown in Table 62 below:

Table 62. LDH activity

Flask	dsRNA concentration	Via density	LDH activity	(LDH activity /106 cells)* mL dil.	% knockdown
1	0 LDH siRNA	2.11	1489	776.3	
2	10 pM LDH siRNA	2.08	1248	660.0	15.0
3	50 pM LDH siRNA	2.08	754	398.8	48.6
4	100 pM LDH siRNA	2.22	560	277.5	64.3
5	500 pM LDH	2.22	335	166.0	78.6

	siRNA				
6	1 nM LDH siRNA	2.16	335	170.6	78.0
7	5 nM LDH siRNA	2.21	363	180.7	76.7

[00652] The results show that LDH activity can be modulated to a range between 15% to greater than 75% inhibition by titrating the concentration of dsRNA. Therefore, use of RNA effector molecules such as the dsRNAs shown herein can be used to achieve a desired expression level of the target gene. In addition, based on earlier experiments (not shown), cells treated at concentrations in which partial inhibition is achieved (for example, at 10-100 pM) are expected to recover from RNA interference more rapidly than those treated at higher concentrations. As such, where it is desirable to have cells recover from inhibition of a target gene faster (i.e., inhibition of gene expression will persist for a shorter period of time), then one can provide a lower concentration of RNA effector molecule (e.g., 3X of the apparent EC_{50} or less, for example 2X the apparent EC50, 1X the apparent EC50, etc).

[00653] The following tables exemplify target genes and siRNA sequences useful with the methods and compositions described herein.

Table 51. Target genes

Target	Description	siRNA SEQ ID NOs
15-lipoxygenase-1	arachidonate lipoxygenase 3	2480018-2480362
Ago2	eukaryotic translation initiation factor 2C, 2	255154-255411
Ago3	eukaryotic translation initiation factor 2C, 3	3103755-3103973
Ago4	eukaryotic translation initiation factor 2C, 4	1326374-1326705
APAFI	apoptotic peptidase activating factor 1	2262408-2262743
ApoE	apolipoprotein E	3172384-3172483
asparagine deamidase	N-terminal Asn amidase	1999410-1999756
glutamine deamidase	WDYHV motif containing 1; aka Protein NH2-terminal glutamine deamidase	2478078-2478376
ATF4	activating transcription factor 4	1552067-1552460
ATF6	activating transcription factor 6	570138-570498
ATF6β	activating transcription factor 6 beta	471680-472070
B4GalT1	UDP-Gal:βGlcNAc β1,4-galactosyltransferase, polypeptide 1	2528454-2528763 additional galactosyl & galactosaminyltransferases elsewhere herein
BAD	BCL2-associated agonist of cell death	3049436-3049721
BAG-1	BCL2-associated athanogene	1683576-1683895
Bcl-w	BCL2-like 2	477629-477999
Bcl-xL	BCL2-like 1	728838-729216
Bid	BH3 interacting domain death agonist	2582517-2582823
Bik	BCL2-interacting killer (apoptosis-inducing)	2899985-2900289

Table 51. Target genes		
BIM/BimL	BCL2-like 11 (apoptosis facilitator)	1960442-1960764
BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	1740754-1741152
calnexin	calnexin	61559-61785
calreticulin	Calreticulin	895691-896051
CASP2	Caspase 2	2718675-2719039
CASP3	Caspase 3	1924836-1925195
CASP6	Caspase 6	2408466-2408843
CASP7	Caspase 7	2301618-2301960
CASP8	Caspase 8	2995593-2995870
CASP9	Caspase 9	1412589-1412860
CCNA2/ Cyclin A2	cyclin A2	1151948-1152332
CCNB1/Cyclin B1	Cyclin B1	1298863-1299236
CCNB2/ Cyclin B2	Cyclin B2	1489394-1489722
CCND1/ Cyclin D1	cyclin D1	139242-139629
CCND2/ Cyclin D2	cyclin D2	960077-960401
CCND3/ Cyclin D3	Cyclin D3	1040554-1040910
CCNE1/ Cyclin E1	cyclin E1	1980613-1981009
CCNE2/ Cyclin E2	Cyclin E2	2904183-2904530
CDK2	cyclin-dependent kinase 2	1193336-1193684
CDK4	cyclin-dependent kinase 4	1609522-1609852
Cmas	cytidine monophosphate N-acetylneuraminic acid synthetase	1633101-1633406
Cofilin (CFL1)	Cofilin	1914036-1914356
cytochrome P4502E1		
cytoplasmic actin capping protein (CapZ)	capping protein (actin filament) muscle Z-line, α 1	235917-236159
dihydrofolate reductase		1739672-1740059
Eri1	exoribonuclease 1	3244117-3244216
Ezrin (VIL2)	Ezrin	339220-339540
fucosyltransferase/ FUT8	Fucosyltransferases	FUT8 dsRNA: 209841-210227 additional seqs elsewhere herein
GLUT1	solute carrier family 2 (facilitated glucose transporter), member 1	438155-438490 additional seqs elsewhere herein
glutaminase		105170-105438
GMDS	GDP mannose dehydratase	1688202-1688519
Gne	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase; UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase	2073971-2074368
GRP94	heat shock protein 90kDa β(Grp94), member 1	180574-180954
HR	Hairless	1110794-1111079
Hsp40	DnaJ (Hsp40) homolog	dsRNA sequences targeting Hsp40 elsewhere herein
interferon receptor	IFNAR1	2436536-2436863
IRE1	endoplasmic reticulum (ER) to nucleus signaling 1	3179284-3179383

Table 51. Target genes		
Laminin A		α5: 48814-49139 α2: 2954307-2954650 α3: 3160721-3160820
lysosomal V-type ATPase		For sequences of the various subunits please see table below
Mcl-1	myeloid cell leukemia sequence 1 (BCL2-related)	312684-312913
N-acetylgalactosaminyt rans-ferase T-4		2876241-2876595, see also, e.g., Table 6
NAD(p)H oxidase		See table elsewhere herein for cytochrome reductases
NADH cytochrome b5 reductase		
NADPH cytochrome c2 reductase		
NAPH cytochrome c reductase		
B4GalT6.	and UDP-Gal:βGlcNAc β1,4-galactosyltransferase, polypeptide 6	3154201-3154224 (sense) and 3154225-3154248
P10	S100 calcium binding protein A10 (calpactin)	3013998-3014274
p115	USO1 vesicle docking protein homolog (yeast)	89340-89737
P14ARF/ p16INK4a	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	2B: 2895015-2895359 2C: 1969649-1970047 2D: 1990790-1991181
P21	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	2659502-2659871
P27	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	3199397-3199496
p53	tumor protein p53; transformation related protein p53	1649857-1650157
P57	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	1A: 2659502-2659871 1B: 2731076-2731440
peptidyl prolyl isomerase	peptidylprolyl isomerase	1074139-1074475, 1085316-1085607, 1127061-1127426, 1649170-1649515, 1780604-1780923, 2197146-2197532, 2253978-2254373, 2261765-2262058, 2275330-2275633,

Table 51. Target genes

		2579547-2579908, 2857424-2857802, 3136158-3136181, 3262205-3262304
PERK	eukaryotic translation initiation factor 2-α kinase 3	1396283-1396617; Kinase 4: 582987-583297; Kinase 1: 1037660-1038052
peroxidase		siRNAs targeting Glutathione peroxidases include: 2439217-2439612 2560559-2560895 2703865-2704225 3151589-3151685 See table below for enzymes possessing peroxidase activity
phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN)	phosphatase and tensin homolog	69091-69404
protein disulfide isomerase		These siRNAs target genes that have protein disulfide isomerase activity: 72748-72996 335875-336225 488676-489039 774355-774677 898511-898822 966735-967056
protein O-fucosyltransferase	protein O-fucosyltransferase 1	2321807-2322122
PUMA	BCL2 binding component 3	1712045-1712425
SLC35A1	solute carrier family 35 (CMP-sialic acid transporter), member 1	3154345-3154368; 1367952-1368265
ST3 β-galactoside α-2,3-sialyltransferase 1		681105-681454
ST3 β-galactoside α-2,3-sialyltransferase 2.		1435989-1436317
ST3 β-galactoside α-2,3-sialyltransferase 3		1131123-1131445
ST3 β-galactoside α-2,3-sialyltransferase 4		707535-707870
ST3 β-galactoside α-2,3-sialyltransferase 5		1155324-1155711

Table 51. Target genes		
ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6		1391079-1391449
TSTA3	tissue specific transplantation antigen P35B	1839578-1839937
xanthine oxidase (XO)	Aka xanthine dehydrogenase	374846-375216
xylose transferase	Xylosyltansferase II	1554774-1555054
α galactosidase		1600968-1601288
β-galactosidase		690601-690989

Table 52: GLUTs (glucose transporters)

SEQ ID NO:	Description	siRNA SEQ ID NOs:
1375	solute carrier family 2 (facilitated glucose transporter), member 1	438155-438490
6869	solute carrier family 2, (facilitated glucose transporter), member 8	2325698-2325997
7909	solute carrier family 2 (facilitated glucose transporter), member 13	2669929-2670303

Table 53: Fucosyltransferases

SEQ ID NO:	consL	Description	siRNA SEQ ID NOs:
676	2680	fucosyltransferase 8	209841-210227
2783	1861	protein O-fucosyltransferase 2	916726-917035
6857	913	protein O-fucosyltransferase 1	2321807-2322122
8126	593	fucosyltransferase 11	2740650-2740952

Table 54. DnaJ (Hsp40) homologs				
SEQ ID NO:	consL	Description	Avg Coverage	siRNA SEQ ID NOs:
1932	2102	Subfamily A, member 1	18.764	628385-628725
893	2541	Subfamily A, member 2	15.853	276519-276904
1925	2104	Subfamily A, member 3	15.15	625909-626254
3157871	528	Subfamily A, member 4	0.656	3215391-3215490
2076	2052	Subfamily B, member 1	9.75	677203-677558
5350	1247	Subfamily B, member 11	17.061	1784585-1784897
5347	1248	Subfamily B, member 12	3.209	1783440-1783810
9545	230	Subfamily B, member 13	0.22	3133435-3133598
3157418	441	Subfamily B, member 14	0.238	3228617-3228716
4158	1511	Subfamily B, member 2	5.045	1381610-1381931
3158137	878	Subfamily B, member 3	1.052	3283549 - 3283648
5405	1236	Subfamily B, member 4	1.568	1804161-1804465
8128	593	Subfamily B, member 5	0.47	2741242-2741540
2619	1902	Subfamily B, member 6	14.116	860762-861101
5149	1289	Subfamily B, member 9	0.929	1715305-1715623
4159	1510	Subfamily C, member 1	3.933	1381932-1382211
546	2787	Subfamily C, member 10	22.023	171304-171555

Table 54. DnaJ (Hsp40) homologs				
SEQ ID NO:	consL	Description	Avg Coverage	siRNA SEQ ID NOs:
1143	2405	Subfamily C, member 11	15.429	360296-360688
3157835	1640	Subfamily C, member 13	0.983	3240717-3240816
412	2946	Subfamily C, member 14	7.271	133746-134002
9442	267	Subfamily C, member 15	0.656	3117145-3117332
1960	2089	Subfamily C, member 16	1.225	637892-638209
6631	962	Subfamily C, member 17	1.346	2243108-2243387
7277	817	Subfamily C, member 18	0.36	2460206-2460591
9036	381	Subfamily C, member 19	1.461	3027351-3027657
2513	1930	Subfamily C, member 2	34.4	825067-825402
2721	1878	Subfamily C, member 21	8.299	895321-895690
5660	1176	Subfamily C, member 22	4.382	1893667-1894030
8661	464	Subfamily C, member 24	2.068	2917681-2918006
6150	1068	Subfamily C, member 25	0.929	2072060-2072449
8171	583	Subfamily C, member 27	0.773	2754733-2755101
3157934	1241	Subfamily C, member 28	2.604	3271096 - 3271195
1054	2449	Subfamily C, member 3	10.89	330430-330812
6648	959	Subfamily C, member 30	1.456	2249119-2249439
7348	800	Subfamily C, member 4	4.236	2483678-2484063
2403	1958	Subfamily C, member 5	5.417	787385-787676
9017	388	Subfamily C, member 6	0.078	3022706-3022949
3188	1749	Subfamily C, member 7	20.562	1055444-1055806
5052	1312	Subfamily C, member 8	41.714	1682260-1682641
7247	827	Subfamily C, member 9	4.989	2450765-2451126

Table 55. Heat Shock proteins				
SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
444	2900	heat shock protein 4	15.95	142820-143094
476	2865	heat shock 105kDa/110kDa protein 1	19.863	151195-151420
485	2858	AHA1, activator of heat shock protein ATPase homolog 2 (yeast)	16.103	153831-154084
579	2758	heat shock protein 90, beta (Grp94), member 1	606.207	180574-180954
594	2744	heat shock protein 90, alpha (cytosolic), class A member 1	93.844	184698-184927
827	2572	heat shock protein 9	28.56	255926-256325
941	2519	heat shock protein 5	729.81	292590-292837
977	2496	heat shock protein 90 alpha (cytosolic), class B member 1	609.471	304274-304591
1543	2232	heat shock protein 1 (chaperonin)	134.366	494743-495086
2029	2068	heat shock protein 8	891.015	660889-661277
2272	1990	heat shock factor 2	2.598	743398-743788

2756	1869	heat shock factor 1	25.227	907582-907889
2974	1807	heat shock protein 2	5.538	982428-982785
3063	1776	heat shock protein 8	38.69	1012333-1012621
3765	1608	heat shock protein 14	20.386	1250279-1250587
4038	1541	heat shock protein 70 family, member 13	2.835	1341514-1341853
4337	1473	HSPA (heat shock 70kDa) binding protein, cytoplasmic cochaperone 1	11.687	1441933-1442264
5002	1323	AHA1, activator of heat shock protein ATPase homolog 1 (yeast)	93.621	1665415-1665746
5756	1155	heat shock factor binding protein 1	28.266	1928608-1928970
7697	715	heat shock protein, α-crystallin-related, B6	1.268	2598077-2598438
8336	539	heat shock protein 1	3.124	2809108-2809434
8405	517	heat shock protein 1 (chaperonin 10)	4.477	2833031-2833420
9679	173	heat shock protein 1B	0.091	3147029-3147080

Table 56. Lysosomal V-type ATPase subunits

SEQ ID NO:	Description	siRNA SEQ ID NOs:
198	ATPase, H+ transporting, lysosomal V1 subunit A	71796-72111
1027	ATPase, H+ transporting, lysosomal V1 subunit B2	321673-321927
1796	ATPase, H+ transporting, lysosomal accessory protein 2	582376-582610
2296	ATPase, H+ transporting, lysosomal V0 subunit A1	751583-751949
2532	ATPase, H+ transporting, lysosomal accessory protein 1	831609-831895
2762	ATPase, H+ transporting, lysosomal V1 subunit H	909697-910010
3329	T-cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 protein A3	1103103-1103418
4324	ATPase, H+ transporting, lysosomal V0 subunit D1	1437602-1437944
4347	ATPase, H+ transporting, lysosomal V0 subunit A2	1445278-1445615
5454	ATPase, H+ transporting, lysosomal V1 subunit E1	1821367-1821755
5620	ATPase, H+ transporting, lysosomal V1 subunit D	1879531-1879860
5788	ATPase, H+ transporting, lysosomal V0 subunit C	1940302-1940675
5816	ATPase, H+ transporting, lysosomal V1 subunit C1	1950210-1950528
6117	ATPase, H+ transporting, lysosomal V1 subunit G1	2059770-2060150
6486	ATPase, H+ transporting, lysosomal V0 subunit B	2192145-2192538
7910	ATPase, H+ transporting, lysosomal V0 subunit E2	2670304-2670626
7976	ATPase, H+ transporting, lysosomal V1 subunit F	2692263-2692620
7987	ATPase, H+ transporting, lysosomal V0 subunit E	2695797-2696168
8582	ATPase, H+ transporting, lysosomal V1 subunit G2	2890746-2891087
3157707	ATPase, H+ transporting, lysosomal V0 subunit D2	3281849-3281948

Table 57. Peroxidase

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
442	2901	heterogeneous nuclear ribonucleoprotein L-like	11.591	142215-142508
1706	2173	catalase	18.084	551058-551444
3107	1768	prostaglandin-endoperoxide synthase 2	0.699	1027449-1027832

Table 57. Peroxidase				
6122	1074	peroxiredoxin 3	15.819	2061664-2062027
6608	967	peroxiredoxin 4	81.791	2235293-2235671
6741	937	peroxiredoxin 6	9.666	2281128-2281515
6816	921	peroxidasin homolog (*Drosophila*)	0.334	2307638-2308007
7213	835	glutathione peroxidase 1	10.976	2439217-2439612
7386	792	peroxiredoxin 1	1.522	2496217-2496481
7582	743	glutathione peroxidase 4	73.452	2560559-2560895
7749	702	peroxiredoxin 2	15.903	2616024-2616366
8011	630	glutathione peroxidase 8 (putative)	15.42	2703865-2704225
8179	582	peroxiredoxin 5	3.766	2757414-2757689
8565	482	glutathione S-transferase kappa 1	1.46	2885542-2885890
8687	461	iodotyrosine deiodinase	0.299	2926039-2926366
9756	131	glutathione peroxidase 3	0.087	3151589-3151685

Table 58. Protein Disulfide Isomerase Activity				
SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
201	3342	thioredoxin-related transmembrane protein 3	6.308	72748-72996
1071	2440	prolyl 4-hydroxylase, beta polypeptide	262.952	335875-336225
1525	2239	protein disulfide isomerase associated 4	31.944	488676-489039
2364	1967	protein disulfide isomerase associated 3	173.819	774355-774677
2730	1874	protein disulfide isomerase associated 6	699.725	898511-898822
2929	1822	protein disulfide isomerase associated 5	42.884	966735-967056

Table 59. Signal Recognition Particle				
SEQ ID NO:	cons L	Description	Avg Cov	siRNA SEQ ID NOs:
348	3031	signal recognition particle 72	13.053	115319-115586
498	2844	signal recognition particle receptor (docking protein)	23.636	157648-157932
1200	2382	signal recognition particle 68	40.31	379331-379670
1535	2235	signal recognition particle 54a	4.713	492211-492502
2108	2042	signal recognition particle 54b	7.508	687895-687922
3277	1725	signal recognition particle 54C	3.004	1085608-1085800
6222	1053	signal recognition particle 9	6.194	2097989-2098388
6901	903	signal recognition particle receptor, B subunit	8.479	2335474-2335804
7846	677	signal recognition particle 14	2.01	2648705-2649066
9140	355	signal recognition particle 19	0.4	3053860-3054133
8427	513	retinitis pigmentosa 9 (human)	0.65	2840748-2841112

Table 60. Example kinase targets		
SEQ ID NO:	Description	siRNA SEQ ID NOs:
2	TAO kinase 1	10148-10532
16	homeodomain interacting protein kinase 1	14439-14801
26	dual-specificity tyrosine-(Y)-phosphorylation	17461-17750

Table 60. Example kinase targets		
	regulated kinase 1a	
67	casein kinase 2, alpha 1 polypeptide	30901-31248
74	mitogen-activated protein kinase kinase kinase kinase 4	33333-33668
80	Rho-associated coiled-coil containing protein kinase 2	35242-35563
92	calcium/calmodulin-dependent serine protein kinase (MAGUK family)	39068-39431
105	cDNA sequence BC033915	43314-43658
131	mitogen-activated protein kinase 9	51635-51907
135	Braf transforming gene	52754-53026
153	serine/arginine-rich protein specific kinase 2	57998-58262
160	ribosomal protein S6 kinase, polypeptide 1	60208-60510
199	protein kinase C, alpha	72112-72439
211	AP2 associated kinase 1	75589-75893
215	AXL receptor tyrosine kinase	76768-77080
249	discoidin domain receptor family, member 2	86688-86974
272	Rho-associated coiled-coil containing protein kinase 1	94052-94292
301	MAP/microtubule affinity-regulating kinase 1	102310-102609
345	glycogen synthase kinase 3 beta	114424-114743
349	adrenergic receptor kinase, beta 1	115587-115982
378	tousled-like kinase 1	124295-124551
416	PCTAIRE-motif protein kinase 1	134792-135023
420	MAP/microtubule affinity-regulating kinase 2	135926-136274
432	cyclin D1	139242-139629
434	mitogen-activated protein kinase kinase kinase 7	139905-140195
448	casein kinase 1, delta	144005-144272
454	PFTAIRE protein kinase 1	145534-145792
455	PRP4 pre-mRNA processing factor 4 homolog B (yeast)	145793-146023
459	serine/threonine kinase 39, STE20/SPS1 homolog (yeast)	146854-147131
490	Fyn proto-oncogene	155354-155611
510	calcium/calmodulin-dependent protein kinase II γ	161048-161267
543	Janus kinase 2	170408-170768
559	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	174646-174897
600	casein kinase 1, gamma 1	186716-187114
634	leucine-rich repeat kinase 1	197327-197719
644	mitogen-activated protein kinase 6	200294-200550
662	calcium/calmodulin-dependent protein kinase II, δ	205498-205717
681	MAP/microtubule affinity-regulating kinase 3	211317-211594
689	budding uninhibited by benzimidazoles 1 homolog (*S. cerevisiae*)	213750-213996
725	LIM motif-containing protein kinase 2	224252-224614
729	homeodomain interacting protein kinase 3	225660-225908
730	microtubule associated serine/threonine kinase 2	225909-226275
732	transforming growth factor, beta receptor I	226652-227037
829	protein kinase, cAMP dependent, catalytic, beta	256726-256960
836	mitogen-activated protein kinase kinase kinase 12	258825-259201
864	intestinal cell kinase	267348-267605

Table 60. Example kinase targets		
870	mitogen-activated protein kinase kinase kinase 3	269115-269501
871	nemo like kinase	269502-269739
873	cyclin G associated kinase	270072-270372
878	mitogen-activated protein kinase 3	271504-271774
907	G protein-coupled receptor kinase 6	281096-281476
929	Rous sarcoma oncogene	288625-288989
969	thymoma viral proto-oncogene 2	301570-301889
1006	large tumor suppressor 2	314156-314545
1049	casein kinase 1, gamma 3	328602-328958
1057	serine/threonine kinase 38	331497-331885
1074	MAP kinase-activated protein kinase 2	336742-337085
1082	tousled-like kinase 2 (*Arabidopsis*)	339541-339778
1083	serine/threonine kinase 40	339779-340105
1094	SCY1-like 1 (*S. cerevisiae*)	343589-343905
1098	PCTAIRE-motif protein kinase 2	344918-345284
1105	triple functional domain (PTPRF interacting)	347214-347540
1158	protein kinase N2	365489-365727
1173	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	370312-370704
1188	WEE 1 homolog 1 (*S. pombe*)	375593-375982
1205	mitogen-activated protein kinase-activated protein kinase 3	380855-381192
1223	conserved helix-loop-helix ubiquitous kinase	386803-387186
1230	mitogen-activated protein kinase 8	388975-389185
1245	bone morphogenetic protein receptor, type 1A	393916-394306
1248	tripartite motif-containing 28	394982-395338
1283	serine/arginine-rich protein specific kinase 1	406749-407114
1310	mitogen-activated protein kinase kinase 4	415843-416086
1320	platelet derived growth factor receptor, β polypeptide	419363-419724
1360	receptor-like tyrosine kinase	433042-433431
1440	TANK-binding kinase 1	460287-460685
1452	DNA segment, Chr 8, ERATO Doi 82, expressed	464366-464673
1472	v-raf murine sarcoma 3611 viral oncogene homolog	471108-471446
1496	CDC-like kinase 3	479192-479450
1498	casein kinase 1, epsilon	479802-480166
1507	serine/threonine kinase 24 (STE20 homolog, yeast)	482939-483243
1534	protein kinase D1	491875-492210
1615	interleukin-1 receptor-associated kinase 2	519606-519900
1623	v-raf-leukemia viral oncogene 1	522454-522805
1638	polo-like kinase 2 (Drosophila)	527681-527996
1640	p21 protein (Cdc42/Rac)-activated kinase 2	528351-528713
1688	serine/threonine kinase 16	544970-545325
1696	ribosomal protein S6 kinase polypeptide 1	547863-548141
1700	transforming growth factor, beta receptor II	549106-549395
1719	ataxia telangiectasia and Rad3 related	555695-555945
1791	insulin-like growth factor I receptor	580583-580928
1793	thymoma viral proto-oncogene 1	581286-581643
1798	eukaryotic translation initiation factor 2 α kinase 4	582987-583297
1802	cyclin-dependent kinase 8	584337-584730

Table 60. Example kinase targets		
1821	ribosomal protein S6 kinase, polypeptide 4	590773-591132
1822	polo-like kinase 1 (*Drosophila*)	591133-591528
1838	proviral integration site 3	596508-596892
1839	WNK lysine deficient protein kinase 1	596893-597187
1842	MAP kinase-interacting serine/threonine kinase 2	597880-598207
1849	NIMA-related expressed kinase 6	600327-600624
1853	BMP2 inducible kinase	601662-602044
1873	protein kinase C, delta	608454-608757
1874	NIMA-related expressed kinase 9	608758-609143
1885	interleukin-1 receptor-associated kinase 1	612534-612817
1953	CDC42 binding protein kinase beta	635482-635834
1956	mitogen-activated protein kinase kinase 3	636446-636831
1967	serum/glucocorticoid regulated kinase 1	640401-640729
1982	mitogen-activated protein kinase kinase kinase 4	645415-645811
1985	serine/threonine kinase 4	646540-646922
2022	p21 protein (Cdc42/Rac)-activated kinase 1	658646-658945
2040	STE20-like kinase (yeast)	664580-664973
2058	PX domain containing serine/threonine kinase	670668-671043
2064	TAO kinase 3	672877-673175
2074	SH3-binding kinase 1	676411-676808
2089	nuclear receptor binding protein 1	681455-681766
2094	polo-like kinase 3 (Drosophila)	683175-683550
2096	mitogen-activated protein kinase 14	683848-684174
2157	macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	704139-704461
2224	protein kinase N1	726766-727146
2252	mitogen-activated protein kinase kinase kinase 5	736639-737018
2281	casein kinase 1, alpha 1	746332-746692
2313	testis specific protein kinase 1	757254-757624
2321	U2AF homology motif (UHM) kinase 1	759990-760335
2348	casein kinase 1, gamma 2	769048-769436
2371	activin A receptor, type 1	776681-777035
2391	TYRO3 protein tyrosine kinase 3	783438-783823
2395	platelet derived growth factor receptor, αpolypeptide	784759-785127
2429	SNF related kinase	796332-796725
2433	met proto-oncogene	797652-798038
2434	mitogen-activated protein kinase kinase 1	798039-798333
2450	receptor (TNFRSF)-interacting serine-threonine kinase 1	803414-803712
2453	cell division cycle 2-like 5 (cholinesterase-related cell division controller)	804372-804761
2498	SCY1-like 2 (*S. cerevisiae*)	819902-820288
2500	Eph receptor A2	820644-820974
2530	misshapen-like kinase 1 (zebrafish)	830880-831232
2567	Unc-51 like kinase 1 (*C. elegans*)	843486-843843
2569	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	844194-844512
2605	protein serine kinase H1	856267-856572
2606	NIMA-related expressed kinase 7	856573-856901
2609	Janus kinase 1	857488-857805

Table 60. Example kinase targets		
2615	c-mer proto-oncogene tyrosine kinase	859390-859712
2649	serine/threonine kinase 25 (yeast)	870722-871034
2656	maternal embryonic leucine zipper kinase	873142-873499
2660	transforming growth factor beta regulated gene 4	874486-874847
2678	mitogen-activated protein kinase kinase kinase 6	880782-881178
2685	c-src tyrosine kinase	883213-883509
2690	protein kinase, cAMP dependent, catalytic, alpha	884918-885283
2697	RIKEN cDNA C230081A13 gene	887214-887504
2727	mitogen-activated protein kinase 1	897474-897851
2728	STE20-related kinase adaptor alpha	897852-898184
2739	LIM-domain containing, protein kinase	901587-901936
2767	mitogen-activated protein kinase kinase kinase 10	911247-911607
2797	mitogen-activated protein kinase 10	921494-921818
2815	serine/threonine kinase 3 (Ste20, yeast homolog)	927749-928072
2821	protein kinase N3	929703-929953
2844	large tumor suppressor	937654-937969
2854	leucine-rich repeat kinase 2	940941-941325
2917	phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 4, p150	962493-962788
2965	protein kinase, DNA activated, catalytic polypeptide	979242-979576
2966	doublecortin-like kinase 1	979577-979919
3005	activin receptor IIA	993008-993293
3016	Unc-51 like kinase 2 (C. elegans)	996609-996900
3028	branched chain ketoacid dehydrogenase kinase	1000498-1000839
3066	mitogen-activated protein kinase 3	1013377-1013718
3072	p21 protein (Cdc42/Rac)-activated kinase 4	1015266-1015566
3110	protein kinase, membrane associated tyrosine/threonine 1	1028441-1028755
3137	eukaryotic translation initiation factor 2 α kinase 1	1037660-1038052
3141	PAS domain containing serine/threonine kinase	1039167-1039558
3145	cyclin D3	1040554-1040910
3170	PTK2 protein tyrosine kinase 2	1049366-1049709
3215	c-abl oncogene 1, receptor tyrosine kinase	1064790-1065134
3234	FAST kinase domains 5	1071097-1071485
3264	ribosomal protein S6 kinase polypeptide 3	1081273-1081650
3293	glycogen synthase kinase 3 alpha	1091000-1091318
3302	integrin linked kinase	1094162-1094466
3325	fer (fms/fps related) protein kinase, testis specific 2	1101741-1102066
3390	cell division cycle 2-like 1	1124002-1124331
3497	CDC-like kinase 2	1159741-1160065
3517	aarF domain containing kinase 1	1166401-1166741
3551	RIKEN cDNA B230120H23 gene	1177903-1178190
3583	checkpoint kinase 1 homolog (S. pombe)	1188354-1188736
3598	cyclin-dependent kinase 2	1193336-1193684
3636	vaccinia related kinase 3	1206468-1206770
3672	MAP kinase-activated protein kinase 5	1218590-1218943
3697	tyrosine kinase, non-receptor, 2	1227011-1227293
3752	calcium/calmodulin-dependent protein kinase 2, β	1245765-1246095
3761	ataxia telangiectasia mutated homolog (human)	1248864-1249255

Table 60. Example kinase targets		
3792	salt inducible kinase 1	1259549-1259840
3803	phosphoinositide-3-kinase, class 3	1263190-1263540
3810	aarF domain containing kinase 2	1265631-1265906
3818	tripartite motif-containing 24	1268181-1268568
3839	MAP kinase-interacting serine/threonine kinase 1	1275270-1275564
3946	polo-like kinase 4 (Drosophila)	1310666-1311034
4001	mitogen-activated protein kinase kinase 2	1329109-1329497
4017	Janus kinase 3	1334368-1334721
4043	CDC like kinase 4	1343146-1343482
4045	SCY1-like 3 (*S. cerevisiae*)	1343876-1344245
4071	NIMA-related expressed kinase 2	1352509-1352861
4151	vaccinia related kinase 2	1379213-1379553
4171	casein kinase 2, alpha prime polypeptide	1385888-1386249
4193	mitogen-activated protein kinase 1	1393467-1393856
4201	eukaryotic translation initiation factor 2 α kinase 3	1396283-1396617
4255	budding uninhibited by benzimidazoles 1 homolog, beta (*S. cerevisiae*)	1414236-1414628
4264	vaccinia related kinase 1	1417312-1417688
4268	STE20-related kinase adaptor beta	1418669-1418996
4275	FAST kinase domains 2	1421149-1421474
4299	cyclin-dependent kinase 9 (CDC2-related kinase)	1429472-1429796
4365	lemur tyrosine kinase 2	1451144-1451458
4404	Yamaguchi sarcoma viral (v-yes) oncogene homolog 1	1464339-1464640
4414	cyclin-dependent kinase 5	1467595-1467925
4488	bone morphogenic protein receptor, type II (serine/threonine kinase)	1492190-1492490
4502	testis-specific kinase 2	1496336-1496660
4632	cell division cycle 7 (*S. cerevisiae*)	1539427-1539781
4652	mitogen-activated protein kinase kinase 5	1545970-1546310
4686	mitogen-activated protein kinase 1	1557428-1557817
4715	ribonuclease L (2', 5'-oligoisoadenylate synthetase-dependent)	1567391-1567708
4744	fibroblast growth factor receptor 1	1577052-1577365
4770	protein kinase D3	1585680-1585976
4839	cyclin-dependent kinase 4	1609522-1609852
4856	protein kinase C, iota	1615321-1615627
4867	ribosomal protein S6 kinase, polypeptide 2	1618874-1619239
4903	tyrosine kinase 2	1631375-1631670
4904	FAST kinase domains 3	1631671-1632058
4928	phosphorylase kinase, gamma 2 (testis)	1639845-1640227
4947	protein kinase, AMP-activated, β 1 non-catalytic subunit	1646526-1646858
4952	tribbles homolog 3 (Drosophila)	1648199-1648515
4980	natriuretic peptide receptor 2	1658017-1658362
5012	NIMA-related expressed kinase 8	1668806-1669200
5119	protein kinase, X-linked	1705097-1705372
5127	interleukin-1 receptor-associated kinase 4	1707814-1708142
5155	protein kinase, AMP-activated, γ 1 non-catalytic subunit	1717347-1717743
5205	serine/threonine kinase 10	1734723-1735086
5258	protein kinase C, eta	1752699-1753060

Table 60. Example kinase targets		
5260	receptor (TNFRSF)-interacting serine-threonine kinase 2	1753377-1753673
5303	protein kinase, AMP-activated, γ 2 non-catalytic subunit	1767887-1768173
5443	CHK2 checkpoint homolog (*S. pombe*)	1817364-1817648
5466	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	1825671-1825984
5513	NIMA-related expressed kinase 1	1842362-1842733
5526	PDZ binding kinase	1846866-1847240
5543	Ttk protein kinase	1852758-1853100
5580	cell division cycle 2 homolog A (*S. pombe*)	1865374-1865693
5636	mitogen-activated protein kinase 7	1885325-1885696
5698	aurora kinase A	1907469-1907831
5753	Eph receptor B3	1927508-1927885
5812	oxidative-stress responsive 1	1948788-1949181
5833	cyclin H	1956302-1956671
5892	inhibitor of kappaB kinase epsilon	1978013-1978395
5902	cell cycle related kinase	1981792-1982170
5944	serine/threonine kinase 38 like	1997111-1997478
5974	tribbles homolog 1 (Drosophila)	2008081-2008383
6029	mixed lineage kinase domain-like	2027899-2028286
6121	discoidin domain receptor family, member 1	2061270-2061663
6141	aurora kinase B	2068620-2068994
6178	mitogen-activated protein kinase kinase kinase 14	2081730-2082108
6215	RIKEN cDNA E130304F04 gene	2095357-2095740
6281	cyclin-dependent kinase-like 2 (CDC2-related kinase)	2118747-2119146
6305	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	2127434-2127800
6404	cyclin-dependent kinase (CDC2-like) 10	2162918-2163302
6480	cyclin-dependent kinase 6	2189891-2190242
6633	protein kinase D2	2243758-2244155
6653	WNK lysine deficient protein kinase 4	2250760-2251118
6731	G protein-coupled receptor kinase 5	2278131-2278499
6882	aurora kinase C	2329723-2330035
6891	cyclin-dependent kinase-like 1 (CDC2-related kinase)	2332108-2332434
6929	RIKEN cDNA 4930444A02 gene	2344573-2344930
6980	p21 protein (Cdc42/Rac)-activated kinase 3	2361621-2361941
7029	ribosomal protein S6 kinase, polypeptide 5	2378152-2378437
7063	CDC-like kinase 1	2389819-2390124
7073	PDLIM1 interacting kinase 1 like	2393123-2393501
7086	salt inducible kinase 2	2397231-2397606
7124	homeodomain interacting protein kinase 2	2409808-2410107
7144	serum/glucocorticoid regulated kinase 3	2416403-2416787
7151	germ cell-specific gene 2	2418878-2419222
7165	cyclin-dependent kinase-like 3	2423119-2423482
7167	fibroblast growth factor receptor 3	2423777-2424112
7224	NIMA-related expressed kinase 4	2443004-2443301
7242	hormonally upregulated Neu-associated kinase	2449048-2449437
7289	inhibitor of kappaB kinase beta	2464074-2464378
7487	serum/glucocorticoid regulated kinase 2	2529508-2529774

Table 60. Example kinase targets		
7501	3-phosphoinositide dependent protein kinase-1	2534260-2534622
7507	lymphocyte protein tyrosine kinase	2536052-2536408
7604	microtubule associated serine/threonine kinase-like	2567713-2568021
7630	serine/threonine kinase 11	2575716-2576017
7661	MAP/microtubule affinity-regulating kinase 4	2585629-2585955
7781	proviral integration site 1	2626615-2627001
7784	serine/threonine kinase 17b (apoptosis-inducing)	2627742-2628087
7797	protein kinase C, epsilon	2632117-2632509
7808	myosin, light polypeptide kinase 2, skeletal muscle	2635957-2636283
7841	NIMA-related expressed kinase 3	2646895-2647246
7917	PTK2 protein tyrosine kinase 2 beta	2672668-2672997
7980	endothelial-specific receptor tyrosine kinase	2693563-2693919
8109	thymoma viral proto-oncogene 3	2735270-2735575
8123	citron	2740025-2740319
8173	NUAK family, SNF1-like kinase, 1	2755489-2755818
8206	activin A receptor, type 1B	2766172-2766565
8328	FAST kinase domains 1	2806153-2806512
8469	activin receptor IIB	2854148-2854509
8556	serine/threonine kinase 30	2882719-2883094
8662	death-associated protein kinase 3	2918007-2918383
8760	testis-specific serine kinase 6	2949013-2949363
8792	RIKEN cDNA A630047E20 gene	2959129-2959498
8890	testis-specific serine kinase 4	2988076-2988379
8946	G protein-coupled receptor kinase 1	3003705-3003945
9035	PAN3 polyA specific ribonuclease subunit homolog (*S. cerevisiae*)	3027117-3027350
9149	mitogen-activated protein kinase 2	3055949-3056195
9202	calcium/calmodulin-dependent protein kinase IV	3067906-3067965
9218	ribosomal protein S6 kinase polypeptide 1	3070827-3071085
9232	apoptosis-associated tyrosine kinase	3074031-3074270
9252	Eph receptor B4	3078422-3078630
9266	serine/threonine/tyrosine kinase 1	3081287-3081520
9338	testis-specific serine kinase 1	3097427-3097661
9460	G protein-coupled receptor kinase 4	3120208-3120400
9526	NUAK family, SNF1-like kinase, 2	3130443-3130616
9577	FMS-like tyrosine kinase 1	3137414-3137564
9643	testis-specific serine kinase 5	3143809-3143951
9672	calcium/calmodulin-dependent protein kinase 1, α	3146563-3146684
9688	tyrosine kinase, non-receptor, 1	3147699-3147819
9721	phosphorylase kinase gamma 1	3149851-3149854
9722	mitogen-activated protein kinase 7	3149855-3149946
3157213	mitogen-activated protein kinase 5	3233617-3233716
3157247	endoplasmic reticulum (ER) to nucleus signaling 1	3179284-3179383
3157267	mitogen-activated protein kinase kinase kinase 2	3185971-3186070
3157347	fibroblast growth factor receptor 4	3276349-3276448
3157427	dual serine/threonine and tyrosine protein kinase	3163684-3163783
3157453	testis expressed gene 14	3276149-3276248
3157487	NIMA-related expressed kinase 11	3167184-3167283
3157527	NIMA-related expressed kinase 5	3275849-3275948

Table 60. Example kinase targets		
3157545	death-associated protein kinase 2	3254417-3254516
3157639	spleen tyrosine kinase	3259705-3259804
3157684	doublecortin-like kinase 2	3170684-3170783
3157692	myosin, light polypeptide kinase	3220991-3221090
3157728	NA	3235317-3235416
3157785	TRAF2 and NCK interacting kinase	3264805-3264904
3157794	tribbles homolog 2 (*Drosophila*)	3204997-3205096
3157808	unc-51-like kinase 3 (*C. elegans*)	3229117-3229216
3157827	insulin receptor	3239817-3239916
3157880	PTK7 protein tyrosine kinase 7	3277549-3277648
3157993	epidermal growth factor receptor	3166784-3166883
3158134	anaplastic lymphoma kinase	3247317-3247416
3158136	receptor tyrosine kinase-like orphan receptor 1	3228817-3228916
3158179	NA	3252117-3252216
3158184	calcium/calmodulin-dependent protein kinase II α	3257905-3258004
3158194	NA	3255705-3255804
3158209	fibroblast growth factor receptor 2	3207458-3207557
3158279	unc-51-like kinase 4 (*C. elegans*)	3273396-3273495
3158375	megakaryocyte-associated tyrosine kinase	3204897-3204996
3158394	bone morphogenetic protein receptor, type 1B	3218291-3218390

Table 61. Cytochrome reductases			
SEQ ID NO:	Description	Avg Cov	siRNA SEQ ID NOs:
1124	P450 (cytochrome) oxidoreductase	18.96	353642-353994
1759	cytochrome b5 reductase 4	14.829	569460-569777
2330	ubiquinol-cytochrome c reductase complex chaperone, CBP3 homolog (yeast)	18.852	763043-763396
3795	ubiquinol-cytochrome c reductase core protein 1	109.161	1260523-1260890
3799	cytochrome b5 reductase 3	78.623	1261910-1262218
3897	cytochrome b reductase 1	1.445	1294703-1295101
4548	ubiquinol cytochrome c reductase core protein 2	74.045	1511637-1511998
5706	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	78.928	1910358-1910701
6495	cytochrome b5 reductase 1	7.465	2195311-2195681
8631	ubiquinol-cytochrome c reductase hinge protein	4.546	2907991-2908330
8675	ubiquinol-cytochrome c reductase binding protein	3.239	2922032-2922391
9127	ubiquinol-cytochrome c reductase, complex III subunit VII	2.023	3050777-3051054

Table 21. Ubiquitin-thiolesterases

SEQ ID NO:	cons L	Description	Avg Cov	siRNA SEQ ID NOs:
	7293	ubiquitin specific peptidase 9, X chromosome	6.127	9772-10147
93	3839	ubiquitin specific peptidase 48	5.077	39432-39822

SEQ ID NO:	cons L	Description	Avg Cov	siRNA SEQ ID NOs:
95	3832	ubiquitin specific peptidase 7	16.622	40175-40559
103	3754	ubiquitin specific peptidase 40	2.183	42743-43018
273	3151	ubiquitin specific peptidase 47	11.289	94293-94582
276	3145	cylindromatosis (turban tumor syndrome)	5.717	95119-95374
335	3057	ubiquitin specific peptidase 8	10.752	111384-111738
514	2833	ubiquitin specific peptidase 10	11.689	161987-162319
598	2741	ubiquitin specific peptidase 15	10.777	185975-186368
625	2714	ubiquitin specific peptidase 25	1.533	194182-194422
834	2567	ubiquitin specific peptidase 38	3.655	258184-258571
931	2523	ubiquitin specific peptidase 4 (proto-oncogene)	13.735	289262-289658
965	2501	ubiquitin specific peptidase 16	11.237	300334-300663
980	2494	ubiquitin specific peptidase 28	6.027	305222-305581
1311	2331	ubiquitin specific peptidase 12	3.674	416087-416477
1499	2245	ubiquitin specific peptidase 33	3.642	480167-480565
1502	2244	ubiquitin specific peptidase 19	7.049	481244-481580
1541	2233	ubiquitin specific peptidase 1	1.24	494093-494468
1612	2205	OTU domain containing 7B	0.437	518572-518901
1660	2188	ubiquitin specific peptidase 54	0.655	535568-535921
1941	2098	ubiquitin specific peptidase 11	3.914	631257-631579
2267	1990	ubiquitin specific peptidase 14	2.01	741691-741995
2275	1989	ubiquitin specific peptidase 39	11.625	744331-744665
2303	1982	ubiquitin specific peptidase 46	1.193	753953-754261
2460	1942	Brca1 associated protein 1	3.462	806747-807089
2596	1909	ubiquitin specific peptidase 21	10.965	853543-853866
2634	1899	ubiquitin specific peptidase 22	1.692	865729-866104
3030	1785	ubiquitin specific peptidase 5 (isopeptidase T)	13.894	1001194-1001562
3074	1774	BRCA1/BRCA2-containing complex, subunit 3	1.488	1015902-1016231
3536	1662	ubiquitin specific peptidase 27, X chromosome	0.685	1172962-1173239
3558	1654	ubiquitin specific peptidase 52	3.654	1180058-1180445
3714	1620	ubiquitin specific peptidase 30	0.966	1232956-1233353
3842	1586	myb-like, SWIRM and MPN domains 1	0.676	1276194-1276510
3915	1570	ubiquitin specific peptidase 3	6.65	1300512-1300831
4057	1535	ubiquitin specific peptidase 18	3.571	1347935-1348245
4072	1530	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	67.811	1352862-1353184
4107	1522	ubiquitin carboxyl-terminal esterase L5	10.895	1364288-1364643
4509	1434	ubiquitin specific peptidase 20	0.904	1498598-1498950
4875	1353	OTU domain containing 5	3.986	1621572-1621944
5615	1187	ubiquitin specific peptidase like 1	1.464	1877785-1878169
5649	1178	STAM binding protein	2.283	1889758-1890088
6996	881	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)	2.405	2367046-2367358
8860	427	ubiquitin carboxyl-terminal esterase L4	0.446	2979143-2979234
8992	395	ataxin 3	0.087	3016154-3016402
9384	291	ubiquitin specific peptidase 53	0.073	3106251-3106450
3157441	263	ubiquitin specific peptidase 50	0.152	3267405-3267504

SEQ ID NO:	cons L	Description	Avg Cov	siRNA SEQ ID NOs:
3157521	192	ubiquitin specific peptidase 37	0.027	3170784-3170883
3157574	1203	ubiquitin specific petidase 45	0.416	3242017-3242116

Table 23. E3 Ubiquitin Protein ligases

SEQ ID NO:	cons L	Description	Avg Cov	siRNA SEQ ID NOs:
9	4809	ubiquitin protein ligase E3 component n-recognin 3	2.236	12279-12498
48	4159	SMAD specific E3 ubiquitin protein ligase 2	7.495	24792-25162
64	3999	itchy, E3 ubiquitin protein ligase	4.833	29919-30278
101	3757	ubiquitin protein ligase E3 component n-recognin 5	9.431	42166-42421
143	3560	ubiquitin protein ligase E3C	11.999	55140-55421
936	2521	ubiquitin protein ligase E3 component n-recognin 2	3.175	290987-291365
1355	2311	HECT domain and ankyrin repeat containing, E3 ubiquitin protein ligase 1	11.689	431371-431703
2414	1956	SMAD specific E3 ubiquitin protein ligase 1	0.804	791272-791663
3279	1724	ubiquitin protein ligase E3 component n-recognin 7 (putative)	2.904	1086176-1086492
3531	1663	ubiquitin protein ligase E3B	1.82	1171311-1171631
3906	1573	WW domain containing E3 ubiquitin protein ligase 2	1.581	1297605-1297894
4078	1528	WW domain containing E3 ubiquitin protein ligase 1	0.308	1354729-1355093
6165	1066	G2/M-phase specific E3 ubiquitin ligase	0.358	2077605-2078002
6645	960	ubiquitin protein ligase E3 component n-recognin 1	0.266	2248043-2248415
6760	934	ubiquitin protein ligase E3A	0.576	2287890-2288245
3157485	2014	ubiquitin protein ligase E3 component n-recognin 4	0.639	3209658-3209757
3157673	192	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2	0.017	3269496-3269595

Table 24. STATs

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
540	2799	signal transducer & activator of transcription 5B	1.323	169415-169753
887	2543	signal transducer & activator of transcription 1	5.548	274540-274924
2234	2001	signal transducer & activator of transcription 6	2.945	730267-730586
2249	1997	signal transducer & activator of transcription 3	0.987	735545-735924
3913	1571	signal transducer & activator of transcription 5A	1.268	1299843-1300222
3157484	433	signal transducer & activator of transcription 2	0.099	3168284-3168383
3157597	252	signal transducer & activator of transcription 4	0.087	3226517-3226616

Table 27. Stress Response Genes

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
74	3956	mitogen-activated protein kinase kinase kinase kinase 4	10.121	33333-33668
221	3285	hypoxia up-regulated 1	59.506	78625-79007
279	3139	methyl CpG binding protein 2	1.23	95910-96141

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
345	3034	glycogen synthase kinase 3 beta	0.647	114424-114743
444	2900	heat shock protein 4	15.95	142820-143094
476	2865	heat shock 105kDa/110kDa protein 1	19.863	151195-151420
485	2858	AHA1, activator of heat shock protein ATPase homolog 2 (yeast)	16.103	153831-154084
579	2758	heat shock protein 90, β(Grp94), member 1	606.207	180574-180954
594	2744	heat shock protein 90, α (cytosolic), class A member 1	93.844	184698-184927
827	2572	heat shock protein 9	28.56	255926-256325
977	2496	heat shock protein 90 alpha (cytosolic), class B member 1	609.5	304274-304591
1384	2293	TNF receptor-associated protein 1	66.2	441242-441639
1489	2250	mitogen-activated protein kinase associated protein 1	9.725	476915-477307
1798	2143	eukaryotic translation initiation factor 2 alpha kinase 4	2.779	582987-583297
1842	2130	MAP kinase-interacting serine/threonine kinase 2	2.895	597880-598207
1967	2087	serum/glucocorticoid regulated kinase 1	4.001	640401-640729
1979	2085	histone deacetylase 5	7.779	644628-644970
2076	2052	DnaJ (Hsp40) homolog, subfamily B, member 1	9.75	677203-677558
2096	2045	mitogen-activated protein kinase 14	7.294	683848-684174
2272	1990	heat shock factor 2	2.598	743398-743788
2297	1984	protein phosphatase 3, catalytic subunit, alpha isoform	4.715	751950-752267
2372	1964	Ser (or Cys) peptidase inhibitor clade H member 1	125.59	777036-777317
2530	1925	misshapen-like kinase 1 (zebrafish)	1.615	830880-831232
2756	1869	heat shock factor 1	25.227	907582-907889
2779	1862	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	19.826	915394-915727
2929	1822	protein disulfide isomerase associated 5	42.884	966735-967056
2974	1807	heat shock protein 2	5.538	982428-982785
3063	1776	heat shock protein 8	38.69	1012333-1012621
3137	1761	eukaryotic translation initiation factor 2 alpha kinase 1	9.682	1037660-1038052
3151	1757	cancer susceptibility candidate 3	6.742	1042529-1042877
3589	1647	calmodulin binding transcription activator 2	0.784	1190341-1190653
3699	1623	transforming, acidic coiled-coil containing protein 3	13.073	1227651-1228044
3754	1611	isocitrate dehydrogenase 2 (NADP+), mitochondrial	8.177	1246485-1246791
3839	1586	MAP kinase-interacting serine/threonine kinase 1	2.216	1275270-1275564
3943	1563	eukaryotic translation initiation factor 2, subnt 1 α	14.063	1309599-1309969
4201	1500	eukaryotic translation initiation factor 2 α kinase 3	2.46	1396283-1396617

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
4434	1453	protein kinase, interferon inducible double stranded RNA dependent activator	5.527	1474052-1474353
4947	1338	protein kinase, AMP-activated, beta 1 non-catalytic subunit	5.753	1646526-1646858
5002	1323	AHA1, activator of heat shock protein ATPase homolog 1 (yeast)	93.621	1665415-1665746
5155	1287	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	12.934	1717347-1717743
5251	1271	antigenic determinant of rec-A protein	1.928	1750245-1750559
5295	1259	nuclear receptor subfamily 4, group A, member 2	0.73	1765734-1766070
5303	1258	protein kinase, AMP-activated, gamma 2 non-catalytic subunit	0.729	1767887-1768173
5406	1236	cold inducible RNA binding protein	32.931	1804466-1804836
5424	1231	SMT3 suppressor of mif two 3 homolog 1 (yeast)	10.803	1810772-1811128
6622	965	pyrroline-5-carboxylate reductase 1	0.9	2239835-2240228
7418	785	myeloid differentiation primary response gene 116	2.514	2506840-2507215
7981	638	Parkinson disease (autosomal recessive, early onset) 7	47.839	2693920-2694252
8048	615	RIKEN cDNA 2310016C08 gene	1.503	2715913-2716256
8085	605	protein phosphatase 1, regulatory (inhibitor) subunit 15b	0.176	2727942-2728269
8155	587	sphingomyelin phosphodiesterase 3, neutral	0.179	2750331-2750645
8336	539	heat shock protein 1	3.124	2809108-2809434
8405	517	heat shock protein 1 (chaperonin 10)	4.477	2833031-2833420
8780	444	HIG1 domain family, member 1A	0.685	2955263-2955620
8954	403	junction-mediating and regulatory protein	0.09	3005715-3006035
9679	173	heat shock protein 1B	0.091	3147029-3147080
9722	149	mitogen-activated protein kinase kinase 7	0.089	3149855-3149946
3157247	594	endoplasmic reticulum (ER) to nucleus signaling 1	0.18	3179284-3179383
3157505	644	crystallin, alpha B	0.99	3280749-3280848
3157706	999	family with sequence similarity 129, member A	0.792	3219891-3219990
3158121	3735	transformation related protein 53 inducible nuclear protein 1	2.567	3197071-3197170
3158350	787	response to stress	0.417	3201697-3201796

Table 28.Glycosyltransferases

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
150	3549	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1	11.757	57147-57422
178	3411	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2	22.835	65737-65999
270	3158	UDP-GalNAc:betaGlcNAc beta 1,3-galactosaminyltransferase, polypeptide 2	4.224	93348-93655

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
310	3102	nicotinamide phosphoribosyltransferase	5.348	104902-105169
439	2903	poly (ADP-ribose) polymerase family, member 1	23.907	141472-141718
676	2680	fucosyltransferase 8	9.927	209841-210227
818	2576	poly (ADP-ribose) polymerase family, member 8	6.624	253302-253609
1075	2439	TCDD-inducible poly(ADP-ribose) polymerase	8.079	337086-337454
1172	2394	exostoses (multiple) 1	13.888	370087-370311
1284	2341	WD repeat and FYVE domain containing 3	0.277	407115-407476
1580	2217	beta 1,3-galactosyltransferase-like	3.289	507336-507709
1671	2185	phosphatidylinositol glycan anchor biosynthesis, class Q	2.327	539094-539385
1720	2167	protein-O-mannosyltransferase 2	1.099	555946-556293
1813	2138	poly (ADP-ribose) polymerase family, member 16	4.303	588191-588503
1869	2123	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)	0.839	607012-607348
1899	2113	glycogen synthase 1, muscle	2.695	617021-617381
1998	2081	exostoses (multiple)-like 3	0.53	650808-651119
2056	2058	liver glycogen phosphorylase	4.632	670012-670314
2088	2048	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	5.651	681105-681454
2167	2021	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	13.01	707535-707870
2174	2019	brain glycogen phosphorylase	3.301	709790-710087
2211	2008	glycosyltransferase-like domain containing 1	3.796	722365-722668
2254	1995	mannoside acetylglucosaminyltransferase 4, isoenzyme B	27.246	737377-737697
2363	1967	exostoses (multiple) 2	12.067	774056-774354
2417	1954	mannoside acetylglucosaminyltransferase 2	5.098	792371-792746
2557	1918	UDP-glucose ceramide glucosyltransferase	1.94	840181-840538
2589	1909	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 3	18.933	851115-851489
2597	1909	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 9	2.935	853867-854128
2696	1886	glycosyltransferase 25 domain containing 1	29.095	886942-887213
2783	1861	protein O-fucosyltransferase 2	28.156	916726-917035
2830	1851	asparagine-linked glycosylation 12 homolog (yeast, alpha-1,6-mannosyltransferase)	9.883	932756-933070
2920	1824	asparagine-linked glycosylation 8 homolog (yeast, alpha-1,3-glucosyltransferase)	6.563	963558-963865
3065	1776	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10	1.546	1013002-1013376
3249	1736	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2	5.258	1075997-1076374
3332	1709	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1	24.577	1104024-1104401
3411	1689	ST3 beta-galactoside alpha-2,3-sialyltransferase 3	3.964	1131123-1131445

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
3472	1674	glycogenin	12.806	1151366-1151643
3484	1672	ST3 beta-galactoside alpha-2,3-sialyltransferase 5	21.148	1155324-1155711
3594	1646	phosphatidylinositol glycan anchor biosynthesis, class M	0.64	1191982-1192311
3711	1621	nicotinate phosphoribosyltransferase domain containing 1	9.212	1231855-1232201
3731	1616	glucan (1,4-alpha-), branching enzyme 1	2.847	1238609-1238920
3887	1577	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 2	5.414	1291326-1291668
3937	1565	exostoses (multiple)-like 2	2.123	1307522-1307889
4007	1548	protein-O-mannosyltransferase 1	1.418	1331135-1331436
4105	1522	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11	1.816	1363583-1363970
4177	1507	RIKEN cDNA A130022J15 gene	1.007	1387950-1388266
4186	1504	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 6	5.237	1391079-1391449
4319	1476	ST3 beta-galactoside alpha-2,3-sialyltransferase 2	1.043	1435989-1436317
4391	1460	dolichyl-phosphate (UDP-N-acetylglucosamine) acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)	10.516	1460002-1460374
4654	1402	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7	0.782	1546609-1546999
4671	1399	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 4	3.652	1552461-1552728
4673	1398	phosphatidylinositol glycan anchor biosynthesis, class V	0.875	1553085-1553453
4701	1392	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5	2.241	1562813-1563108
4795	1370	asparagine-linked glycosylation 1 homolog (yeast, beta-1,4-mannosyltransferase)	4.698	1594394-1594762
4883	1350	glycosyltransferase 8 domain containing 1	12.347	1624267-1624637
4914	1345	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6	0.514	1635173-1635561
4945	1339	mannoside acetylglucosaminyltransferase 5	0.5	1645857-1646201
5003	1323	poly (ADP-ribose) polymerase family, member 6	1.689	1665747-1666131
5314	1256	phosphatidylinositol glycan anchor biosynthesis, class A	1.768	1771843-1772168
5410	1235	queuine tRNA-ribosyltransferase 1	3.554	1805877-1806240
5523	1206	xylosylprotein beta1,4-galactosyltransferase, polypeptide 7 (galactosyltransferase I)	4.56	1845828-1846182
5541	1201	phosphatidylinositol glycan anchor biosynthesis, class C	1.816	1852108-1852474
5577	1195	poly (ADP-ribose) polymerase family, member 2	2.269	1864411-1864683
5594	1191	mannoside acetylglucosaminyltransferase 1	3.072	1870192-1870557
5596	1190	uridine monophosphate synthetase	2.109	1870945-1871338
5603	1189	like-glycosyltransferase	1.088	1873387-1873696

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
5740	1158	protein O-linked mannose beta1,2-N-acetylglucosaminyltransferase	2.323	1922712-1923111
5782	1148	UDP-Gal:betaGal beta 1,3-galactosyltransferase, polypeptide 6	2.721	1938009-1938394
5811	1143	UDP-GalNAc:betaGlcNAc beta 1,3-galactosaminyltransferase, polypeptide 1	1.658	1948459-1948787
6018	1098	phosphatidylinositol glycan anchor biosynthesis, class B	0.881	2023895-2024261
6204	1057	methylthioadenosine phosphorylase	15.667	2091342-2091736
6220	1053	asparagine-linked glycosylation 5 homolog (yeast, dolichyl-phosphate beta-glucosyltransferase)	4.737	2097263-2097647
6257	1043	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase-like 1	0.564	2110626-2111006
6374	1019	asparagine-linked glycosylation 11 homolog (yeast, alpha-1,2-mannosyltransferase)	1.981	2151968-2152316
6415	1008	glycosyltransferase 8 domain containing 3	0.363	2166772-2167170
6428	1006	core 1 synthase, glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase, 1	0.85	2171365-2171714
6531	983	hypoxanthine guanine phosphoribosyl transferase 1	40.474	2207724-2208109
6806	924	purine-nucleoside phosphorylase 1	10.99	2304356-2304474
6857	913	protein O-fucosyltransferase 1	0.441	2321807-2322122
6893	904	asparagine-linked glycosylation 2 homolog (yeast, alpha-1,3-mannosyltransferase)	0.997	2332768-2333127
6925	899	dolichol-phosphate (beta-D) mannosyltransferase 1	3.276	2343195-2343568
6955	891	asparagine-linked glycosylation 9 homolog (yeast, alpha 1,2 mannosyltransferase)	1.514	2353366-2353756
7217	834	poly (ADP-ribose) polymerase family, member 14	0.115	2440491-2440873
7484	767	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1	0.387	2528454-2528763
7778	694	RFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	1.377	2625536-2625911
7893	663	phosphatidylinositol glycan anchor biosynthesis, class P	5.595	2664400-2664764
8007	632	asparagine-linked glycosylation 6 homolog (yeast, alpha-1,3,-glucosyltransferase)	1.15	2702432-2702775
8072	608	dolichol-phosphate (beta-D) mannosyltransferase 2	1.511	2724089-2724407
8110	598	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	0.277	2735576-2735965
8126	593	fucosyltransferase 11	0.72	2740650-2740952
8137	591	asparagine-linked glycosylation 13 homolog (*S. cerevisiae*)	1.131	2744301-2744619
8277	553	adenine phosphoribosyl transferase	7.251	2789152-2789451
8302	547	poly (ADP-ribose) polymerase family, member 11	0.182	2797670-2797988
8323	541	ADP-ribosyltransferase 3	0.457	2804437-2804812
8510	493	UDP-Gal:betaGlcNAc beta 1,3-	0.099	2867869-2868208

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
		galactosyltransferase, polypeptide 1		
8536	489	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 4	0.096	2876241-2876595
8900	417	UDP glucuronosyltransferase 1 family, polypeptide A6B	0.382	2990930-2991111
9154	351	UDP glucuronosyltransferase 1 family, polypeptide A6A	0.106	3057120-3057211
9275	322	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 4	0.228	3083416-3083607
3157421	431	glycosyltransferase 8 domain containing 2	0.2	3173684-3173783
3157495	1014	phosphatidylinositol glycan anchor biosynthesis, class H	1.147	3183184-3183283
3157929	501	ADP-ribosyltransferase 2b	0.579	3280549-3280648
3157944	155	beta-1,4-N-acetyl-galactosaminyl transferase 2	0.038	3175384-3175483
3157960	2282	ST8 α-N-acetyl-neuraminide α-2,8-sialyltransferase 4	1.629	3246817-3246916
3158019	362	ABO blood group (transferase A, α1-3-N-acetylgalactosaminyltransferase, transferase B, α1-3-galactosyltransferase)	0.204	3185571-3185670
3158211	343	ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 4	0.282	3260605-3260704
3158222	726	asparagine-linked glycosylation 10 homolog B (yeast, α-1,2-glucosyltransferase)	0.262	3163121-3163220

Table 29. GTPase activators

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
15	4557	RIKEN cDNA B230339M05 gene	3.965	14108-14438
58	4061	TBC1 domain family, member 2B	20.58	27984-28289
102	3754	neurofibromatosis 1	1.523	42422-42742
128	3628	regulator of G-protein signaling 17	3.266	50726-50999
231	3253	SLIT-ROBO Rho GTPase activating protein 2	3.644	81642-81883
288	3130	ArfGAP w/ SH# domain, ankyrin repeat & PH domain1	5.511	98329-98712
339	3047	active BCR-related gene	7.246	112574-112969
382	2979	breakpoint cluster region	3.754	125289-125540
385	2977	GTPase activating RANGAP domain-like 1	1.897	126120-126355
422	2926	Rho GTPase activating protein 18	15.948	136578-136825
469	2875	ralA binding protein 1	6.921	149400-149662
574	2762	GTPase activating protein & VPS9 domains 1	3.958	179030-179286
651	2697	USP6 N-terminal like	3.097	202215-202493
743	2635	signal-induced proliferation-associated 1 like 1	5.005	230159-230551
766	2610	Rho GTPase activating protein 21	4.9	236928-237164
872	2550	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1	0.806	269740-270071
877	2547	Rho GTPase activating protein 22	46.084	271221-271503

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
919	2530	IQ motif containing GTPase activating protein 2	7.731	285026-285361
1013	2474	ArfGAP with FG repeats 1	7.52	316623-316999
1019	2471	TBC1 domain family, member 1	3.523	318888-319270
1021	2471	Rho GTPase activating protein 24	7.769	319636-320032
1180	2390	G protein-coupled receptor kinase-interactor 1	9.642	372750-373054
1202	2381	G protein-coupled receptor kinase-interactor 2	1.721	380043-380305
1209	2378	tuberous sclerosis 2	2.396	382192-382530
1231	2366	ADP-ribosylation factor GTPase activating protein 2	13	389186-389510
1237	2364	rabaptin, RAB GTPase binding effector protein 1	1.86	391313-391594
1251	2357	G-protein signalling modulator 2 (AGS3-like, *C. elegans*)	25.263	396073-396448
1391	2292	ecotropic viral integration site 5 like	2.064	443532-443866
1408	2285	TBC1 domain family, member 15	5.501	449214-449575
1410	2285	Rho GTPase activating protein 12	1.14	449921-450284
1449	2267	guanosine diphosphate (GDP) dissociation inhibitor 1	25.652	463287-463618
1479	2253	TBC1 domain family, member 10b	11.038	473445-473815
1513	2240	RAN GTPase activating protein 1	12.173	484741-485095
1562	2226	small G protein signaling modulator 3	9.371	501162-501548
1634	2197	Rho GTPase activating protein 29	3.76	526292-526588
1642	2193	IQ motif containing GTPase activating protein 1	0.799	529103-529460
1649	2191	ADP-ribosylation factor GTPase activating protein 1	17.61	531693-532043
1752	2158	ArfGAP with GTPase domain, ankyrin repeat and PH domain 3	11.364	567066-567372
1803	2141	Rho GTPase activating protein 17	3.223	584731-585028
1858	2125	TBC1 domain family, member 9B	5.288	603350-603639
1886	2116	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3	4.242	612818-613159
1922	2106	stromal membrane-associated protein 1	12.305	624987-625343
1926	2104	Rho guanine nucleotide exchange factor (GEF) 1	5.042	626255-626602
2031	2067	TBC1 domain family, member 25	6.666	661569-661914
2165	2022	RIKEN cDNA A230067G21 gene	0.566	706803-707157
2223	2004	Ras and Rab interactor 2	5.703	726472-726765
2289	1985	amyotrophic lateral sclerosis 2 (juvenile) homolog (human)	0.792	749132-749432
2291	1985	TBC1 domain family, member 17	6.336	749822-750199
2301	1983	ADP-ribosylation factor GTPase activating protein 3	5.309	753270-753612
2309	1981	RAB GTPase activating protein 1-like	1.389	755934-756259
2365	1966	stromal membrane-associated GTPase-activating protein 2	7.748	774678-775049
2419	1954	RAB3 GTPase activating protein subunit 1	1.494	793063-793349
2479	1938	oligophrenin 1	2.039	813214-813607
2534	1925	signal-induced proliferation associated gene 1	3.696	832257-832632
2559	1917	guanosine diphosphate (GDP) dissociation inhibitor 2	4.745	840859-841143

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
2621	1902	Rac GTPase-activating protein 1	19.316	861408-861766
2622	1902	RAS p21 protein activator 3	2.103	861767-862055
2695	1886	TBC1 domain family, member 22a	1.294	886641-886941
2854	1845	leucine-rich repeat kinase 2	1.495	940941-941325
2862	1841	ArfGAP w/ coiled-coil, ankyrin repeat & PH domains 2	1.693	943650-943952
3038	1783	Rho GDP dissociation inhibitor (GDI) alpha	85.766	1003934-1004232
3084	1771	myosin IXb	1.071	1019313-1019670
3134	1761	resistance to inhibitors of cholinesterase 8 homolog (C. elegans)	5.191	1036587-1036933
3144	1759	disabled homolog 2 (Drosophila) interacting protein	1.484	1040220-1040553
3163	1754	rabaptin, RAB GTPase binding effector protein 2	3.591	1046902-1047174
3512	1667	RAS p21 protein activator 4	1.866	1164603-1164943
3637	1637	Rho GTPase activating protein 25	4.095	1206771-1207157
3644	1635	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2	2.011	1209078-1209429
3676	1627	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1	0.823	1219997-1220302
3750	1612	SLIT-ROBO Rho GTPase activating protein 3	1.234	1245082-1245453
3760	1610	TBC1 domain family, member 20	6.983	1248542-1248863
3805	1596	signal-induced proliferation-associated 1 like 2	1.048	1263925-1264323
3902	1573	GIPC PDZ domain containing family, member 1	31.917	1296244-1296541
3911	1571	TBC1 domain family, member 23	0.785	1299237-1299523
4133	1516	ALS2 C-terminal like	1.264	1373305-1373600
4479	1441	DEP domain containing 1B	2.389	1489111-1489393
4536	1428	Rho GTPase activating protein 1	2.875	1507506-1507890
4552	1425	Rho GTPase activating protein 6	0.435	1512969-1513333
4775	1373	ecotropic viral integration site 5	1.536	1587335-1587660
4892	1348	ADP-ribosylation factor-like 2 binding protein	13.977	1627434-1627798
4971	1331	WD repeat domain 67	0.743	1654864-1655263
5128	1294	TBC1 domain family, member 10c	1.46	1708143-1708504
5234	1274	TBC1 domain family, member 4	0.291	1744511-1744853
5247	1272	choroidermia	0.842	1749109-1749507
5475	1218	DEP domain containing 1a	0.524	1828873-1829271
5704	1165	Rho GTPase activating protein 10	4.456	1909622-1909976
5893	1127	RIKEN cDNA 4933428G20 gene	0.385	1978396-1978755
6057	1091	TBC1 domain family, member 7	8.529	2037948-2038347
6189	1061	SH3-domain binding protein 1	2.587	2085849-2086155
6387	1016	development & differentiation enhancing factor 2	0.44	2156641-2157022
6449	1001	TBC1 domain family, member 14	0.485	2178913-2179271
6597	969	G-protein signalling modulator 3 (AGS3-like, *C. elegans*)	10.243	2231290-2231663
6629	963	ankyrin repeat domain 27 (VPS9 domain)	0.299	2242342-2242728
6789	928	Rho GTPase activating protein 19	0.204	2298285-2298665
7012	877	TBC1 domain family, member 24	0.285	2372442-2372763

SEQ ID NO:	consL	Description	Avg Cov	siRNA SEQ ID NOs:
7028	874	T-cell lymphoma invasion and metastasis 2	0.333	2377813-2378151
7443	777	TBC1 domain family, member 8	0.219	2515218-2515579
7553	749	choroideremia-like	0.203	2551113-2551507
7888	664	StAR-related lipid transfer (START) domain containing 13	0.12	2662630-2662978
7967	642	RNA binding protein 1	2.033	2689665-2689951
8020	627	Rho GTPase-activating protein	0.115	2706942-2707263
8021	626	RAS p21 protein activator 2	0.141	2707264-2707590
8342	537	Rho GTPase activating protein 28	0.108	2811107-2811423
8393	522	proline rich 5 (renal)	0.462	2828643-2828993
8701	458	TBC1 domain family, member 13	0.867	2930463-2930783
8702	458	family with sequence similarity 13, member B	0.273	2930784-2931124
8792	441	RIKEN cDNA A630047E20 gene	0.215	2959129-2959498
8865	425	CDC42 GTPase-activating protein	0.055	2980492-2980833
8990	396	GTPase activating RANGAP domain-like 3	0.117	3015728-3016007
9085	369	glucocorticoid receptor DNA binding factor 1	0.084	3040212-3040461
9146	354	ArfGAP with FG repeats 2	0.239	3055161-3055411
9161	349	RAB GTPase activating protein 1	0.083	3058415-3058689
9322	307	Ras and Rab interactor 1	0.073	3093895-3094135
9483	252	G-protein signalling modulator 1 (AGS3-like, *C. elegans*)	0.075	3123987-3124129
9653	186	Rho GTPase activating protein 27	0.054	3144717-3144852
9665	179	regulator of G-protein signaling 2	0.059	3145905-3146047
3157157	1019	Rho guanine nucleotide exchange factor (GEF) 19	0.439	3188671-3188770
3157282	1366	regulator of G protein signaling 7	1.027	3244017-3244116
3157556	1034	SLIT-ROBO Rho GTPase activating protein 1	0.398	3185171-3185270
3157624	439	Rho GTPase activating protein 9	0.242	3186071-3186170
3157647	369	Rho GTPase activating protein 20	0.059	3273896-3273995
3157800	319	Ras and Rab interactor 3	0.082	3255305-3255404
3157893	356	muscle-related coiled-coil protein	0.173	3166984-3167083
3158205	1690	TBC1D12: TBC1 domain family, member 12	2.618	3168384-3168483
3158329	1467	synapse defective 1, Rho GTPase, homolog 1 (*C. elegans*)	1.092	3213858-3213957
3158404	1495	NA	0.581	3227117-3227216

Table 65. GTPases

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
47	4181	eukaryotic translation initiation factor 5B	7.249	24507-24791
121	3653	G1 to S phase transition 1	4.531	48461-48813
309	3103	guanine nucleotide binding protein (G protein), beta 1	308.482	104658-104901
333	3061	guanine nucleotide binding protein (G protein), alpha inhibiting 3	12.233	110847-111128
491	2850	eukaryotic translation elongation factor 2	331.312	155612-155855

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
498	2844	signal recognition particle receptor ('docking protein')	23.636	157648-157932
758	2618	elongation factor Tu GTP binding domain containing 1	10.74	234313-234699
869	2551	Ras-related GTP binding C	52.673	268890-269114
872	2550	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1	0.806	269740-270071
874	2549	elongation factor Tu GTP binding domain containing 2	13.667	270373-270590
938	2520	G elongation factor, mitochondrial 1	60.355	291757-292001
1104	2424	ras homolog gene family, member Q	4.127	346976-347213
1333	2322	dynamin 2	10.975	423531-423830
1378	2296	mitofusin 1	1.418	439108-439451
1397	2290	RAS-related C3 botulinum substrate 1	73.806	445639-445879
1487	2250	guanine nucleotide binding protein, alpha q polypeptide	1.455	476277-476517
1520	2239	optic atrophy 1 homolog (human)	2.52	487010-487405
1709	2172	guanine nucleotide binding protein (G protein), alpha inhibiting 2	70.605	552132-552530
1769	2154	EH-domain containing 1	11.604	572945-573251
1816	2137	dynamin 1-like	4.171	589195-589429
1944	2097	cell division cycle 42 homolog (*S. cerevisia*e)	189.607	632324-632630
2009	2076	guanine nucleotide binding protein, α 13	2.993	654543-654775
2124	2034	ras homolog gene family, member A	135.612	693012-693333
2488	1937	GTP binding protein 2	5.681	816419-816817
2525	1927	myxovirus (influenza virus) resistance 2	8.118	829145-829432
2560	1916	EH-domain containing 2	3.355	841144-841487
2575	1913	Hbs1-like (S. cerevisiae)	2.621	846230-846577
2700	1885	GUF1 GTPase homolog (S. cerevisiae)	4.872	888158-888500
2834	1851	neuroblastoma ras oncogene	2.46	934198-934494
2857	1844	guanine nucleotide binding protein, alpha transducing 1	2.474	942072-942447
2918	1824	eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked	14.911	962789-963172
3009	1792	eukaryotic elongation factor, selenocysteine-tRNA-specific	3.035	994350-994678
3041	1782	GTP binding protein 1	3.109	1004869-1005198
3244	1737	tubulin, alpha 1B	543.754	1074476-1074632
3372	1701	guanine nucleotide binding protein (G protein), beta 4	5.812	1117711-1118107
3427	1687	RAB5A, member RAS oncogene family	21.29	1136305-1136633
3455	1678	guanine nucleotide binding protein, alpha O	0.792	1145689-1145997
3661	1631	tubulin, beta 5	61.529	1214795-1215127
3670	1628	atlastin GTPase 3	1.006	1217824-1218196
3715	1620	tubulin, gamma 1	36.02	1233354-1233745
3812	1594	tubulin, alpha 1A	100.894	1266291-1266518
3829	1591	guanine nucleotide binding protein	74.137	1271846-1272244

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
		(G protein) β2		
3862	1582	guanine nucleotide binding protein, α11	4.154	1282881-1283160
3992	1550	tubulin, α1C	191.248	1326132-1326373
4044	1539	tubulin, β2C	81.933	1343483-1343875
4168	1509	G elongation factor, mitochondrial 2	1.773	1384797-1385138
4180	1507	mitofusin 2	4.551	1389006-1389340
4212	1498	RAB5C, member RAS oncogene family	34.285	1400104-1400434
4325	1476	eukaryotic translation elongation factor 1 α2	3.269	1437945-1438305
4398	1459	tubulin, alpha 4A	8.154	1462310-1462667
4458	1447	GTP binding protein 3	3.549	1482368-1482685
4496	1437	GNAS (guanine nucleotide binding protein, alpha stimulating) complex locus	670.983	1494365-1494682
4559	1425	RAS-related protein-1a	12.202	1515193-1515550
4689	1394	mitochondrial translational initiation factor 2	1.08	1558510-1558896
4774	1373	guanylate binding protein 2	1.175	1586947-1587334
4912	1345	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	3.151	1634477-1634773
5185	1283	Tu translation elongation factor, mitochondrial	19.719	1727760-1728085
5399	1238	RAN, member RAS oncogene family	61.287	1802120-1802418
5621	1186	ras homolog gene family, member G	3.894	1879861-1880245
5703	1165	guanine nucleotide binding protein, α12	3.153	1909321-1909621
5909	1123	RAB34, member of RAS oncogene family	14.774	1984213-1984547
6358	1022	EH-domain containing 3	1.748	2146156-2146534
6499	987	guanine nucleotide binding protein (G protein), α inhibiting 1	0.658	2196424-2196754
6599	969	epsilon-tubulin 1	0.387	2232061-2232442
6669	955	ras homolog gene family, member U	0.296	2256530-2256915
6843	915	RAB13, member RAS oncogene family	14.169	2316888-2317279
7557	748	ADP-ribosylation factor related protein 1	2.613	2552598-2552994
7670	721	myxovirus (influenza virus) resistance 1	0.687	2588615-2588951
7944	647	ras homolog gene family, member J	0.275	2681897-2682206
7975	639	tubulin, beta 3	2.093	2692217-2692262
8248	561	tubulin, beta 4	0.376	2779208-2779248
8318	541	T-cell specific GTPase	0.193	2802893-2803167
8330	540	atlastin GTPase 2	0.154	2806902-2807275
8367	530	ADP-ribosylation factor-like 4A	0.247	2819826-2820225
8407	517	guanine nucleotide binding protein, alpha stimulating, olfactory type	0.16	2833728-2833995
8423	514	guanine nucleotide binding prot (G protein), β 3	0.305	2839381-2839711
8694	459	tubulin, alpha 3A	0.369	2928173-2928397
8711	456	guanylate binding protein 5	0.254	2933557-2933829
8739	451	tubulin, alpha 8	0.315	2942782-2943028
9004	392	tubulin, beta 2A	2.629	3019264-3019285
9250	330	tubulin, beta 2B	0.402	3078180-3078203
9400	283	guanine nucl binding prot. (G protein), γ 3	0.168	3109391-3109586
9520	237	RAS-like, family 2, locus 9	0.34	3129635-3129703
9605	205	RAS-related C3 botulinum substrate 2	0.555	3140533-3140548

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
3157235	968	Ras-like without CAAX 2	1.124	3191071-3191170
3157288	395	RAB5B, member RAS oncogene family	0.422	3248917-3249016
3157535	325	interferon inducible GTPase 1	0.11	3164284-3164383
3157709	573	guanine nucl binding prot, α transducing 2	0.496	3265005-3265104
3157755	370	interferon gamma induced GTPase	0.182	3283049-3283148
3157887	312	dynamin 3	0.068	3263705-3263804
3158025	336	RAB37, member of RAS oncogene family	0.154	3216491-3216590
3158055	477	tubulin, alpha-like 3	0.154	3281749-3281848
3158311	545	RAS, dexamethasone-induced 1	0.595	3272596-3272695

Table 66: Cytoskeleton

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
19	4458	platelet-activating factor acetylhydrolase, isoform 1b, subunit 1	4.915	15430-15711
33	4278	bicaudal D homolog 2 (Drosophila)	8.144	19767-20155
38	4237	hook homolog 3 (Drosophila)	2.298	21465-21748
44	4201	SH3-domain kinase binding protein 1	6.615	23443-23756
60	4019	MYC binding protein 2	2.593	28599-28954
76	3941	neuron navigator 1	0.619	34046-34322
125	3643	eukaryotic translation initiation factor 3, subunit A	40.603	49822-50120
140	3579	actinin, alpha 1	23.486	54297-54516
146	3553	microtubule-actin crosslinking factor 1	3.329	56027-56372
165	3467	radixin	17.836	61786-62020
173	3427	protein phosphatase 4, regulatory subunit 2	12.016	64291-64567
174	3423	myosin phosphatase Rho interacting protein	10.215	64568-64841
214	3308	microtubule-associated protein, RP/EB family, member 2	9.685	76455-76767
269	3164	filamin, beta	2.477	93056-93347
272	3152	Rho-associated coiled-coil containing protein kinase 1	3.17	94052-94292
278	3141	abl-interactor 1	4.255	95601-95909
284	3135	fermitin family homolog 2 (Drosophila)	27.426	97199-97521
289	3129	testis specific gene A14	3.473	98713-99031
301	3110	MAP/microtubule affinity-regulating kinase 1	3.607	102310-102609
322	3079	plectin 1	3.784	108038-108268
324	3075	parvin, alpha	6.971	108553-108801
380	2981	topoisomerase (DNA) II binding protein 1	7.196	124761-125048
400	2954	moesin	56.571	130562-130798
408	2949	protein Tyr phosphatase, non-receptor type 14	0.65	132677-132944
446	2899	actinin alpha 4	23.469	143309-143623
451	2894	kinetochore associated 1	2.501	144746-145029
477	2865	spindlin 1	18.581	151421-151677
480	2863	erythrocyte protein band 4.1-like 2	9.783	152372-152645
505	2836	myosin, heavy polypeptide 9, non-muscle	1.62	159627-159938

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
508	2836	filamin, alpha	21.729	160437-160654
518	2828	FERM domain containing 4A	2.061	163151-163399
527	2815	spectrin beta 2	2.13	165820-166071
528	2814	spastin	4.005	166072-166288
543	2791	Janus kinase 2	4.149	170408-170768
562	2773	uveal autoantigen with coiled-coil domains and ankyrin repeats	14.958	175535-175851
573	2763	calmodulin 1	15.152	178775-179029
581	2754	tubulin, gamma complex associated protein 5	3.189	181260-181476
617	2725	dynactin 1	7.088	191583-191944
621	2718	catenin (cadherin associated protein), alpha 1	30.996	192742-193116
659	2690	kinesin family member 15	9.379	204514-204832
664	2688	tetratricopeptide repeat domain 21B	5.893	205946-206342
666	2688	adducin 1 (alpha)	12.01	206742-207131
706	2661	retinoic acid induced 14	5.867	218315-218707
730	2639	microtubule associated serine/threonine kinase 2	9.069	225909-226275
749	2626	WD repeat domain 1	18.988	231778-232036
763	2614	capping protein (actin filament) muscle Z-line, alpha 1	5.404	235917-236159
766	2610	Rho GTPase activating protein 21	4.9	236928-237164
806	2584	spermatid perinuclear RNA binding protein	1.175	249509-249890
811	2582	ajuba	12.735	251195-251502
812	2581	outer dense fiber of sperm tails 2	4.42	251503-251729
830	2569	Fgfr1 oncogene partner	3.18	256961-257251
831	2569	microtubule-associated protein 7 domain containing 1	16.621	257252-257564
841	2564	kinesin family member 5B	3.218	260377-260685
848	2561	ARP1 actin-related protein 1 homolog A, centractin alpha (yeast)	27.559	262622-262828
860	2555	cortactin	45.514	265970-266319
889	2543	katanin p80 (WD40-containing) subunit B 1	12.112	275290-275634
905	2536	ADP-ribosylation factor-like 8B	2.122	280457-280707
962	2503	expressed sequence AW555464	2.283	299312-299692
972	2498	ARP3 actin-related protein 3 homolog (yeast)	166.603	302483-302872
993	2485	ARP2 actin-related protein 2 homolog (yeast)	26.066	309842-310230
997	2483	abl-interactor 2	1.097	311332-311712
1006	2477	large tumor suppressor 2	3.379	314156-314545
1021	2471	Rho GTPase activating protein 24	7.769	319636-320032
1026	2466	FERM domain containing 4B	2.223	321426-321672
1062	2447	septin 2	12.767	333080-333462
1069	2440	dishevelled, dsh homolog 1 (*Drosophila*)	21.381	335136-335489
1081	2436	ezrin	31.498	339220-339540
1085	2434	Wiskott-Aldrich syndrome-like (human)	1.492	340449-340691
1094	2430	SCY1-like 1 (*S. cerevisiae*)	12.863	343589-343905
1097	2428	sarcolemma associated protein	1.377	344524-344917
1103	2426	dystonin	1.863	346599-346975
1117	2418	drebrin 1	25.781	351269-351567
1126	2412	spectrin alpha 2	4.506	354395-354680

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
1133	2410	Rpgrip1-like	0.564	357006-357378
1175	2393	inositol polyphosphate phosphatase-like 1	3.628	371083-371386
1189	2386	talin 1	2.605	375983-376311
1208	2379	CDC14 cell division cycle 14 homolog A (*S. cerevisiae*)	2.141	381807-382191
1247	2359	microtubule-associated protein, RP/EB family, member 1	18.63	394632-394981
1255	2354	centrosomal protein 110	0.814	397494-397774
1278	2344	FERM domain containing 6	4.1	404997-405390
1279	2343	TRIO and F-actin binding protein	34.395	405391-405739
1296	2335	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein	13.338	411043-411389
1314	2327	centrosomal protein 135	0.683	417267-417520
1327	2324	centrosomal protein 55	19.363	421520-421872
1333	2322	dynamin 2	10.975	423531-423830
1349	2315	erythrocyte protein band 4.1-like 1	3.165	429287-429612
1353	2311	centrosomal protein 63	8.32	430642-430998
1359	2308	smoothelin	3.137	432687-433041
1366	2303	cofilin 2, muscle	5.323	435213-435610
1392	2291	tubulin folding cofactor E-like	2.632	443867-444266
1404	2287	A kinase (PRKA) anchor protein (gravin) 12	7.444	447806-448127
1434	2273	expressed sequence AI314180	9.004	458166-458524
1458	2262	Alstrom syndrome 1 homolog (human)	0.712	466342-466731
1466	2259	coiled-coil domain containing 85B	25.622	469052-469416
1505	2243	growth arrest-specific 2 like 1	14.146	482255-482606
1506	2243	WAS/WASL interacting protein family, member 1	5.215	482607-482938
1513	2240	RAN GTPase activating protein 1	12.173	484741-485095
1519	2239	KRIT1, ankyrin repeat containing	9.236	486770-487009
1530	2238	tropomodulin 3	3.292	490452-490783
1531	2237	twinfilin, actin-binding protein, homolog 1 (*Drosophila*)	14.634	490784-491124
1557	2227	annexin A11	55.567	499580-499921
1558	2227	parvin, beta	4.466	499922-500228
1565	2224	ZW10 homolog (*Drosophila*), centromere/kinetochore protein	12.629	502292-502621
1566	2224	coronin, actin binding protein 1C	4.605	502622-502971
1577	2218	transforming, acidic coiled-coil containing protein 2	1.821	506354-506697
1582	2214	family with sequence similarity 83, member D	12.849	508106-508316
1593	2210	rho/rac guanine nucleotide exchange factor (GEF) 2	3.451	511846-512237
1610	2205	ankyrin 2, brain	0.639	517928-518264
1673	2184	adducin 3 (gamma)	5.724	539781-540145
1682	2180	microtubule associated monoxygenase, calponin and LIM domain containing 1	6.737	542784-543124
1767	2154	programmed cell death 6 interacting protein	24.668	572196-572546
1820	2137	slingshot homolog 3 (Drosophila)	2.567	590404-590772

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
1831	2134	dystroglycan 1	3.205	594147-594505
1850	2128	nephronophthisis 4 (juvenile) homolog (human)	2.545	600625-600911
1886	2116	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3	4.242	612818-613159
1903	2112	nuclear distribution gene E-like homolog 1 (*A. nidulans*)	8.837	618380-618725
1910	2110	macrophage erythroblast attacher	48.23	620748-621108
1939	2098	leucine zipper, putative tumor suppressor 2	14.187	630655-630915
1940	2098	kinesin family member C3	6.785	630916-631256
2010	2075	myosin XVIIIA	1.283	654776-655088
2020	2072	vasodilator-stimulated phosphoprotein	13.006	658006-658374
2023	2072	zyxin	12.684	658946-659253
2033	2066	nucleoporin 85	17.448	662254-662568
2050	2061	engulfment and cell motility 2, ced-12 homolog (*C. elegans*)	7.176	668000-668354
2095	2045	CAP, adenylate cyclase-associated protein 1 (yeast)	88.915	683551-683847
2105	2042	CD2-associated protein	0.744	686855-687170
2124	2034	ras homolog gene family, member A	135.612	693012-693333
2136	2031	midline 2	0.659	697033-697389
2160	2024	lethal giant larvae homolog 1 (Drosophila)	2.293	705082-705399
2175	2018	dishevelled 2, dsh homolog (Drosophila)	3.722	710088-710457
2191	2013	ARP8 actin-related protein 8 homolog (*S. cerevisiae*)	8.289	715461-715836
2204	2010	actin filament associated protein 1	1.126	719890-720203
2205	2010	CDC42 effector protein (Rho GTPase binding) 1	4.544	720204-720579
2212	2008	thyroid hormone receptor interactor 10	30.196	722669-723012
2220	2005	tropomyosin 4	428.406	725519-725834
2232	2001	gene model 114	3.412	729587-729910
2235	2001	septin 7	3.112	730587-730976
2295	1984	microcephaly, primary autosomal recessive 1	0.629	751244-751582
2346	1973	calmodulin 3	14.014	768392-768693
2354	1970	protein phosphatase 1, regulatory subunit 9B	2.194	771093-771432
2375	1964	amyloid beta precursor protein (cytoplasmic tail) binding protein 2	13.369	778032-778283
2379	1963	protein regulator of cytokinesis 1	14.63	779205-779513
2387	1962	intraflagellar transport 80 homolog (*Chlamydomonas*)	0.991	782001-782399
2416	1954	kinesin family member C1	16.341	792040-792370
2430	1949	anillin, actin binding protein	2.848	796726-797054
2441	1946	CLIP associating protein 2	1.013	800461-800731
2466	1941	centrosomal protein 170	0.772	808772-809083
2479	1938	oligophrenin 1	2.039	813214-813607
2482	1938	leucine rich repeat containing 49	3.959	814326-814699
2506	1931	Mid1 interacting protein 1 (gastrulation specific G12-like (zebrafish))	129.96	822665-823028
2510	1930	Bardet-Biedl syndrome 4 (human)	5.356	824110-824394

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
2512	1930	formin homology 2 domain containing 1	2.963	824760-825066
2520	1929	drebrin-like	40.695	827385-827727
2543	1922	beclin 1, autophagy related	22.681	835365-835694
2546	1921	actin, gamma, cytoplasmic 1	284.261	836348-836704
2551	1919	coiled-coil and C2 domain containing 2A	0.604	838097-838446
2578	1912	hook homolog 2 (Drosophila)	4.1	847312-847598
2583	1910	inner centromere protein	4.499	848988-849386
2605	1907	protein serine kinase H1	2.142	856267-856572
2609	1905	Janus kinase 1	7.769	857488-857805
2621	1902	Rac GTPase-activating protein 1	19.316	861408-861766
2644	1897	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	46.955	869071-869380
2691	1887	growth arrest specific 2	2.282	885284-885579
2745	1872	mitotic arrest deficient 1-like 1	4.132	903571-903958
2764	1866	DDB1 and CUL4 associated factor 12	5.371	910380-910622
2775	1862	actin, beta	57.391	913994-914315
2805	1856	enabled homolog (Drosophila)	2.768	924287-924598
2816	1854	coronin, actin binding protein 1B	56.328	928073-928458
2841	1849	tubulin, gamma complex associated protein 3	3.057	936562-936886
2844	1847	large tumor suppressor	0.394	937654-937969
2880	1837	actin related protein 2/3 complex, subunit 5	60.269	949749-950130
2896	1832	centromere protein E	1.871	955437-955745
2943	1818	LIM and SH3 protein 1	13.57	971615-971919
3042	1782	sphingosine-1-phosphate phosphatase 1	3.922	1005199-1005578
3050	1780	centrosomal protein 68	0.822	1007927-1008310
3069	1775	centlein, centrosomal protein	0.588	1014347-1014609
3082	1772	pleckstrin homology domain containing, family H (with MyTH4 domain) member 3	3.954	1018621-1018991
3084	1771	myosin IXb	1.071	1019313-1019670
3104	1768	capping protein (actin filament) muscle Z-line, α 2	15.011	1026343-1026702
3147	1758	dynein cytoplasmic 2 heavy chain 1	0.317	1041205-1041524
3170	1752	PTK2 protein tyrosine kinase 2	5.096	1049366-1049709
3172	1752	FYVE, RhoGEF and PH domain containing 1	5.286	1050013-1050360
3199	1747	vimentin	514.871	1059326-1059717
3207	1744	ring finger protein 19A	1.513	1062111-1062496
3211	1742	phosphodiesterase 4D interacting prot (myomegalin)	1.285	1063460-1063768
3215	1742	c-abl oncogene 1, receptor tyrosine kinase	0.436	1064790-1065134
3223	1741	CDC42 effector prot (Rho GTPase binding) 3	6.317	1067503-1067844
3230	1739	destrin	50.913	1069789-1070099
3263	1730	tubulin-specific chaperone E	13.488	1080945-1081272
3306	1717	CLIP associating protein 1	0.948	1095379-1095748
3341	1708	sorbin and SH3 domain containing 3	7.794	1107024-1107409
3502	1669	microtubule-associated protein 6	3.649	1161307-1161624
3505	1668	katanin p60 (ATPase-containing) subunit A1	32.182	1162218-1162611
3541	1661	membrane protein, palmitoylated	15.267	1174530-1174867
3577	1650	cell division cycle 25 homolog B (*S. pombe*)	1.866	1186395-1186715

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
3583	1649	checkpoint kinase 1 homolog (*S. pombe*)	3.146	1188354-1188736
3590	1647	capping protein (actin filament) muscle Z-line, beta	60.716	1190654-1190998
3593	1647	serologically defined colon cancer antigen 8	3.621	1191627-1191981
3609	1642	tubulin, delta 1	13.501	1197043-1197421
3643	1635	metastasis suppressor 1	0.4	1208709-1209077
3692	1625	family with sequence similarity 82, member A2	4.761	1225295-1225616
3715	1620	tubulin, gamma 1	36.02	1233354-1233745
3720	1619	CDK5 regulatory subunit associated protein 2	1.713	1235062-1235355
3724	1618	catenin (cadherin associated protein), α-like 1	0.699	1236365-1236728
3774	1605	family with sequence similarity 110, member B	2.345	1253240-1253580
3781	1603	profilin 2	23.497	1255751-1256078
3796	1599	phosphatidylinositol transfer protein, membrane-associated 2	0.365	1260891-1261174
3846	1585	centrosomal protein 72	5.434	1277509-1277820
3850	1585	actin related protein 2/3 complex, subunit 1A	46.114	1278978-1279372
3898	1574	twinfilin, actin-binding protein, homolog 2 (*Drosophila*)	27.133	1295102-1295393
3901	1574	FYVE, RhoGEF and PH domain containing 6	0.595	1295966-1296243
3910	1572	cyclin B1	25.641	1298863-1299236
3933	1566	ARP10 actin-related protein 10 homolog (*S. cerevisiae*)	11.257	1306422-1306806
3946	1562	polo-like kinase 4 (Drosophila)	2.986	1310666-1311034
3949	1562	Ena-vasodilator stimulated phosphoprotein	3.874	1311646-1311940
4009	1547	ELMO domain containing 2	0.601	1331721-1332074
4014	1545	protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	82.162	1333415-1333732
4017	1545	Janus kinase 3	1.252	1334368-1334721
4036	1541	diaphanous homolog 1 (Drosophila)	1.436	1340818-1341199
4088	1525	ectodermal-neural cortex 1	2.166	1357871-1358264
4103	1522	HAUS augmin-like complex, subunit 4	20.991	1362890-1363204
4160	1510	fibronectin type 3 and SPRY domain-containing protein	2.066	1382212-1382607
4163	1510	glycophorin C	7.299	1383318-1383614
4176	1507	WASP family 1	1.25	1387619-1387949
4180	1507	mitofusin 2	4.551	1389006-1389340
4181	1507	protein Tyr phosphatase, non-receptor type 13	0.677	1389341-1389646
4199	1500	cytoskeleton associated protein 2	1.674	1395624-1396011
4202	1500	intraflagellar transport 57 homolog (*Chlamydomonas*)	4.102	1396618-1396929
4220	1496	centrosomal protein 57	2.62	1402802-1403129
4238	1493	nucleoporin 62	5.816	1408710-1409085
4239	1493	tripartite motif-containing 54	10.739	1409086-1409394
4251	1492	UBX domain protein 6	21.107	1412861-1413233
4257	1491	LIM domain and actin binding 1	0.916	1414950-1415263
4260	1489	TRAF3 interacting protein 1	1.646	1415915-1416305
4285	1483	dynactin 4	1.16	1424542-1424937

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
4370	1466	shroom family member 3	0.482	1452845-1453240
4386	1462	growth arrest specific 8	4.02	1458247-1458599
4408	1457	influenza virus NS1A binding protein	0.809	1465620-1465977
4457	1447	erythrocyte protein band 4.1	0.529	1481994-1482367
4484	1440	sarcoglycan, epsilon	14.957	1490848-1491203
4498	1437	slingshot homolog 1 (Drosophila)	1.043	1494976-1495267
4503	1435	ARP1 actin-related protein 1 homolog B, centractin beta (yeast)	3.69	1496661-1496992
4550	1426	PDZ and LIM domain 1 (elfin)	53.065	1512325-1512634
4552	1425	Rho GTPase activating protein 6	0.435	1512969-1513333
4564	1423	paxillin	2.436	1517047-1517389
4570	1422	coactosin-like 1 (*Dictyostelium*)	22.98	1519097-1519422
4604	1415	CAP-GLY domain containing linker protein 2	1.613	1530649-1531015
4612	1413	cysteine and glycine-rich protein 1	17.093	1533366-1533652
4616	1412	microtubule associated monoxygenase, calponin and LIM domain containing -like 1	0.37	1534571-1534894
4667	1399	family with sequence similarity 110, member A	4.673	1551090-1551435
4729	1387	regulator of chromosome condensation 2	9.39	1571986-1572324
4732	1386	sirtuin 2 (silent mating type information regulation 2, homolog) 2 (*S. cerevisiae*)	9.325	1573015-1573411
4775	1373	ecotropic viral integration site 5	1.536	1587335-1587660
4778	1373	tropomyosin 1, alpha	14.432	1588306-1588667
4811	1366	coiled-coil domain containing 99	1.214	1599899-1600288
4852	1357	syntrophin, basic 2	0.315	1614004-1614358
4869	1354	transforming growth factor beta 1 induced transcript 1	2.305	1619537-1619815
4892	1348	ADP-ribosylation factor-like 2 binding protein	13.977	1627434-1627798
4903	1347	tyrosine kinase 2	0.405	1631375-1631670
4907	1346	CDC42 small effector 2	1.468	1632810-1633100
4913	1345	ninein-like	0.788	1634774-1635172
4941	1339	catenin (cadherin associated protein), beta 1	0.495	1644372-1644747
4956	1336	ADP-ribosylation factor-like 6 interacting protein 5	32.187	1649516-1649856
4987	1326	actin related protein 2/3 complex, subunit 4	80.61	1660460-1660839
5010	1321	protein phosphatase 4, catalytic subunit	90.194	1668127-1668488
5024	1319	pre-B-cell leukemia transcription factor interacting protein 1	2.045	1672920-1673252
5066	1310	centrosomal protein 97	0.234	1687017-1687411
5084	1303	Sfi1 homolog, spindle assembly associated (yeast)	0.686	1693415-1693807
5091	1302	proline-serine-threonine phosphatase-interacting protein 1	12.697	1695903-1696267
5104	1300	nuclear distribution gene C homolog (*Aspergillus*)	102.462	1699971-1700369
5108	1299	actin, alpha 2, smooth muscle, aorta	3.284	1701331-1701660
5171	1285	fuzzy homolog (*Drosophila*)	6.367	1722855-1723213
5186	1283	neurofibromatosis 2	0.719	1728086-1728462

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
5198	1282	centrosomal protein 120	2.153	1732348-1732733
5208	1279	nucleolar and spindle associated protein 1	2.386	1735724-1736042
5253	1270	dynein cytoplasmic 2 light intermediate chain 1	9.834	1750866-1751258
5274	1266	protein Tyr phosphatase, non-receptor type 21	0.472	1758166-1758517
5370	1243	HAUS augmin-like complex, subunit 7	59.234	1791926-1792280
5402	1237	myristoylated Ala rich protein kinase C substrate	3.148	1803092-1803482
5448	1226	RIKEN cDNA F630043A04 gene	2.085	1819143-1819511
5503	1212	stomatin (Epb7.2)-like 2	17.579	1838816-1839204
5654	1177	SMEK homolog 1, suppressor of mek1 (*Dictyostelium*)	0.871	1891648-1892008
5678	1171	actin related protein 2/3 complex, subunit 2	15.986	1900301-1900676
5698	1166	aurora kinase A	16.855	1907469-1907831
5701	1166	telomeric repeat binding factor 1	2.789	1908582-1908967
5716	1164	cofilin 1, non-muscle	107.826	1914036-1914356
5771	1151	TNFRSF1A-associated via death domain	11.061	1934043-1934332
5773	1151	protein tyrosine phosphatase 4a1	0.279	1934685-1935079
5774	1151	centrobin, centrosomal BRCA2 interacting prot	1.021	1935080-1935410
5785	1148	dynactin 5	5.2	1939165-1939526
5791	1147	microtubule-associated protein 1S	6.328	1941401-1941793
5864	1133	kinesin family member 18A	1.465	1967839-1968177
5882	1129	calmodulin 2	263.807	1974401-1974748
5943	1115	PDZ and LIM domain 7	17.513	1996784-1997110
5944	1115	serine/threonine kinase 38 like	0.267	1997111-1997478
6060	1089	cell division cycle associated 8	7.204	2039068-2039461
6067	1087	sorbin and SH3 domain containing 1	1.201	2041684-2042038
6068	1087	tropomodulin 1	0.607	2042039-2042410
6084	1083	bridging integrator 3	4.997	2047682-2048036
6099	1080	actin related protein 2/3 complex, subunit 5-like	28.479	2053256-2053599
6141	1070	aurora kinase B	6.311	2068620-2068994
6179	1062	CDC42 effector prot (Rho GTPase binding) 4	0.921	2082109-2082464
6258	1043	gene trap ROSA b-geo 22	28.608	2111007-2111388
6278	1038	tubulin tyrosine ligase-like family, member 5	0.196	2117720-2118059
6291	1035	formin binding protein 1	0.384	2122377-2122769
6318	1030	centrin 2	4.69	2131765-2132103
6338	1027	FERM domain containing 8	1.308	2139056-2139394
6354	1023	centrosomal protein 70	0.548	2144764-2145134
6460	997	ELMO/CED-12 domain containing 3	3.427	2182595-2182941
6467	995	Leber congenital amaurosis 5 (human)	0.247	2185145-2185497
6500	987	phosphodiesterase 4D, cAMP specific	0.47	2196755-2197145
6516	984	dystrophin, muscular dystrophy	0.119	2202415-2202812
6526	983	huntingtin interacting protein 1 related	0.441	2205988-2206301
6534	981	discs, large (*Drosophila*) homolog-associated protein 5	3.759	2208846-2209155
6553	976	RIKEN cDNA 2810433K01 gene	2.289	2215581-2215976
6581	971	HAUS augmin-like complex, subunit 1	5.105	2225453-2225779
6599	969	epsilon-tubulin 1	0.387	2232061-2232442
6626	964	centrosomal protein 290	0.132	2241296-2241579

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
6651	958	family with sequence similarity 82, member B	0.84	2250023-2250412
6713	943	centrosomal protein 250	0.433	2271720-2272085
6735	938	tropomyosin 3, gamma	2.656	2279590-2279877
6766	934	family with sequence similarity 82, member A1	0.931	2290005-2290384
6782	929	nuclear distribution gene E homolog 1 (*A. nidulans*)	7.884	2295836-2296146
6806	924	purine-nucleoside phosphorylase 1	10.99	2304356-2304474
6822	920	tropomyosin 2, beta	4.355	2309610-2309944
6832	917	RIKEN cDNA 2700060E02 gene	9.838	2313045-2313404
6834	917	v-abl Abelson MLV oncogene homolog 2 (arg, Abelson-related gene)	0.618	2313687-2314064
6882	908	aurora kinase C	14.218	2329723-2330035
6898	903	spindle assembly 6 homolog (*C. elegans*)	0.224	2334515-2334801
6953	892	nucleotide binding protein 2	2.113	2352711-2353003
6991	884	dynactin 6	9.052	2365341-2365718
7003	880	neural precursor cell expressed, developmentally down-regulated gene 1	0.33	2369333-2369684
7007	879	diacylglycerol kinase, theta	0.274	2370707-2371011
7040	872	CDC42 small effector 1	2.926	2382000-2382296
7096	860	slingshot homolog 2 (Drosophila)	0.517	2400517-2400895
7125	853	profilin 1	11.177	2410108-2410492
7146	848	RIKEN cDNA 2410017P07 gene	1.326	2417114-2417508
7175	841	baculoviral IAP repeat-containing 5	0.966	2426437-2426713
7208	836	leucine rich repeat and coiled-coil domain containing 1	0.248	2437517-2437910
7236	829	DNA segment, Chr 15, Wayne State University 169, expressed	0.268	2446945-2447339
7249	826	RIKEN cDNA 4922501C03 gene	0.438	2451461-2451761
7291	813	HAUS augmin-like complex, subunit 2	3.496	2464662-2464966
7323	806	dynein light chain LC8-type 1	4.71	2475322-2475644
7330	803	MAD2L1 binding protein	3.685	2477746-2478077
7365	796	cDNA sequence BC023882	0.603	2489301-2489640
7368	795	RIKEN cDNA 6720456B07 gene	3.581	2490278-2490569
7378	793	tubulin folding cofactor B	8.324	2493603-2493993
7379	793	ankyrin repeat, family A (RFXANK-like), 2	0.45	2493994-2494317
7426	782	engulfment and cell motility 1, ced-12 homolog (C. elegans)	0.528	2509515-2509793
7472	769	palladin, cytoskeletal associated protein	0.53	2524218-2524585
7486	767	melanophilin	0.258	2529148-2529507
7488	766	WAS protein family, member 2	0.188	2529775-2530138
7552	749	mitogen-activated protein kinase 1 interacting protein 1	1.45	2550744-2551112
7588	742	vinculin	0.23	2562670-2562963
7654	724	dynamin binding protein	0.201	2583481-2583787
7756	700	Rap1 interacting factor 1 homolog (yeast)	0.083	2618117-2618471
7794	691	giant axonal neuropathy	0.587	2631132-2631429
7826	683	Mediterranean fever	0.311	2642002-2642302
7889	664	ubiquitously expressed transcript	1.147	2662979-2663371

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
7899	659	ADP-ribosylation factor-like 3	2.999	2666529-2666853
7902	658	intraflagellar transport 20 homolog (*Chlamydomonas*)	4.021	2667596-2667912
7904	657	gamma-aminobutyric acid receptor associated protein	2.814	2668257-2668616
7937	649	trichoplein, keratin filament binding	0.484	2679418-2679802
7975	639	tubulin, beta 3	2.093	2692217-2692262
7978	639	BCL2 modifying factor	0.17	2692923-2693205
8020	627	Rho GTPase-activating protein	0.115	2706942-2707263
8031	622	B9 protein domain 2	12.725	2710630-2711005
8052	613	ARP6 actin-related protein 6 homolog (yeast)	0.57	2717297-2717635
8079	606	ADP-ribosylation factor-like 2	4.584	2726337-2726723
8108	598	thymosin, beta 4, X chromosome	24.043	2734875-2735269
8123	594	citron	0.131	2740025-2740319
8147	588	ankyrin 1, erythroid	0.072	2747574-2747883
8176	583	dynactin 3	1.37	2756466-2756744
8301	547	UBX domain protein 11	0.465	2797362-2797669
8335	539	par-3 (partitioning defective 3) homolog (*C. elegans*)	0.154	2808716-2809107
8358	532	myomesin 1	0.149	2816775-2817100
8401	519	erythrocyte protein band 4.1-like 5	0.187	2831535-2831924
8402	519	ciliary rootlet coiled-coil, rootletin	0.102	2831925-2832268
8451	506	catenin (cadherin associated protein), alpha 2	0.122	2848720-2849102
8516	492	filamin C, gamma	0.062	2869737-2870106
8610	475	erythrocyte protein band 4.1-like 4a	0.123	2900673-2901022
8638	469	formin 1	0.04	2910464-2910758
8660	465	kinesin family member 2A	0.256	2917368-2917680
8677	463	pericentriolar material 1	0.077	2922701-2923049
8680	462	4HAUS augmin-like complex, subunit 8	1.099	2923769-2924049
8771	446	calcium binding and coiled-coil domain 2	1.247	2952270-2952623
8808	439	centrin 3	1.05	2963461-2963764
8846	430	thymosin, beta 10	5.35	2975022-2975322
8852	429	actin related protein M1	0.429	2976880-2977149
8881	421	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	1.474	2985485-2985777
8907	416	formin binding protein 1-like	0.166	2992924-2993272
8931	410	kinesin family member 1B	0.059	2999526-2999824
8932	409	SAC3 domain containing 1	0.452	2999825-3000109
9057	376	FYVE, RhoGEF and PH domain containing 4	0.233	3032941-3033212
9073	372	spectrin alpha 1	0.087	3037049-3037302
9085	369	glucocorticoid receptor DNA binding factor 1	0.084	3040212-3040461
9137	356	FERM, RhoGEF and pleckstrin domain protein 2	0.091	3053199-3053462
9147	353	tetratricopeptide repeat domain 8	0.154	3055412-3055707
9161	349	RAB GTPase activating protein 1	0.083	3058415-3058689
9222	337	inversin	0.261	3071675-3071916
9322	307	Ras and Rab interactor 1	0.073	3093895-3094135

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
9361	297	tubulin cofactor A	1.057	3102113-3102283
9367	295	Fc receptor, IgG, low affinity IIb	0.189	3103313-3103351
9473	256	engulfment and cell motility 3, ced-12 homolog (*C. elegans*)	0.119	3122400-3122588
9486	251	ninein	0.027	3124390-3124544
9587	211	tensin 4	0.089	3138556-3138633
9617	200	tropomodulin 2	0.02	3141512-3141584
9677	173	radial spoke head 9 homolog (*Chlamydomonas*)	0.187	3146825-3146949
9701	161	Rho family GTPase 1	0.152	3148560-3148596
9750	135	actin-binding LIM protein 2	0.039	3151347-3151451
9753	132	actin, alpha 1, skeletal muscle	0.093	3151503-3151520
3157155	394	adducin 2 (beta)	0.13	3183484-3183583
3157158	478	envoplakin	0.075	3268105-3268204
3157230	310	LIM domain binding 3	0.065	3227217-3227316
3157293	264	formin homology 2 domain containing 3	0.259	3166084-3166183
3157294	353	family with sequence similarity 33, member A	0.31	3266905-3267004
3157319	402	HAUS augmin-like complex, subunit 5	0.183	3232617-3232716
3157357	735	PDZ and LIM domain 2	1.175	3190471-3190570
3157369	1826	RIKEN cDNA 2310014H01 gene	2.285	3250317-3250416
3157379	252	P140 gene	0.045	3193771-3193870
3157397	305	receptor-associated protein of the synapse	0.188	3284249-3284348
3157398	818	protein Tyr phosphatase, non-receptor type 4	0.196	3205097-3205196
3157429	809	sarcoglycan, α (dystrophin-associated glycoprotein)	2.184	3188371-3188470
3157432	395	myosin VIIA	0.054	3266305-3266404
3157485	2014	ubiquitin protein ligase E3 component n-recognin 4	0.639	3209658-3209757
3157505	644	crystallin, alpha B	0.99	3280749-3280848
3157523	803	centromere protein V	3.696	3267205-3267304
3157536	307	FYVE, RhoGEF & PH domain containing 3	0.099	3205697-3205796
3157684	329	doublecortin-like kinase 2	0.081	3170684-3170783
3157726	995	desmin	1.2	3159421-3159520
3157766	167	radial spoke head 4 homolog A (*Chlamydomonas*)	0.117	3201497-3201596
3157768	326	myosin regulatory light chain interacting protein	0.109	3182784-3182883
3157794	2378	tribbles homolog 2 (Drosophila)	1.6	3204997-3205096
3157801	379	tropomodulin 4	0.308	3221891-3221990
3157821	390	tubulin tyrosine ligase-like family, member 11	0.203	3159121-3159220
3157887	312	dynamin 3	0.068	3263705-3263804
3157957	150	erythrocyte protein band 4.2	0.043	3187271-3187370
3157963	1734	symplekin	1.095	3217191-3217290
3158016	395	FERM domain containing 5	0.095	3184871-3184970
3158035	383	septin 1	0.279	3259205-3259304
3158061	191	protein Tyr phosphatase, non-receptor type 3	0.03	3234617-3234716
3158125	849	actin related protein 2/3 complex, subunit 3	23.491	3268805-3268904
3158127	427	tubulin tyrosine ligase-like family, member 3	0.294	3262005-3262104
3158142	421	myosin binding protein C, slow-type	0.554	3202897-3202996

SEQ ID NO:	consL	Description	Avg Covg	siRNA SEQ ID NOs:
3158154	347	microtubule-associated protein tau	0.08	3245217-3245316
3158176	705	IAP promoted placental gene	0.395	3167984-3168083
3158226	185	tripartite motif-containing 36	0.119	3176384-3176483
3158232	888	Wiskott-Aldrich syndrome homolog (human)	0.603	3278849-3278948
3158242	3612	SMEK homolog 2, suppressor of mek1 (*Dictyostelium*)	9.065	3210258-3210357
3158328	1531	RAB11 family interacting protein 3 (class II)	0.975	3221991-3222090
3158344	550	cortactin binding protein 2	0.187	3165384-3165483
3158346	482	NA	0.075	3269396-3269495
3158380	464	tubulin Tyr ligase-like family, member 6	0.277	3185771-3185870
3158411	1049	erythrocyte protein band 4.9	0.588	3254017-3254116

CLAIMS

1. A method for producing an immunogenic agent in a large scale host cell culture, comprising:

   (a) contacting a host cell in a large scale host cell culture with at least a first RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell,

   (b) maintaining the host cell culture for a time sufficient to modulate expression of the at least one first target gene, wherein the modulation of expression improves production of an immunogenic agent in the host cell;

   (c) isolating the immunogenic agent from the host cell;

   wherein the large scale host cell culture is at least 1 Liter in size, and wherein the host cell is contacted with at least a first RNA effector molecule by addition of the RNA effector molecule to a culture medium of the large scale host cell culture such that the target gene expression is transiently inhibited.

2. A method for producing an immunogenic agent in a large scale host cell culture, comprising:

   (a) contacting a host cell in a large scale host cell culture with at least a first RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell,

   (b) maintaining the host cell culture for a time sufficient to modulate expression of the at least one first target gene, wherein the modulation of expression improves production of an immunogenic agent in the host cell;

   (c) isolating the immunogenic agent from the host cell;

   wherein the host cell is contacted with at least a first RNA effector molecule by addition of the RNA effector molecule to a culture medium of the large scale host cell culture multiple times throughout production of the immunogenic agent such that the target gene expression is transiently inhibited.

3. The method of any of claims 1 to 2, wherein the host cell in the large scale host cell culture is contacted with a plurality of RNA effector molecules, wherein the plurality of RNA effector molecules modulate expression of at least one target gene, at least two target genes, or a plurality of target genes.

4. A method for production of an immunogenic agent in a cell, the method comprising:

(a) contacting a host cell with a plurality of RNA effector molecules, wherein the two or more RNA effector molecules modulate expression of a plurality of target genes;

(b) maintaining the cell for a time sufficient to modulate expression of the plurality of target genes, wherein the modulation of expression improves production of the immunogenic agent in the cell; and

(c) isolating the immunogenic agent from the cell,

wherein the plurality of target genes comprises at least Bax, Bak, and LDH.

5. The method of claim 4, wherein the host cell is contacted with the plurality of RNA effector molecules by addition of the RNA effector molecule to a culture medium of the large scale host cell culture such that the target gene expression is transiently inhibited.

6. The method of any of claims 1 to 5, wherein the RNA effector molecule, or plurality of RNA effector molecules, comprises a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least part of a target gene, and wherein said region of complementarity is 10-30 nucleotides in length.

7. The method of any of claims 1 to 6, wherein the contacting step is performed by continuous infusion of the RNA effector molecule, or plurality of RNA effector molecules, into the culture medium used for maintaining the host cell culture to produce the immunogenic agent.

8. The method of any of claims 1 to 7, wherein the modulation of expression is inhibition of expression, and wherein the inhibition is a partial inhibition.

9. The method of claim 7, wherein the partial inhibition is no greater than a percent inhibition selected from the group consisting of: 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, and 85%.

10. The method of any of claims 1 to 6 or 8-9, wherein the contacting step is repeated at least once.

11. The method of any of claims 1 to 6 or 8-9, wherein the contacting step is repeated multiple times at a frequency selected from the group consisting of: 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 84 hr, 96 hr, and 108 hr.

12. The method of any of claims 1 to 11, wherein the modulation of expression is inhibition of expression and wherein the contacting step is repeated multiple times, or continuously infused, to maintain an average percent inhibition of at least 50% for the target gene(s) throughout the production of the immunogenic agent.

13. The method of claim 12, wherein the average percent inhibition is selected from the group consisting of at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100%.

14. The method of any of claims 1 to 13, wherein the RNA effector molecule is contacted at a concentration of less than 100 nM.

15. The method of any of claims 1 to 14, wherein the RNA effector molecule is contacted at a concentration of less than 20 nM.

16. The method of any of claims 1 to 15, wherein said contacting a host cell in a large scale host cell culture with a RNA effector molecule is done at least 6 hr, at least 12 hr, at least 18 hr, at least 36 hr, at least 48 hr, at least 60 hr, at least 72 hr, at least 96 hr, or at least 120 hr, or at least 1 week, before isolation of the immunogenic agent or prior to harvesting the supernatant.

17. The method of any of claims 1 to 16, wherein the RNA effector molecule is composition formulated in a lipid formulation.

18. The method of any of claims 1 to 17, wherein the RNA effector molecule is a composition formulated in a non-lipid formulation.

19. The method of any of claims 1 to 18, wherein the RNA effector molecule is not shRNA.

20. The method of any of claims 1 to 19, wherein the RNA effector molecule is siRNA.

21. The method of any of claims 1 to 20, wherein the RNA effector molecule is chemically modified.

22. The method of any of claims 1 to 21, wherein the RNA effector molecule is not chemically modified.

23. The method of any of claims 1 to 22, further comprising monitoring at least one measurable parameter selected from the group consisting of cell density, medium pH, oxygen levels, glucose levels, lactic acid levels, temperature, and protein production.

24. The method of any of claims 2 to 23, wherein each of the plurality of different RNA effector molecules is added simultaneously or at different times.

25. The method of any of claims 2 to 23, wherein each of the plurality of different RNA effector molecules is added at the same or different concentrations.

26. The method of any of claims 2 to 6 or 8 to 25, wherein the plurality of different RNA effector molecules is added at the same or different frequencies.

27. The method of any of claims 1 to 26, further comprising contacting the cell with a second agent.

28. The method of claim 27, wherein the second agent is selected from the group consisting of: an antibody, a growth factor, an apoptosis inhibitor, a kinase inhibitor, a phosphatase inhibitor, a protease inhibitor, and a histone demethylating agent.

29. The method of claim 28, wherein the kinase inhibitor is selected from the group consisting of: a MAP kinase inhibitor, a CDK inhibitor, and K252a.

30. The method of claim 28, wherein the phosphatase inhibitor is selected from the group consisting of: sodium vanadate and okadaic acid.

31. The method of claim 28, wherein the histone demethylating agent is 5-azacytidine.

32. The method of any of claims 1 to 31, wherein the immunogenic agent is a polypeptide.

33. The method of any of claims 1 to 31, wherein the immunogenic agent is a virus.

34. The method of claim 33, wherein the virus is PCV.

35. The method of any of claims 1 to 34, wherein the cell is contacted with the RNA effector molecule at a phase of cell growth selected from the group consisting of: stationary phase, early log phase, mid-log phase, late-log phase, lag phase, and death phase.

36. The method of any of claims 1 to 35, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, comprises a duplex region.

37. The method of any of claims 1 to 36, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is 15-30 nucleotides in length.

38. The method of any of claims 1 to 37, the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is 17-28 nucleotides in length.

39. The method of any one of claims 1 to 38, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, comprises at least one modified nucleotide.

40. The method of any of claims 1 to 39, wherein the cell is a plant cell, a fungal cell, or an animal cell.

41. The method of any of claims 1 to 40, wherein the cell is a mammalian cell.

42. The method of claim 41, wherein the mammalian cell is a human cell.

43. The method of claim 42, wherein the human cell is an adherent cell selected from the group consisting of: SH-SY5Y cells, IMR32 cells, LAN5 cells, HeLa cells, MCFlOA cells, 293T cells, and SK-BR3 cells.

44. The method of claim 42, wherein the human cell is a primary cell selected from the group consisting of: HuVEC cells, HuASMC cells, HKB-Il cells, and hMSC cells.

45. The method of claim 42, wherein the human cell is selected from the group consisting of: U293 cells, HEK 293 cells, PERC6® cells, Jurkat cells, HT-29 cells, LNCap.FGC cells, A549 cells, MDA MB453 cells, HepG2 cells, THP-I cells, MCF7 cells, BxPC-3 cells, Capan-1 cells, DU145 cells, and PC-3 cells.

46. The method of claim 41, wherein the mammalian cell is a rodent cell selected from the group consisting of: BHK21 cells, BHK(TK$^-$) cells, NS0 cells, Sp2/0 cells, EL4 cells, CHO cells, CHO cell derivatives, NIH/3T3 cells, 3T3-Ll cells, ES-D3 cells, H9c2 cells, C2C12 cells, Madin Darby canine kidney (MDCK) cells and miMCD 3 cells.

47. The method of claim 46, wherein the CHO cell derivative is selected from the group consisting of: CHO-Kl cells, CHO-DUKX, CHO-DUKX B1, and CHO-DG44 cells.

48. The method of claim 42, wherein the cell is selected from the group consisting of: PERC6 cells, HT-29 cells, LNCaP-FGC cells A549 cells, MDA MB453 cells, HepG2 cells, THP-1 cells, miMCD-3 cells, HEK 293 cells, HeLaS3 cells, MCF7 cells, Cos-7 cells, BxPC-3 cells, DU145 cells, Jurkat cells, PC-3 cells, and Capan-1 cells,

49. The method of claim 41, wherein the cell is a rodent cell selected from the group consisting of: BHK21, BHK(TK$^-$), NS0 cells, Sp2/0 cells, U293 cells, EL4 cells, CHO cells, and CHO cell derivatives.

50. The method of any of claims 1 to 49, wherein the cell further comprises a genetic construct encoding the immunogenic agent.

51. The method of any of claims 1 to 50, wherein the cell further comprises a genetic construct encoding a viral receptor.

52. The method of any of claims 1 to 51, wherein the target gene encodes a protein that affects protein glycosylation.

53. The method of any of claims 1 to 52, wherein the target gene encodes the immunogenic agent.

54. The method of any of claims 1 to 53, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is added at a concentration selected from the group consisting of 0.1 nM, 0.5 nM, 0.75 nM, 1nM, 2 nM, 5 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 75 nM, and 100 nM.

55. The method of any of claims 1 to 53, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is added at an amount of 50 molecules per cell, 100 molecules/cell, 200 molecules/cell, 300 molecules/cell, 400 molecules/cell, 500 molecules/ cell, 600 molecules/cell, 700 molecules/ cell, 800 molecules/cell, 900 molecules/cell, 1000 molecules/cell, 2000 molecules/cell, or 5000 molecules/cell.

56. The method of any of claims 1 to 53, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is added at a concentration selected from the group consisting of: 0.01 fmol/10^6 cells, 0.1 fmol/10^6 cells, 0.5 fmol/10^6 cells, 0.75 fmol/10^6 cells, 1 fmol/10^6 cells, 2 fmol/10^6 cells, 5 fmol/10^6 cells, 10 fmol/10^6 cells, 20 fmol/10^6 cells, 30 fmol/10^6 cells, 40 fmol/10^6 cells, 50 fmol/10^6 cells, 60 fmol/10^6 cells, 100 fmol/10^6 cells, 200 fmol/10^6 cells, 300 fmol/10^6 cells, 400 fmol/10^6 cells, 500 fmol/10^6 cells, 700 fmol/10^6 cells, 800 fmol/10^6 cells, 900 fmol/10^6 cells, and 1 pmol/10^6 cells.

57. The method of any of claims 1-56, wherein the at least first RNA effector molecule, or at least one of the plurality of RNA effector molecules, is selected from the group consisting of siRNA, miRNA, dsRNA, saRNA, shRNA, piRNA, tkRNAi, eiRNA, pdRNA, a gapmer, an antagomir, a ribozyme, and any combination thereof.

58. The method of any of claims 1 to 57, wherein the method further comprises contacting the cell with at least one additional RNA effector molecule, or agent, that modulates a

cellular process selected from the group consisting of: carbon metabolism and transport, apoptosis, RNAi uptake and/or efficiency, reactive oxygen species production, control of cell cycle, protein folding, protein pyroglutamation, protein deamidation, protein glycosylation, disulfide bond formation, protein secretion, gene amplification, viral replication, viral infection, viral particle release, control of cellular pH, and protein production.

59. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene, is selected from the group consisting of: GLUT1, GLUT2, GLUT3, GLUT4, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN), and lactate dehydrogenase (LDH), and wherein the modulation of expression improves production of a immunogenic agent in the cell by modulating carbon metabolism or transport in the cell.

60. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is lactate dehydrogenase (LDH) and the RNA effector molecule comprises a sequence selected from SEQ ID NOs:3152540-3152603.

61. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene selected from the group consisting of: Bcl-G, Bax, Bak, Bok, Bad, Bid, Bik, Blk, Hrk, BNIP3, PUMA, NOXA, BimL, Bcl-2, Bcl-xL, Bcl-B, Bcl-w, Boo, Mcl-1, CASP2, CASP3, CASP6, CASP7, CASP8, CASP9, and CASP10; and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating apoptosis of the cell.

62. The method of claim any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is Bak and the RNA effector molecule comprises a sequence selected from SEQ ID NOs:3152412-3152475.

63. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is Bax and the RNA effector molecule comprises a sequence selected from SEQ ID NOs:3152476-3152539.

64. The method of claim 16 or 17, wherein the RNA effector molecule significantly decreases the fraction of cells that enter early apoptosis.

65. The method of claim 3, wherein the plurality of target genes are at least Bax and Bak.

66. The method of claim 3, wherein the plurality of target genes are at least Bax, Bac, and LDH.

67. The method of any of claims 4, 5, 65, or 66, wherein the RNA effector molecule, a portion of which is complementary to Bax comprises a sequence selected from SEQ ID NOs:3152476-3152539, wherein the RNA effector molecule, a portion of which is complementary to Bak, comprises a sequence selected fromSEQ ID NOs:3152412-3152475.

68. The method of claim 4 or 66, wherein the RNA effector molecule, a portion of which is complementary to LDH, comprises a sequence selected from SEQ ID NOs:3152540-3152603

69. The method of any of claims 1 to 3, or 6 to 58, wherein the expression of at least two target genes is modulated and the at least two target genes are selected from the group consisting of: Bcl-G, Bax, Bak, Bok, Bad, Bid, Bik, Blk, Hrk, BNIP3, PUMA, NOXA, and BimL .

70. The method of claim any of claims 1 to 3, 6 to 58, further comprising contacting the cell with a RNA effector molecule comprising a sequence complementary to lactate dehydrogenase (LDH).

71. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene selected from the group consisting of: Ago1, Ago2, Ago3, Ago4, HIWI1, HIWI2, HIWI3, HILI, interferon receptor, ApoE, Eri1 and mannose/GalNAc-receptor, and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating RNAi uptake and/or efficacy in the cell.

72. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of NAD(p)H oxidase, peroxidase, constitutive neuronal nitric oxide synthase (cnNOS), myeloperoxidase (MPO), xanthine oxidase (XO), 15-lipoxygenase-1, NADPH cytochrome c2 reductase, NAPH cytochrome c reductase, NADH cytochrome b5 reductase, and cytochrome P4502E1, and wherein the modulation of expression improves production of the immunogenic agent in the cell by inhibiting production of reactive oxygen species in the cell.

73. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: MuLV protein, MVM protein, Reo-3 protein, PRV protein, and vesivirus protein; and wherein the modulation of expression improves production of the immunogenic agent in the cell by inhibiting viral infection of the cell.

74. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is xylose transferase.

75. The method of claim 73, wherein the at least one target gene is a vesivirus protein and the at least one RNA effector molecule comprises at least one strand that comprises at least 16 contiguous nucleotides of an oligonucleotide having a sequence selected from SEQ ID NOs:3152604-3152713.

76. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: CCNA1, CCNA2, CCNB1, CCNB2, CCNB3, CCND1, CCND2, CCND3, CCNE1, CCNE2, cyclin B, cyclin D, cyclin E, CDK2, CDK4, P10, P21, P27, p53, P57, p16INK4a, P14ARF, and CDK4, and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating the cell cycle of the cell.

77. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: IRE1, PERK, ATF4, ATF6, eIF2alpha, GRP78, GRP94, Bip, Hsp40, HSP47, HSP60, Hsp70, HSP90, HSP100, protein disulfide isomerase, peptidyl prolyl isomerase, calreticulin, calnexin, Erp57, and BAG-1; and wherein the modulation of expression improves production of the protein in the cell by enhancing folding of the protein.

78. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is a methionine sulfoxide reductase gene in the host cell, and wherein the modulation of expression improves production of the protein in the cell by inhibiting modification of the protein by methionine oxidation.

79. The method of any of claims 1 to 3, or 6 to 58, wherein the target gene is a glutaminyl cyclase gene in the host cell, and wherein the modulation of expression improves production of the protein in the cell by inhibiting modification of the protein by pyroglutamation.

80. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: asparagine deamidase and glutamine deamidase; and wherein the modulation of expression improves production of the protein in the cell by inhibiting modification of the protein by deamidation.

81. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of dolichyl-diphosphooligosaccharide-protein

glycosyltransferase, UDP glycosyltransferase, UDP-Gal:βGlcNAcβ1,4-galactosyltransferase, UDP-galactose-ceramide galactosyltransferase, fucosyltransferase, protein O-fucosyltransferase, N-acetylgalactosaminytransferase T-4, O-GlcNAc transferase, oligosaccharyl transferase, O-linked N-acetylglucosamine transferase, α-galactosidase, and β-galactosidase; and wherein the modulation of expression improves production of the protein in the cell by modulating glycosylation of the protein.

82. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of protein disulfide isomerase and sulfhydryl oxidase; and wherein the modulation of expression improves production of the protein in the cell by modulating disulfide bond formation in the protein.

83. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of gamma-secretase, p115, a signal recognition particle (SRP) protein, secretin, and a kinase; and wherein the modulation of expression improves production of the protein in the cell by modulating secretion of the protein.

84. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is a dehydrofolate reductase gene in the host cell, wherein the modulation of expression improves production of the protein in the cell by enhancing gene amplification in the cell.

85. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is a gene of a virus or a target gene of a cell, thereby producing an immunogenic agent from a host cell having a reduced viral load.

86. The method of claim 85, wherein said virus is selected from the group consisting of: vesivirus, MMV, MuLV, PRV, and Reo-3.

87. The method of claim 85, wherein said at least one target gene encodes a viral protein.

88. The method of claim 85, wherein said at least one target gene encodes a non-viral protein.

89. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: pro-oxidant enzymes, BIK, BAD, BIM, HRK, BCLG, HR, NOXA, PUMA, BOK, BOO, BCLB, CASP2, CASP3, CASP6, CASP7, CASP8, CASP9, CASP10, BAX, BAK, BCL2, p53, APAFI, and HSP70; and wherein the modulation of expression improves production of the immunogenic agent in the cell by enhancing the viability of the cell.

90. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: CCNA1, CCNA2, CCNB1, CCNB2, CCNB3, CCND1, CCND2, CCND3, CCNE1, CCNE2, cyclin B, cyclin D, cyclin E, CDK2, CDK4, P10, P21, P27, p53, P57, p16INK4a, P14ARF, CDK4, Bcl-G, Bax, Bak, Bok, Bad, Bid, Bik, Blk, Hrk, BNIP3, PUMA, NOXA, BimL, Bcl-2, Bcl-xL, Bcl-B, Bcl-w, Boo, Mcl-1, A1, CASP2, CASP3, CASP6, CASP7, CASP8, CASP9, CASP10, GLUT1, GLUT2, GLUT3, GLUT4, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN), and lactate dehydrogenase (LDH); and wherein the modulation of expression improves production of the immunogenic agent in the cell by enhancing the specific productivity of the cell.

91. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: GLUT1, GLUT2, GLUT3, GLUT4, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN), lactate dehydrogenase (LDH), CCNA1, CCNA2, CCNB1, CCNB2, CCNB3, CCND1, CCND2, CCND3, CCNE1, CCNE2, cyclin B, cyclin D, cyclin E, CDK2, CDK4, P10, P21, P27, p53, P57, p16INK4a, P14ARF, and CDK4; wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating nutrient requirements of the cell.

92. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: lactate dehydrogenase and lysosomal V-type ATPase; and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating the pH of the cell.

93. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of: cytoplasmic actin capping protein (CapZ), Ezrin (VIL2), Laminin A, and Cofilin (CFL1); and wherein the modulation of gene expression improves production of the immunogenic agent in the cell by modulating actin dynamics of the cell

94. The method of claim 93, wherein at least one RNA effector molecule inhibits expression of the target gene Cofilin.

95. The method of claim 93, wherein at least one RNA effector molecule increases expression of a target gene selected from the group consisting of: cytoplasmic actin capping protein (CapZ), Ezrin (VIL2), and Laminin A.

96. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is a gene of a host cell latent virus, an adventitious virus, a host cell endogenous retrovirus, or a host cell binding-ligand of such virus.

97. The method of claim 96, wherein the target gene is a gene of an endogenous retrovirus (ERV) selected from HERV-K, pt01-Chr10r-17119458, pt01-Chr5-53871501, BaEV, GaLV, HERV-T, ERV-3, HERV-E, HERV-ADP, HERV-I, MER4like, HERV-FRD, HERV-W, HERVH-RTVLH2, HERVH-RGH2, HERV-Hconsensus, HERV-Fc1, hg15-chr3-152465283, HERVL66, HSRV, HFV, HERV-S, HERV-L, HERVL40, HERVL74, HTLV-1, HTLV-2, HIV-1, HIV-2, MPMV, MMTV, HML1, HML2, HML3, HML4, HML7, HML8, HML5, HML10, HML6, HML9, MMTV, FLV, PERV, BLV, EIAV, JSRV, gg01-chr7-7163462, gg01-chrU-52190725, gg01-Chr4-48130894, ALV, gg01-chr1-15168845, gg01-chr4-77338201, gg01-ChrU-163504869, gg01-chr7-5733782, Python-molurus, WDSV, SnRV, Xen1, Gypsy, and Ty1.

98. The method of claim 96, wherein the target gene is a gene of a latent virus selected from the group consisting of C serotype adenovirus, avian adenovirus, avian adenovirus-associated virus, human herpesvirus-4 (EBV), and circovirus.

99. The method of claim 98, wherein the latent virus is a circovirus, and the target gene is the *rep* gene of porcine circovirus type 1 (PCV1) or circovirus type 2 (PCV2).

100. The method of claim 98, wherein the latent virus is EBV and the target gene is latent membrane protein (LMP)-2A.

101. The method of claim 96, wherein the target gene is a gene of an adventitious virus selected from the group consisting of: exogenous retrovirus, human immunodeficiency virus type 1 (HIV-1), HIV-2, human T-cell lymphotropic virus type I (HTLV-I), HTLV-II, human hepatitis A (HHA), HHB, HHC, human cytomegalovirus, EBV, herpesvirus, human herpesvirus 6 (HHV6), HHV7, HHV8, human parvovirus B19, reovirus, polyoma (JC/BK) virus, SV40, human coronavirus, papillomavirus, human papillomavirus, influenza A, B, and C viruses, human enterovirus, human parainfluenza virus, human respiratory syncytial virus, vesivirus, porcine circovirus, lymphocytic choriomeningitis virus (LCMV), lactate dehydrogenase virus, porcine parvovirus, adeno-associated virus, reovirus, rabies virus, leporipoxviruse, avian leukosis virus (ALV), hantaan virus, Marburg virus, SV20, Semliki Forest virus, feline sarcoma virus, porcine parvovirus, mouse hepatitis virus (MHV), murine leukemia virus (MuLV), pneumonia virus of mice (PVM), Theiler's encephalomyelitis virus, murine minute virus, mouse

adenovirus (MAV); mouse cytomegalovirus, mouse rotavirus (EDIM), Kilham rat virus, Toolan's H-1 virus, Sendai virus, rat coronavirus, pseudorabies virus, Cache Valley virus, bovine viral diarrhoea virus, bovine parainfluenza virus type 3, bovine respiratory syncytial virus, bovine adenovirus, bovine parvovirus, infectious bovine rhinotracheitis virus, bovine herpesvirus, bovine reovirus, bluetongue virus, bovine polyoma virus, bovine circovirus, vaccinia, orthopoxviruses other than vaccinia, pseudocowpox virus, and leporipoxvirus.

102. The method of claim 96, wherein target gene is a host cell binding ligand for an endogenous virus, a latent virus, or an adventitious virus.

103. The method of claim 102, wherein the target gene is SLC35A1, Gne, Cmas, B4GalT1, or B4GalT6.

104. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of FUT8, TSTA3, and GMDS; and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating fucosylation.

105. The method of claim 104, further comprising contacting a host cell with at least one RNA effector molecule that targets a gene that encodes a sialytransferase.

106. The method of claim 105, wherein the sialytransferase is selected from the group consisting of ST3 β-galactoside α-2,3-sialyltransferase 1, ST3 β-galactoside α-2,3-sialyltransferase 4, ST3 β-galactoside α-2,3-sialyltransferase 3, ST3 β-galactoside α-2,3-sialyltransferase 5, ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6, and ST3 β-galactoside α-2,3-sialyltransferase 2.

107. The method of any of claims 1 to 3, or 6 to 58, wherein the at least one target gene is selected from the group consisting of glutaminase and glutamine dehydrogenase; and wherein the modulation of expression improves production of the immunogenic agent in the cell by modulating ammonia buildup.

108. The method of any of claims 1 to 108, further comprising contacting the host cell with at least one RNA effector molecule that modulates expression of glutaminase.

109. The method of any of claims 1 to 108, further comprising contacting the host cell with at least one RNA effector molecule that modulates expression of glutamine synthetase.

110. A composition comprising: at least one RNA effector molecule, a portion of which is complementary to at least one target gene of a host cell, and a cell medium suitable for culturing the host cell, wherein the RNA effector molecule is capable of modulating expression of the target gene and the modulation of expression enhances production of an immunogenic agent, wherein the at least one RNA effector molecule is an siRNA that comprises an antisense strand comprising at least 16 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs:9772-3152339 and SEQ ID NOs:3161121-3176783.

111. The composition of claim 110, comprising two or more RNA effector molecules, wherein the two or more RNA effector molecules are each complementary to different target genes.

112. A composition comprising: a plurality of RNA effector molecules, wherein a portion of each RNA effector molecule is complementary to at least one target gene of a host cell, and wherein the composition is capable of modulating expression of Bax, Bak, and LDH, and the modulation of expression enhances production of an immunogenic agent.

113. The composition of claim 110 or 112, further comprising at least one additional RNA effector molecule or agent

114. The composition of 110 or 112, wherein the at least one RNA effector molecule is siRNA.

115. The composition of claim 110 or 112, wherein the at least one RNA effector molecule comprises a duplex region.

116. The composition of claim 110 or 112, wherein the at least one RNA effector molecule is 15-30 nucleotides in length.

117. The composition of claim 110 or 112, wherein the at least one RNA effector molecule is 17-28 nucleotides in length.

118. The composition of claim 110 or 112, wherein the at least one RNA effector molecule comprises a modified nucleotide.

119. The composition of claim 110, wherein the cell medium is a serum-free medium.

120. The composition of any of claims 110 to 119, wherein the composition is formulated in a non-lipd formulation.

121. The composition of claim 110 to 119, wherein the composition is formulated in a lipid formulation.

122. The composition of any one of claims 121, wherein the lipid in the formulation comprises a cationic or non-ionic lipid.

123. The composition of any of claims 110 to 122, wherein the composition further comprises one or more cell culture media supplements.

124. The composition of claims 110 to 123, wherein the at least one RNA effector molecule comprises a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least part of a target gene, and wherein said region of complementarity is 10 to 30 nucleotides in length.

125. A kit for enhancing production of an immunogenic agent by a cultured cell, comprising:

(a) a substrate comprising one or more assay surfaces suitable for culturing the cell under conditions in which the immunogenic agent is produced;

(b) one or more RNA effector molecules, wherein at least a portion of each RNA effector molecule is complementary to a target gene; and

(c) a reagent for detecting the immunogenic agent or production thereof by the cell,

wherein the one or more RNA effector molecules is an siRNA comprising an antisense strand that comprises at least 16 contiguous nucleotides of the nucleotide sequence selected from the group consisting of: SEQ ID NOs:9772-3152339 and SEQ ID NOs:3161121-3176783.

126. The kit of claim 125, wherein the one or more assay surfaces further comprises a matrix for supporting the growth and maintenance of host cells.

127. The kit of claim 125, wherein the one or more RNA effector molecules are deposited on the substrate.

128. The kit of claim 125, further comprising a carrier for promoting uptake of the RNA effector molecules by the host cell.

129. The kit of claim 128, wherein the carrier comprises a cationic lipid composition.

130. The kit of claim 128, wherein the carrier is deposited on the substrate.

131. The kit of claim 125, further comprising cell culture media suitable for culturing the host cell.

132. The kit of claim 125, further comprising instructions for culturing a host cell in the presence of one or more RNA effector molecules and assaying the cell for production of the immunogenic agent.

133. A kit for optimizing production of an immunogenic agent by cultured cells, comprising:

(a) a microarray substrate comprising a plurality of assay surfaces, the assay surfaces being suitable for culturing the cells under conditions in which the immunogenic agent is produced;

(b) one or more RNA effector molecules, wherein at least a portion of each RNA effector molecule is complementary to a target gene; and

(c) a reagent for detecting the effect of the one or more RNA effector molecules on production of the immunogenic agent.

wherein the one or more RNA effector molecules is an siRNA comprising an antisense strand that comprises at least 16 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NOs:9772-3152339 and SEQ ID NOs:3161121-3176783.

134. The kit of claim 133, wherein the substrate is a multi-well plate or biochip.

135. The kit of claim 133, wherein the substrate is a two-dimensional microarray plate or biochip.

136. The kit of claim 133, wherein the one or more RNA effector molecules are deposited on the assay surfaces of the substrate.

137. The kit of claim 135, wherein a plurality of different RNA effector molecules are deposited on assay surfaces across a first dimension of the microarray.

138. The kit of claim 137, wherein the plurality of RNA effector molecules are each complementary to a different target gene.

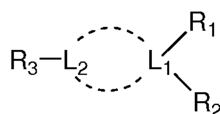
139. The kit of claim wherein the different target genes are Bax, Bak, and LDH.

140. The kit of claim 137, wherein a plurality of RNA effector molecules are each complementary to a different region of the same target gene.

141. The kit of claim 137, wherein each of the RNA effector molecules comprising the plurality is deposited at varying concentrations on assay surfaces along the second dimension of the microarray.

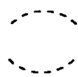
142. The method of any of claims 1-109, wherein the RNA effector molecule, a portion of which is complementary to the target gene, is a corresponding siRNA that comprises an antisense strand comprising at least 16 contiguous nucleotides of a nucleotide sequence, wherein the nucleotide sequence is set forth in the tables herein.

143. The method of claim 121, wherein the lipid formulation comprises a lipid having the following formula:



wherein:

R_1 and R_2 are each independently for each occurrence optionally substituted C_{10}-C_{30} alkyl, optionally substituted C_{10}-C_{30} alkoxy, optionally substituted C_{10}-C_{30} alkenyl, optionally substituted C_{10}-C_{30} alkenyloxy, optionally substituted C_{10}-C_{30} alkynyl, optionally substituted C_{10}-C_{30} alkynyloxy, or optionally substituted C_{10}-C_{30} acyl;

 represents a connection between L_2 and L_1 which is:

(1) a single bond between one atom of L_2 and one atom of L_1, wherein

L_1 is $C(R_x)$, O, S or N(Q);

L_2 is $-CR_5R_6-$, -O-, -S-, -N(Q)-, $=C(R_5)-$, -C(O)N(Q)-, -C(O)O-, -N(Q)C(O)-, -OC(O)-, or -C(O)-;

(2) a double bond between one atom of L_2 and one atom of L_1; wherein

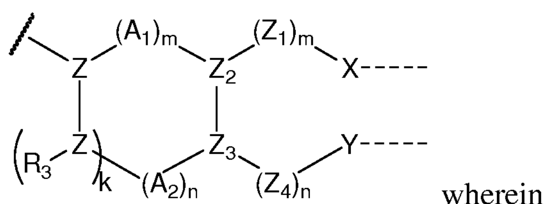
L_1 is C;

L_2 is $-CR_5=$, -N(Q)=, -N-, -O-N=, -N(Q)-N=, or -C(O)N(Q)-N=;

(3) a single bond between a first atom of L_2 and a first atom of L_1, and a single bond between a second atom of L_2 and the first atom of L_1, wherein

L_1 is C;

L_2 has the formula



wherein

X is the first atom of L_2, Y is the second atom of L_2, - - - - - represents a single bond to the first atom of L_1, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, -OS(O)(Q_2)O-, and -OP(O)(Q_2)O-;

Z_1 and Z_4 are each, independently, -O-, -S-, -CH_2-, -CHR^5-, or -CR^5R^5-;

Z_2 is CH or N;

Z_3 is CH or N;

or Z_2 and Z_3, taken together, are a single C atom;

A_1 and A_2 are each, independently, -O-, -S-, -CH_2-, -CHR^5-, or -CR^5R^5-;

each Z is N, C(R_5), or C(R_3);

k is 0, 1, or 2;

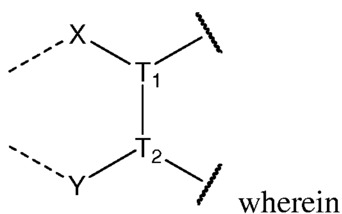
each m, independently, is 0 to 5;

each n, independently, is 0 to 5;

where m and n taken together result in a 3, 4, 5, 6, 7 or 8 member ring;

(4) a single bond between a first atom of L_2 and a first atom of L_1, and a single bond between the first atom of L_2 and a second atom of L_1, wherein

(A) L_1 has the formula:



wherein

X is the first atom of L_1, Y is the second atom of L_1, - - - - - represents a single bond to the first atom of L_2, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, -OS(O)(Q_2)O-, and -OP(O)(Q_2)O-;

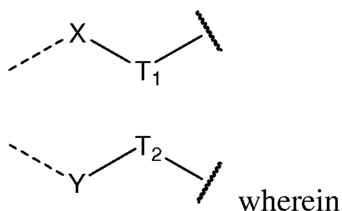
T_1 is CH or N;

T_2 is CH or N;

or T_1 and T_2 taken together are C=C;

L_2 is CR_5; or

(B) L_1 has the formula:



wherein

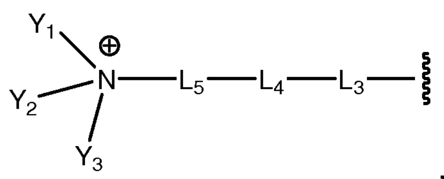
X is the first atom of L_1, Y is the second atom of L_1, - - - - - represents a single bond to the first atom of L_2, and X and Y are each, independently, selected from the group consisting of -O-, -S-, alkylene, -N(Q)-, -C(O)-, -O(CO)-, -OC(O)N(Q)-, -N(Q)C(O)O-, -C(O)O, -OC(O)O-, -OS(O)(Q_2)O-, and -OP(O)(Q_2)O-;

T_1 is -CR_5R_5-, -N(Q)-, -O-, or -S-;

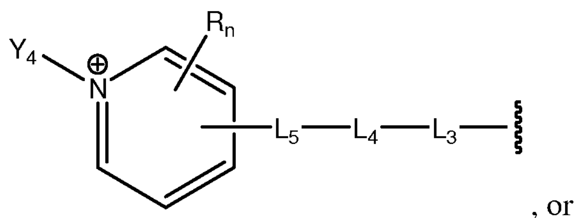
T_2 is -CR_5R_5-, -N(Q)-, -O-, or -S-;

L_2 is CR_5 or N;

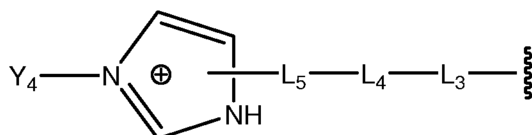
R_3 has the formula:



,



, or



wherein

each of Y_1, Y_2, Y_3, and Y_4, independently, is alkyl, cycloalkyl, aryl, aralkyl, or alkynyl; or

any two of Y_1, Y_2, and Y_3 are taken together with the N atom to which they are attached to form a 3- to 8- member heterocycle; or

Y_1, Y_2, and Y_3 are all be taken together with the N atom to which they are attached to form a bicyclic 5- to 12- member heterocycle;

each R_n, independently, is H, halo, cyano, hydroxy, amino, alkyl, alkoxy, cycloalkyl, aryl, heteroaryl, or heterocyclyl;

L_3 is a bond, -N(Q)-, -O-, -S-, -$(CR_5R_6)_a$-, -C(O)-, or a combination of any two of these;

L_4 is a bond, -N(Q)-, -O-, -S-, -$(CR_5R_6)_a$-, -C(O)-, or a combination of any two of these;

L_5 is a bond, -N(Q)-, -O-, -S-, -$(CR_5R_6)_a$-, -C(O)-, or a combination of any two of these;

each occurrence of R_5 and R_6 is, independently, H, halo, cyano, hydroxy, amino, alkyl, alkoxy, cycloalkyl, aryl, heteroaryl, or heterocyclyl; or two R_5 groups on adjacent carbon atoms are taken together to form a double bond between their respective carbon atoms; or two R_5 groups on adjacent carbon atoms and two R_6 groups on the same adjacent carbon atoms are taken together to form a triple bond between their respective carbon atoms;

each a, independently, is 0, 1, 2, or 3;

wherein

an R_5 or R_6 substituent from any of L_3, L_4, or L_5 is optionally taken with an R_5 or R_6 substituent from any of L_3, L_4, or L_5 to form a 3- to 8- member cycloalkyl, heterocyclyl, aryl, or heteroaryl group; and

any one of Y_1, Y_2, or Y_3, is optionally taken together with an R_5 or R_6 group from any of L_3, L_4, and L_5, and atoms to which they are attached, to form a 3- to 8- member heterocyclyl group;

each Q, independently, is H, alkyl, acyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl or heterocyclyl; and

each Q_2, independently, is O, S, N(Q)(Q), alkyl or alkoxy.

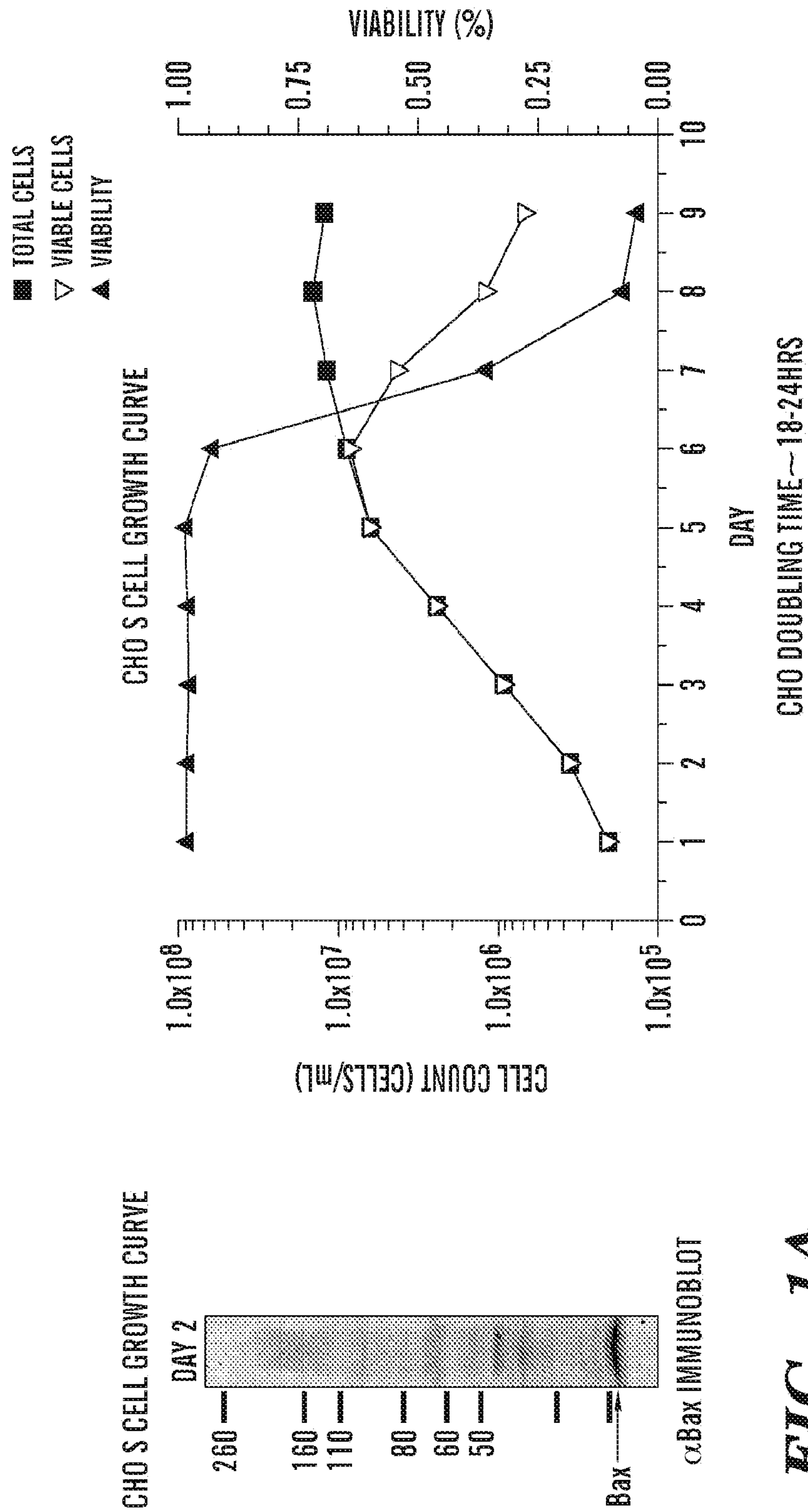
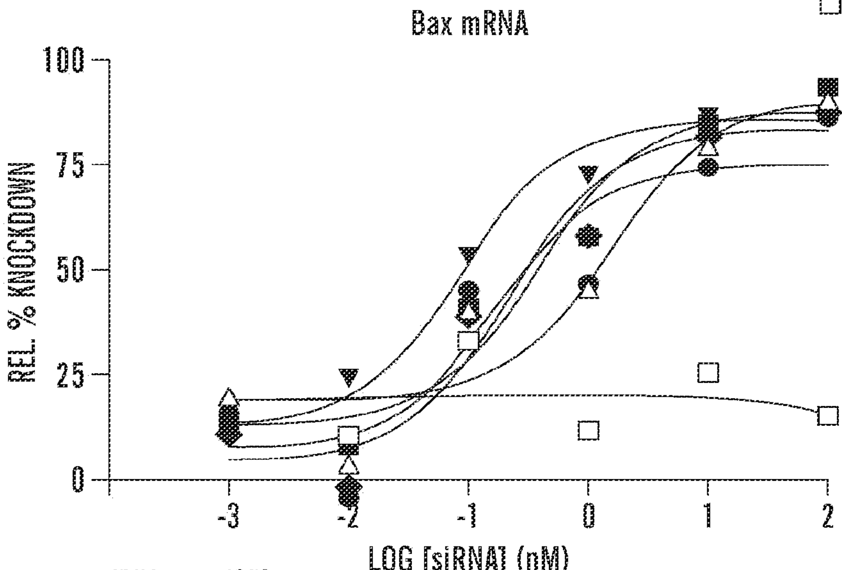


FIG. 1A

FIG. 1B

IC50 VALUES OF Bax & Bak siRNAs in CHO CELLS

Bax mRNA

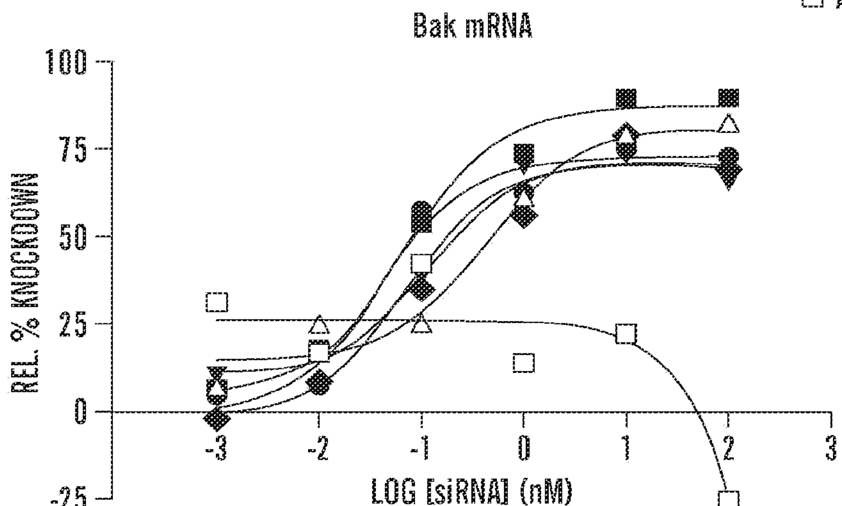


siRNA	IC50
A7	0.38nM
B2	1.46nM
B4	0.08nM
B11	0.22nM
C6	0.17nM

FIG. 2A

IC50 VALUES OF Bax & Bak siRNAs in CHO CELLS

Bak mRNA



siRNA	IC50
A2	0.07nM
A10	0.38nM
A11	0.14nM
B9	0.08nM
C7	0.04nM

FIG. 2B

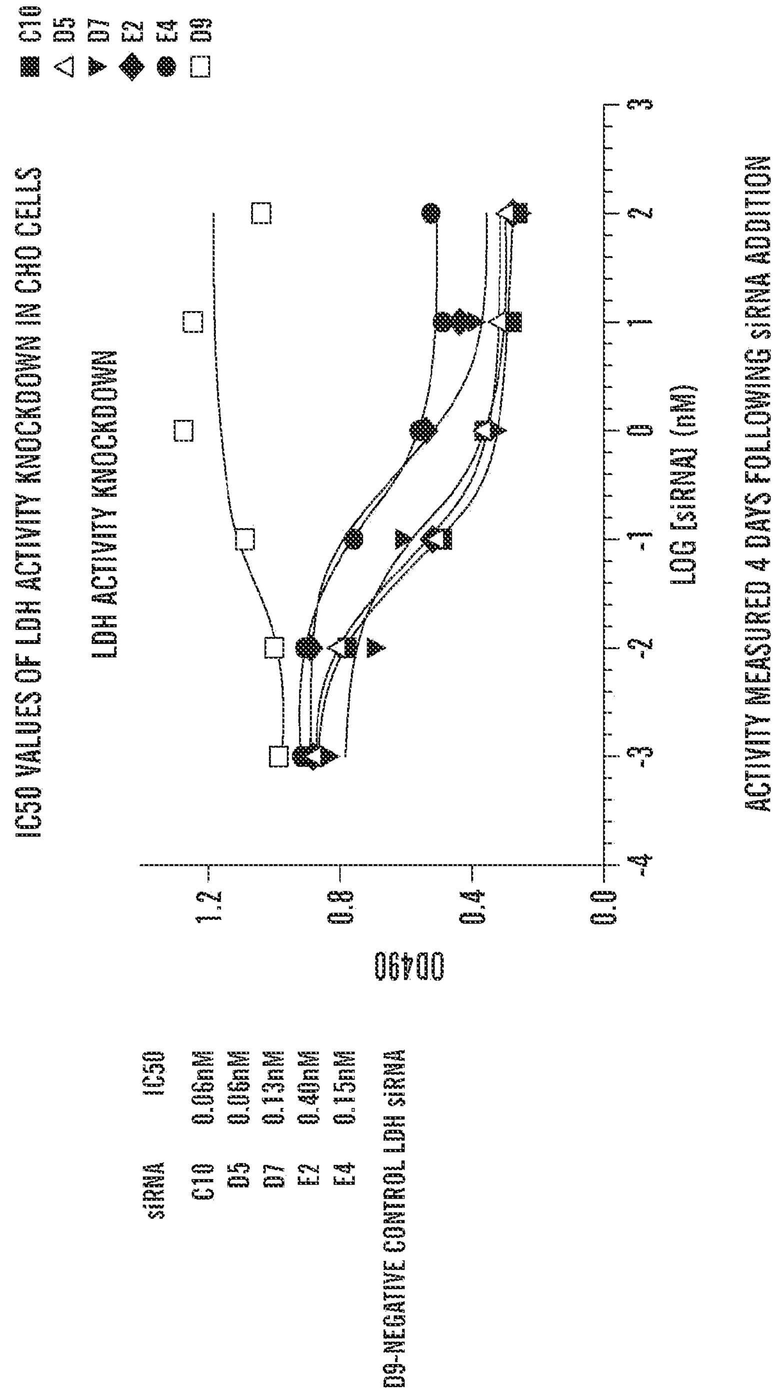


FIG. 3

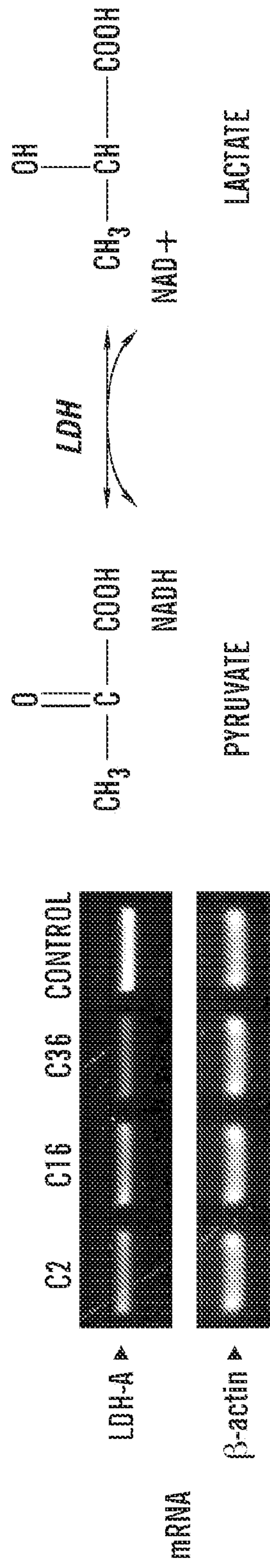


FIG. 4A

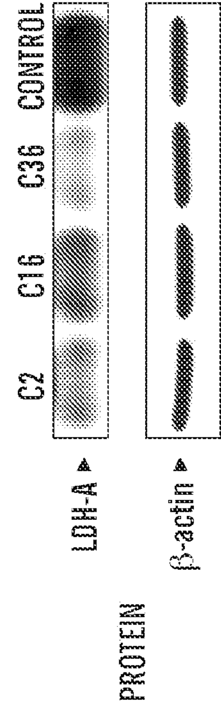


FIG. 4B

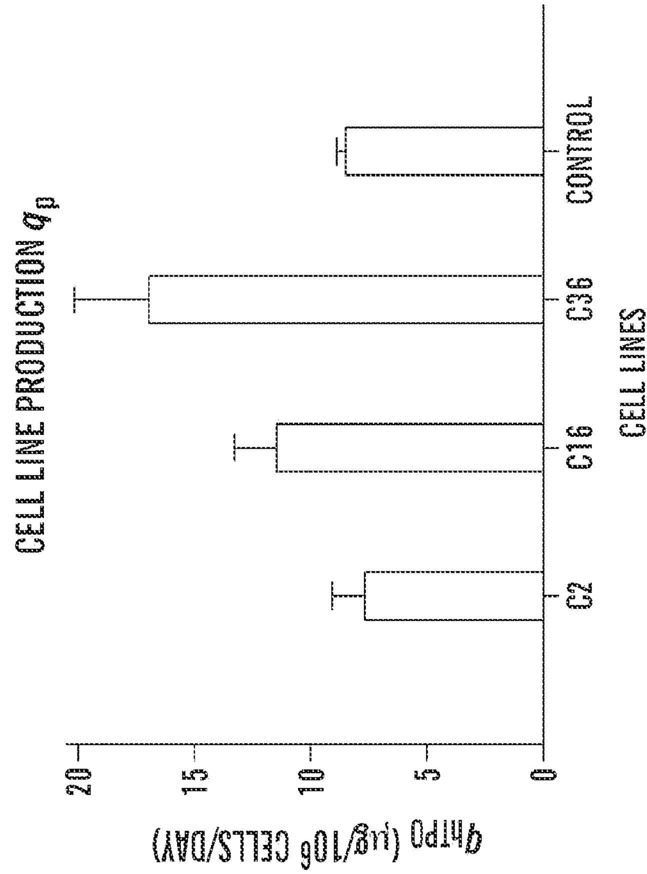


FIG. 4D

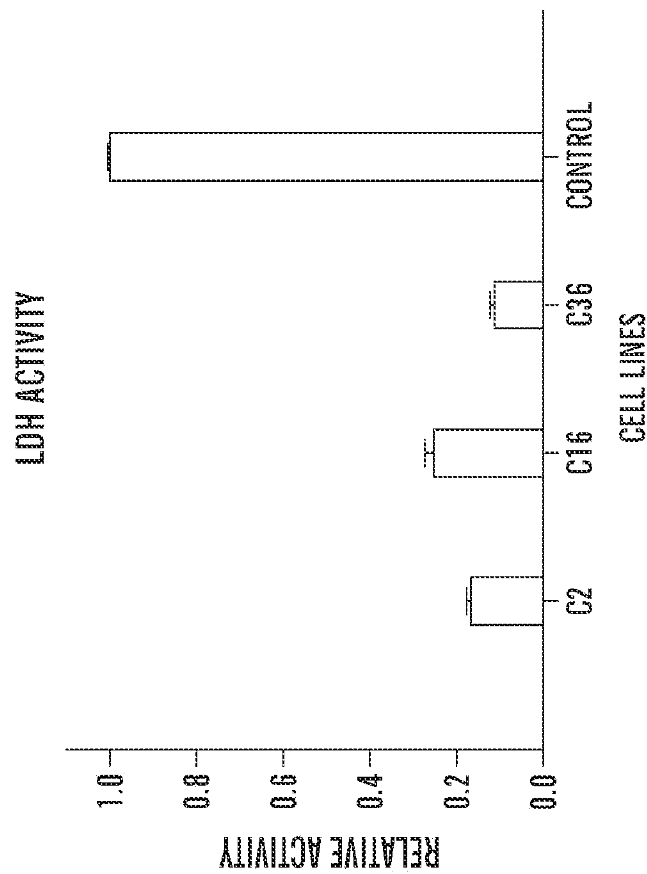


FIG. 4C

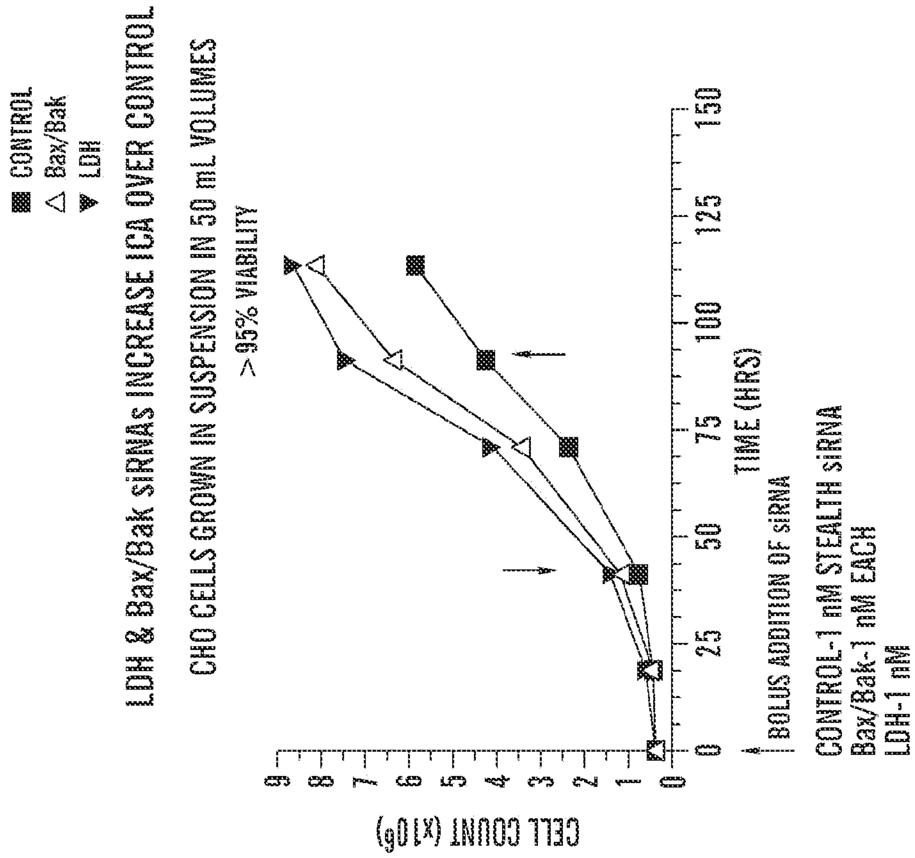


FIG. 5B

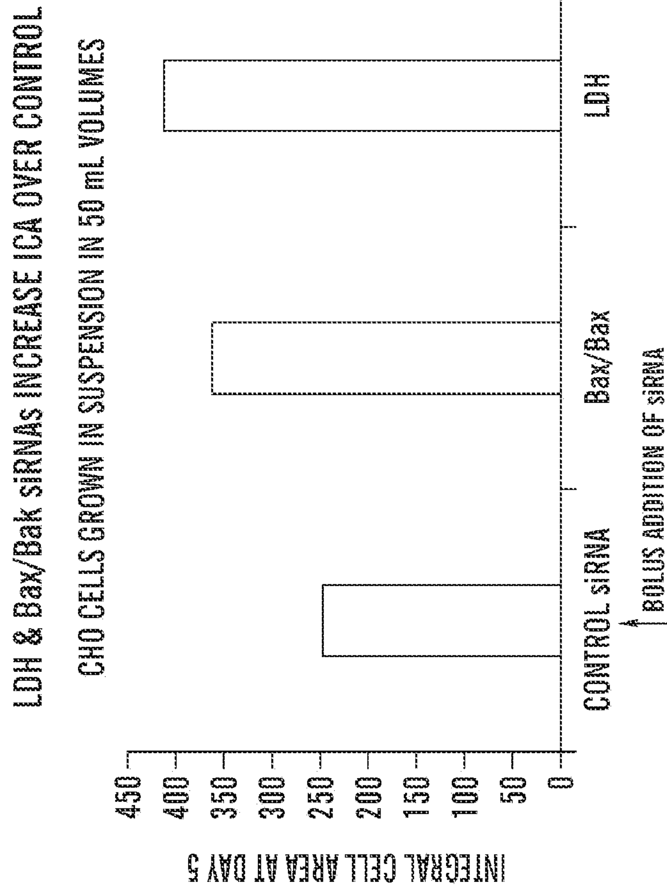


FIG. 5A

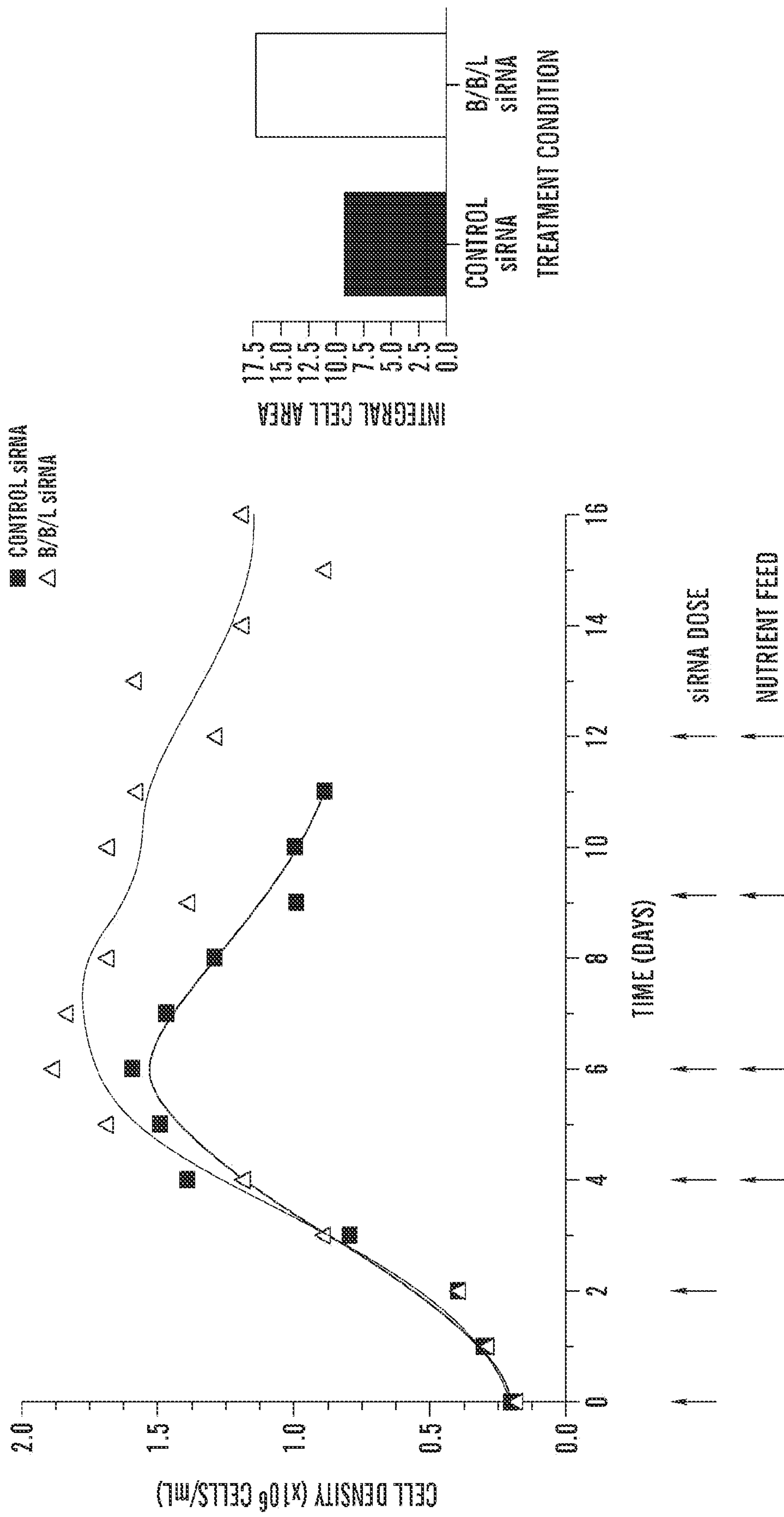


FIG. 6

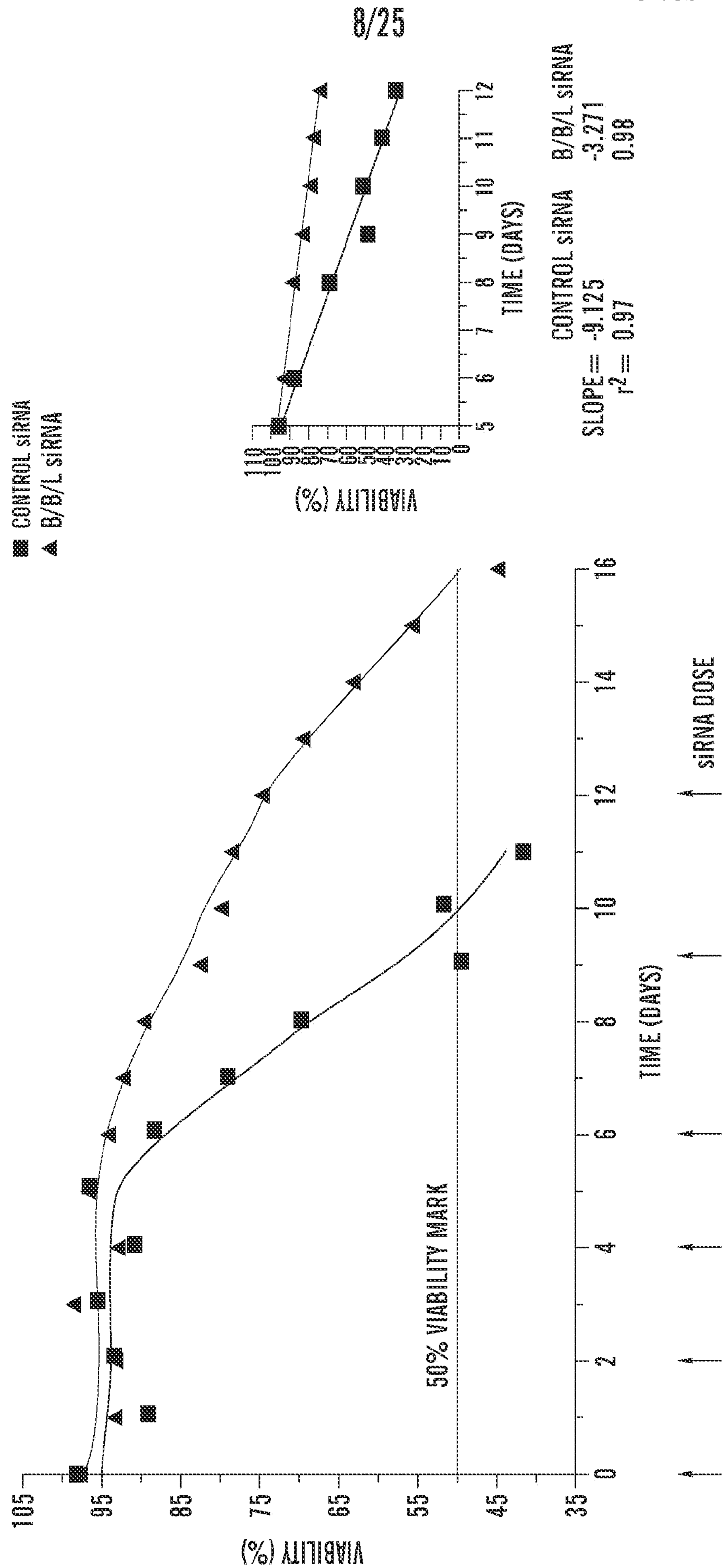


FIG. 7

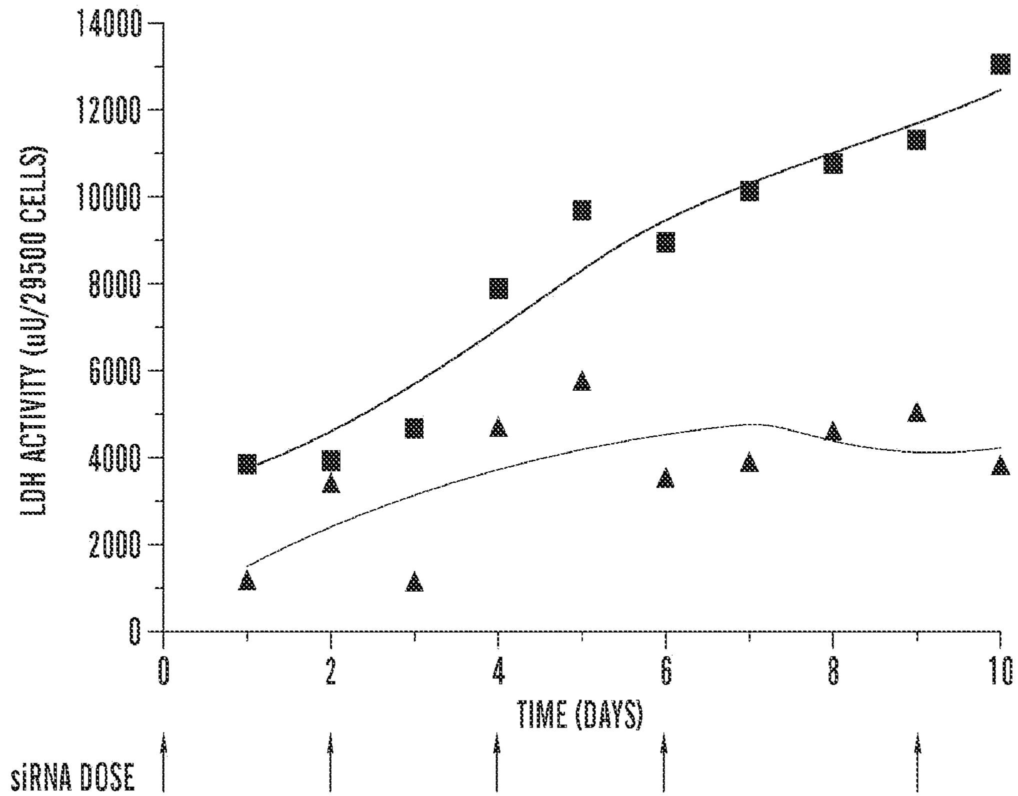


FIG. 8

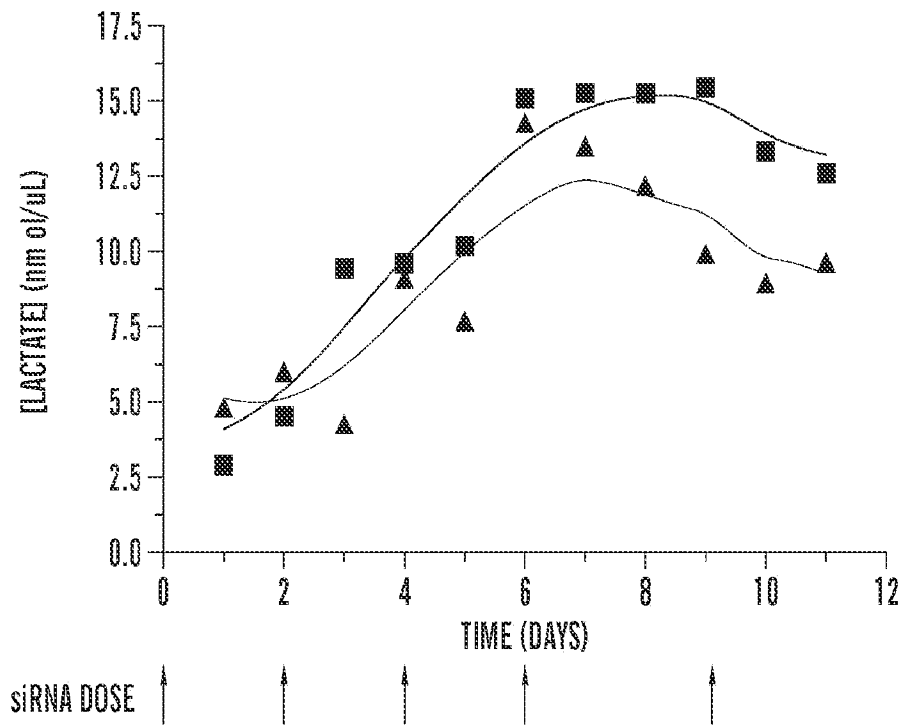


FIG. 9

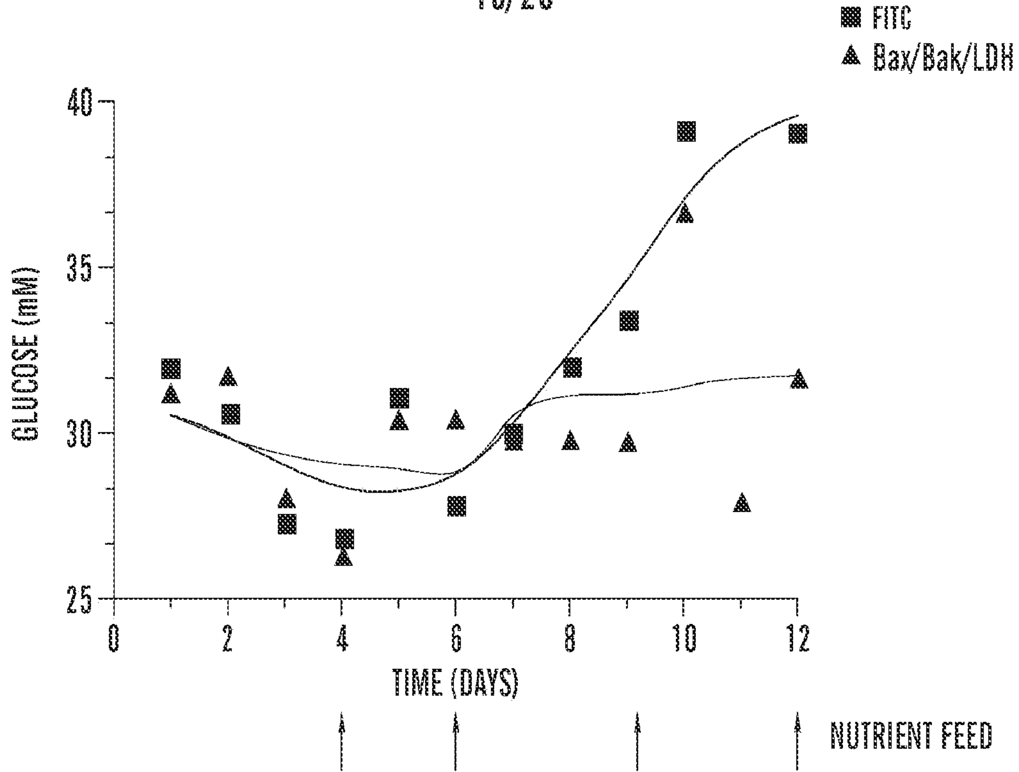


FIG. 10

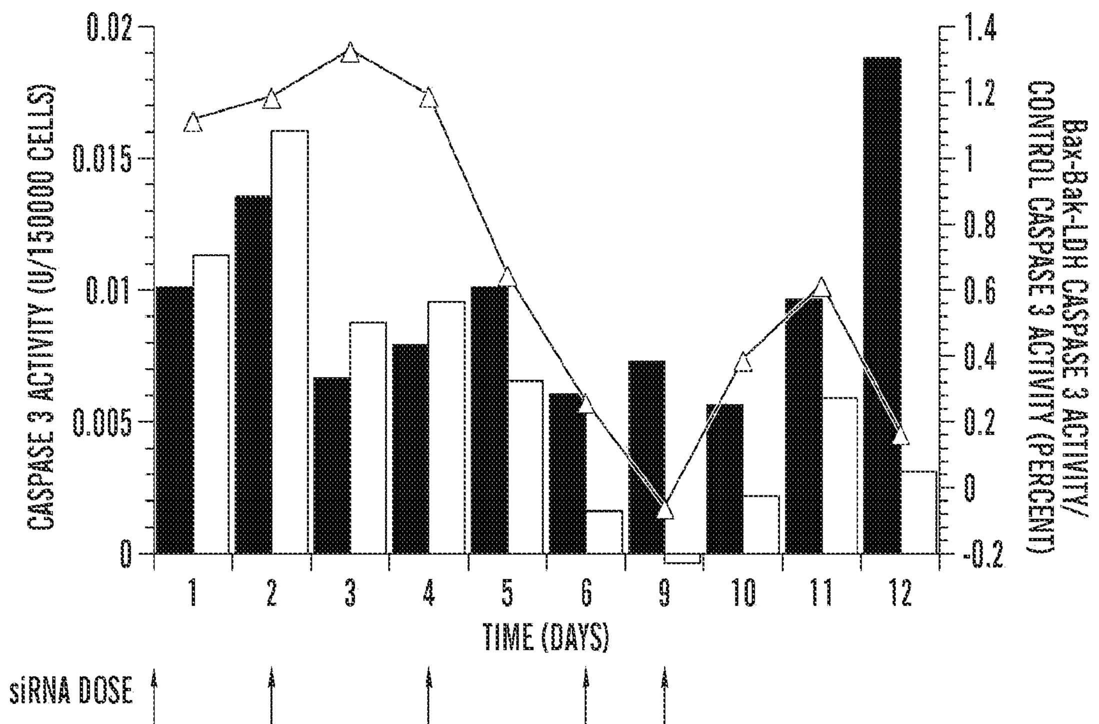


FIG. 11

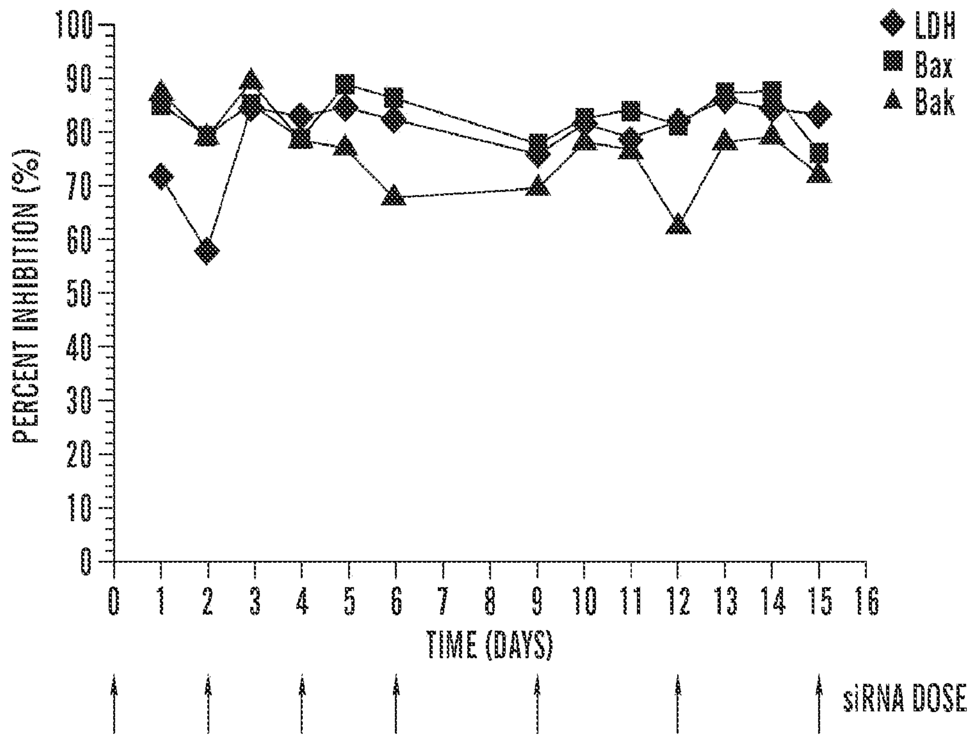


FIG. 12

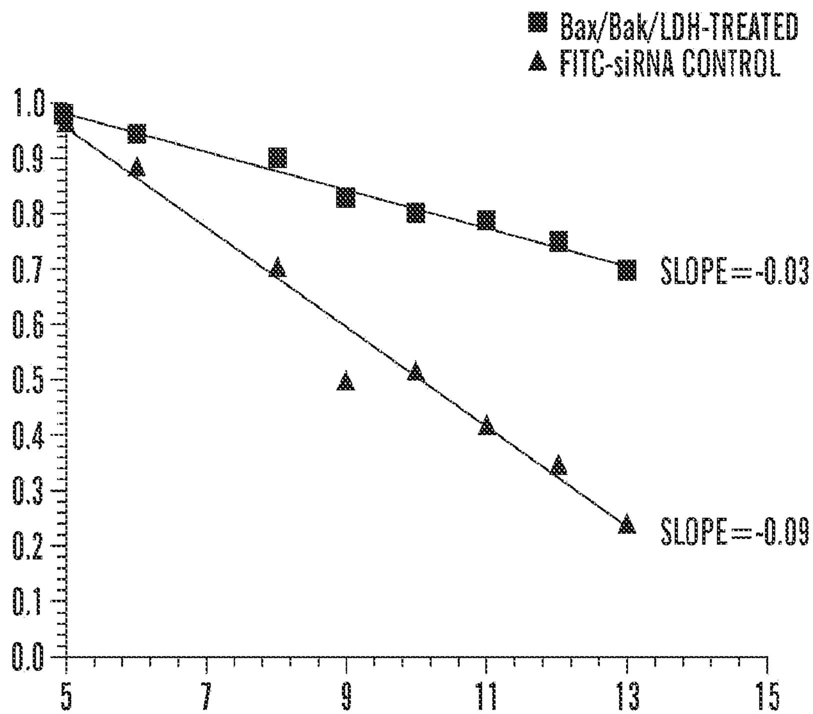


FIG. 13

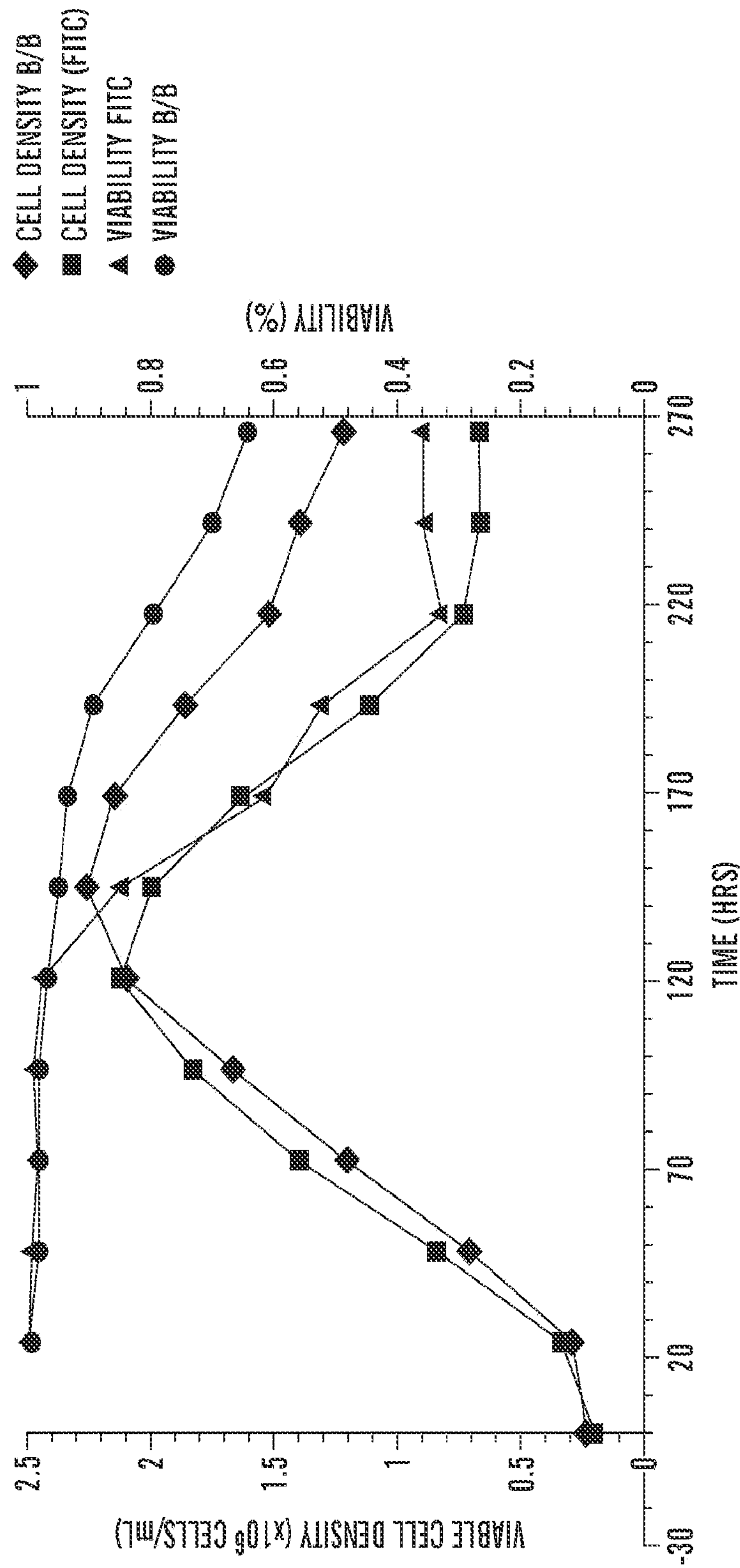


FIG. 14

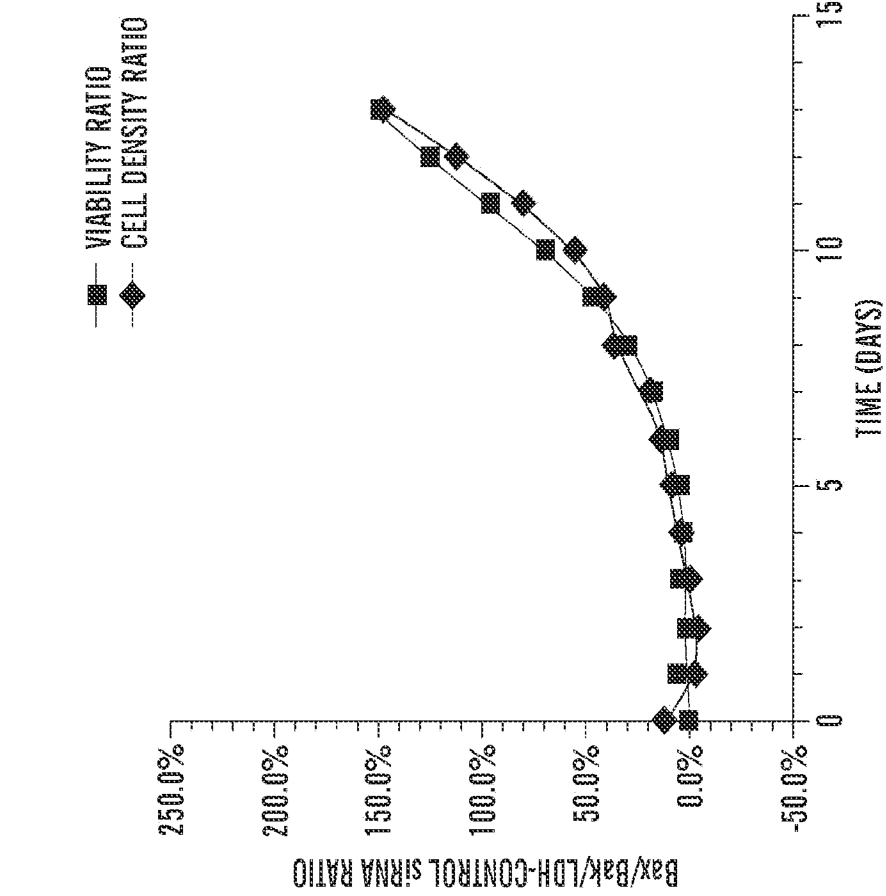


FIG. 15B

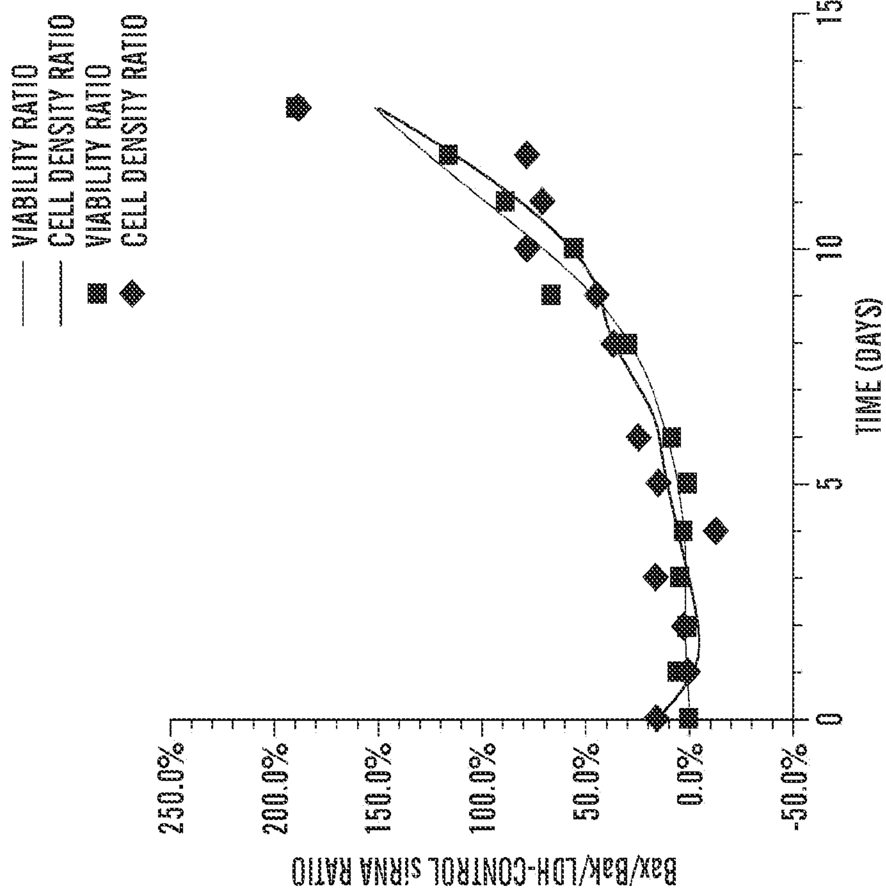


FIG. 15A

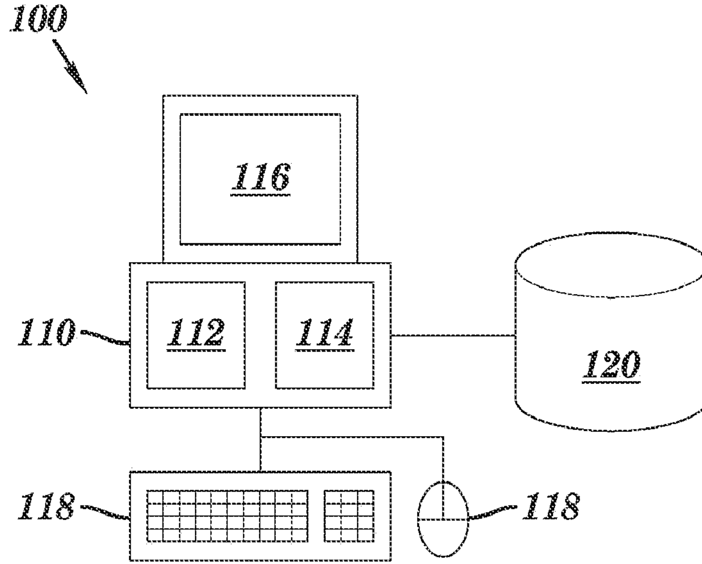


FIG. 16

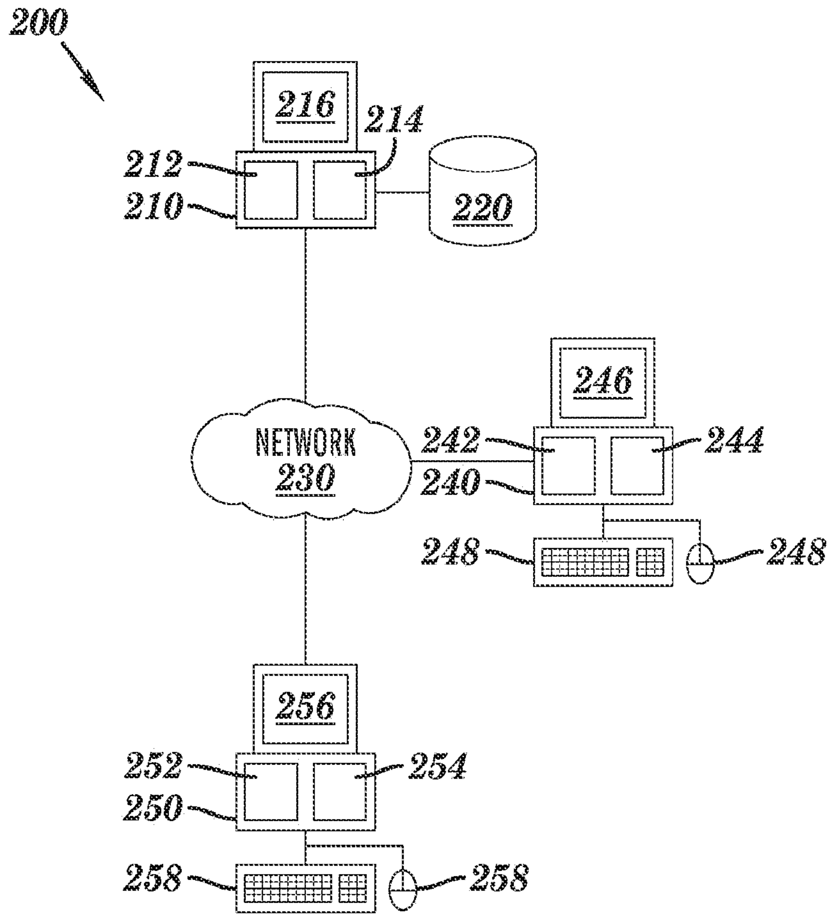


FIG. 17

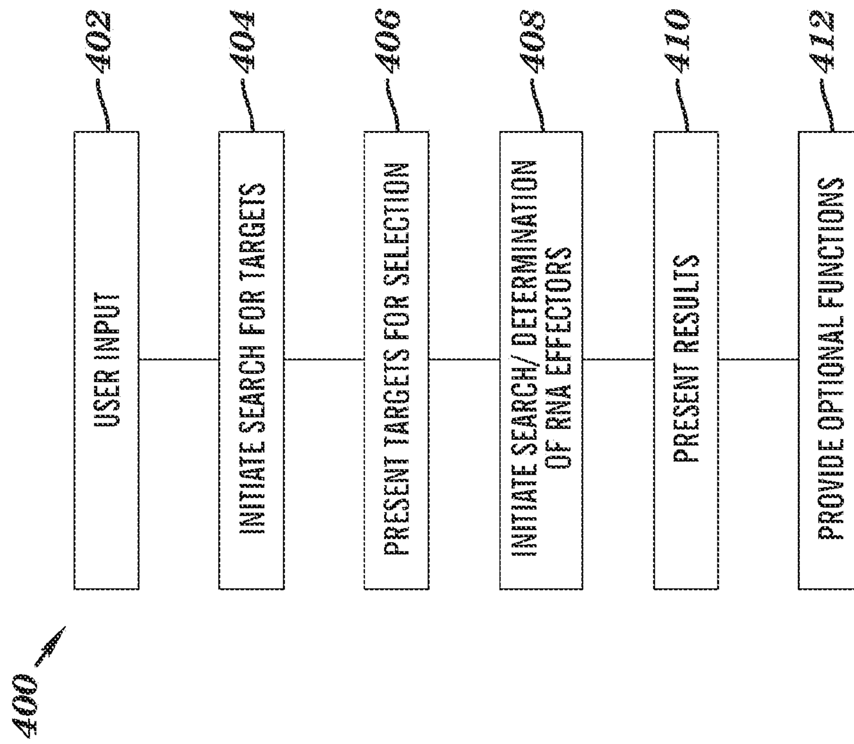


FIG. 19

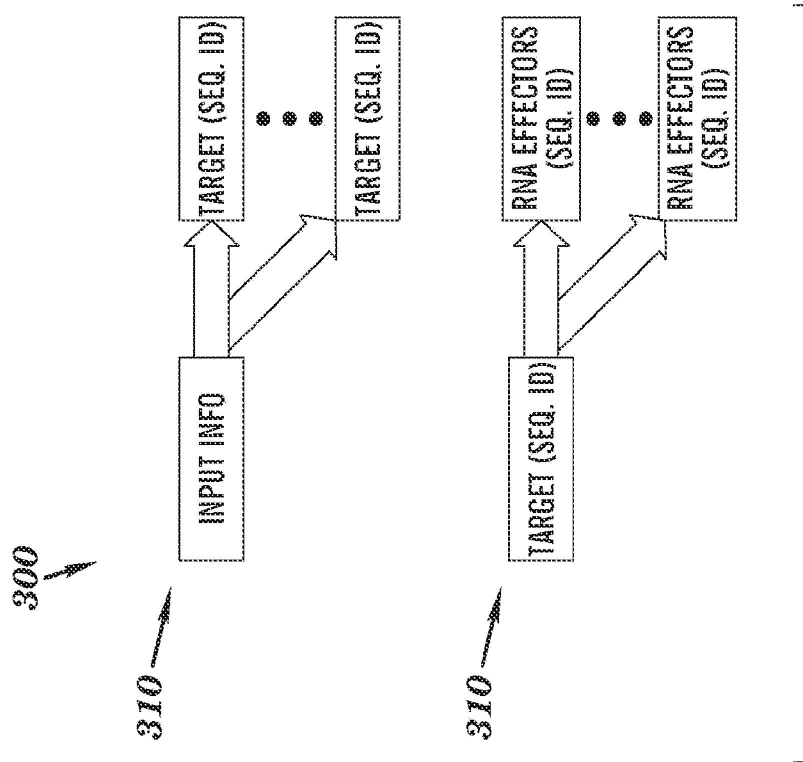


FIG. 18

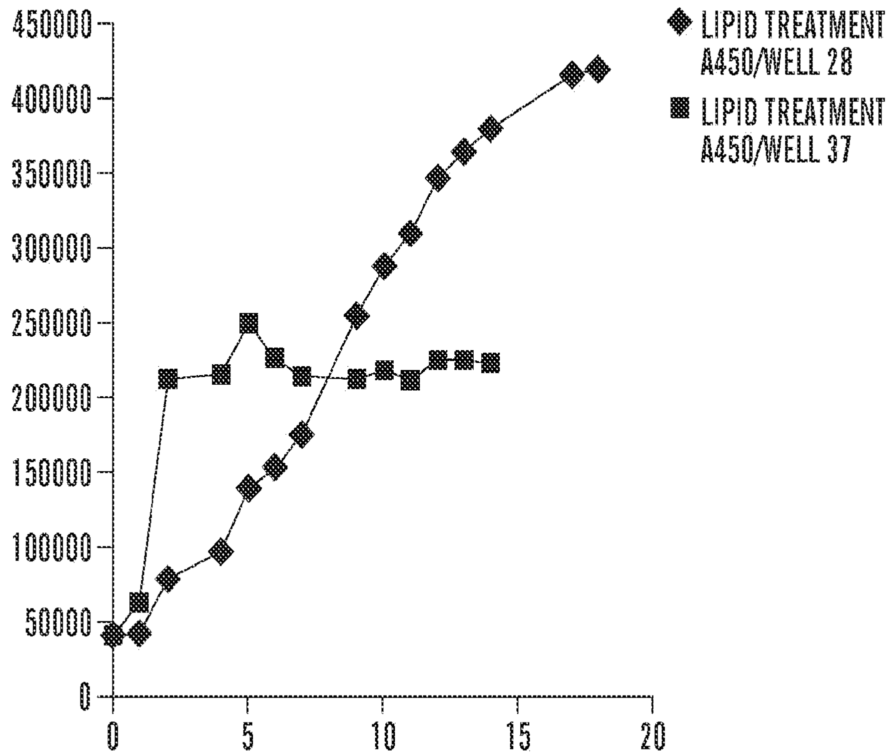


FIG. 20

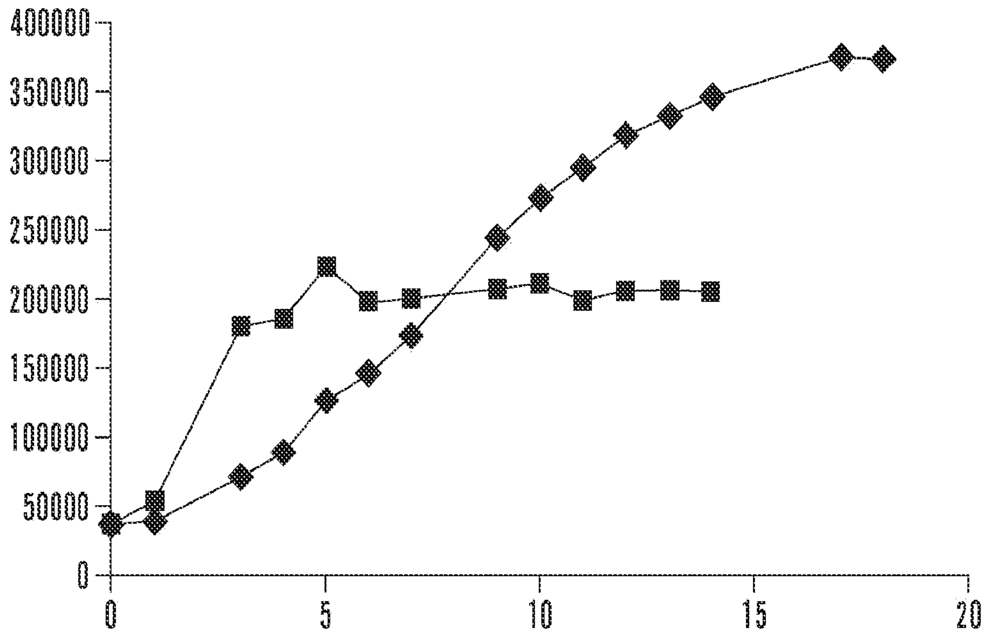


FIG. 21

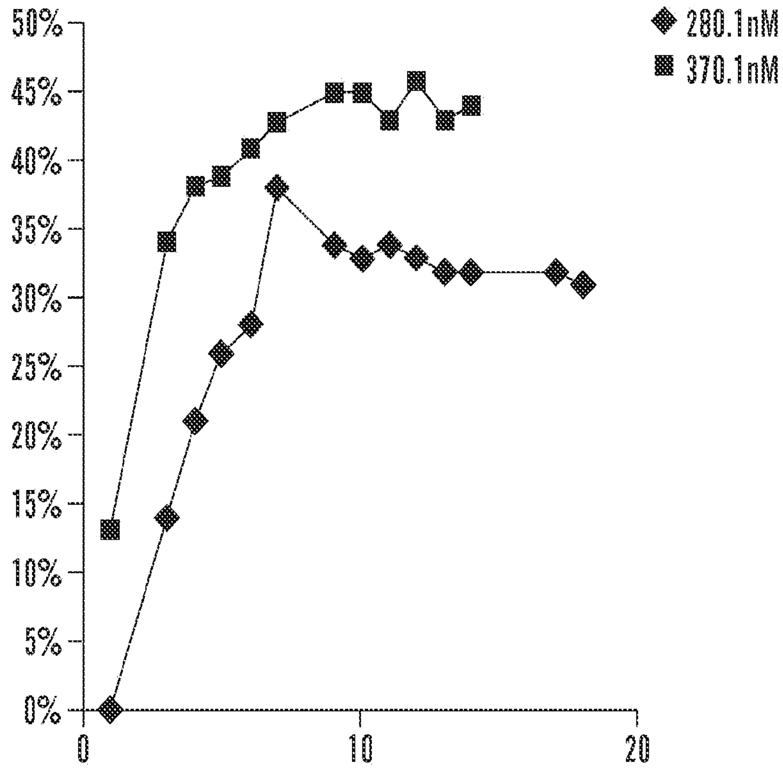


FIG. 22A

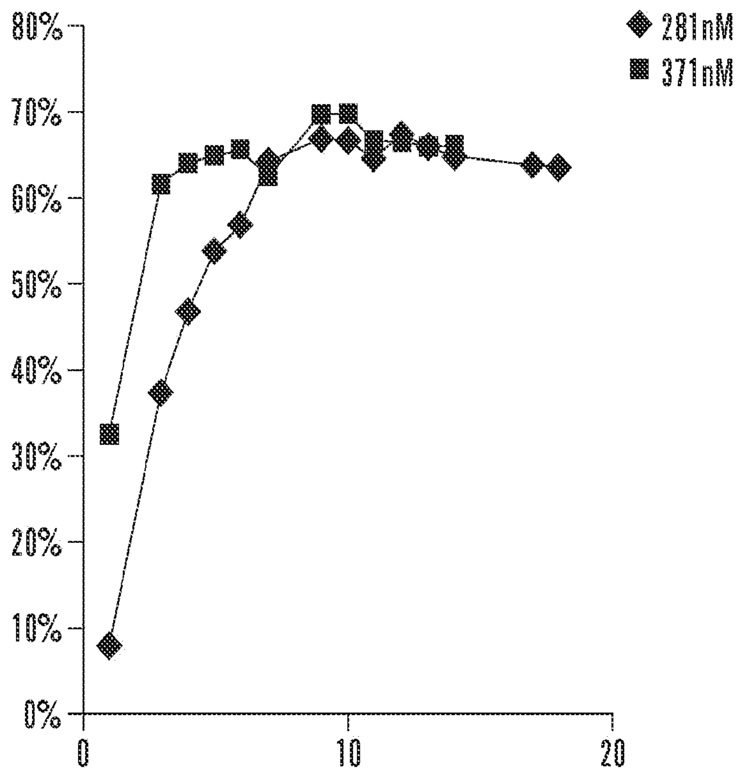


FIG. 22B

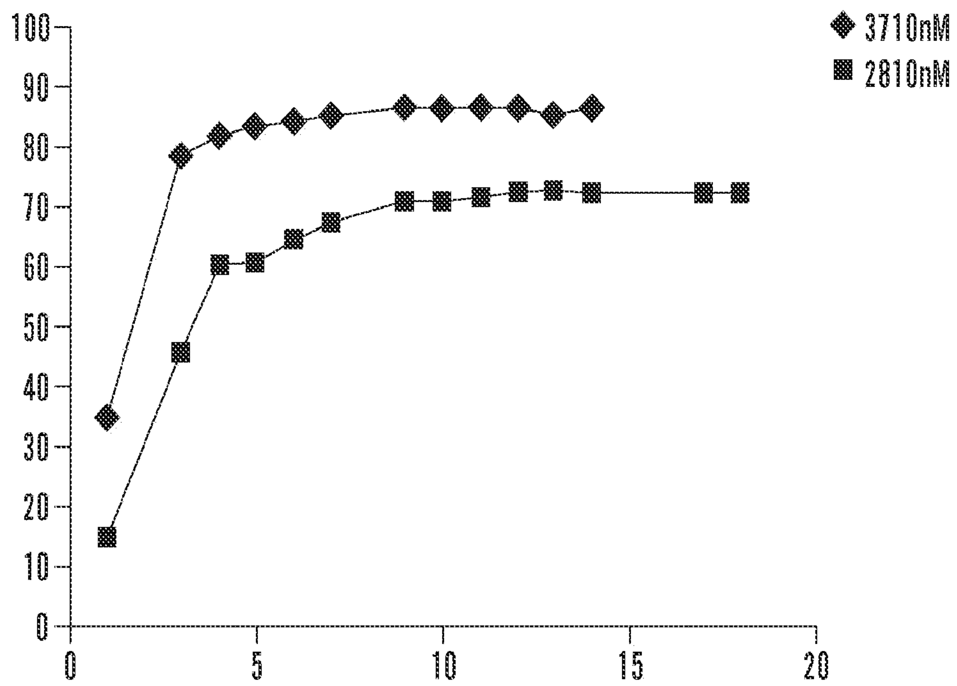


FIG. 22C

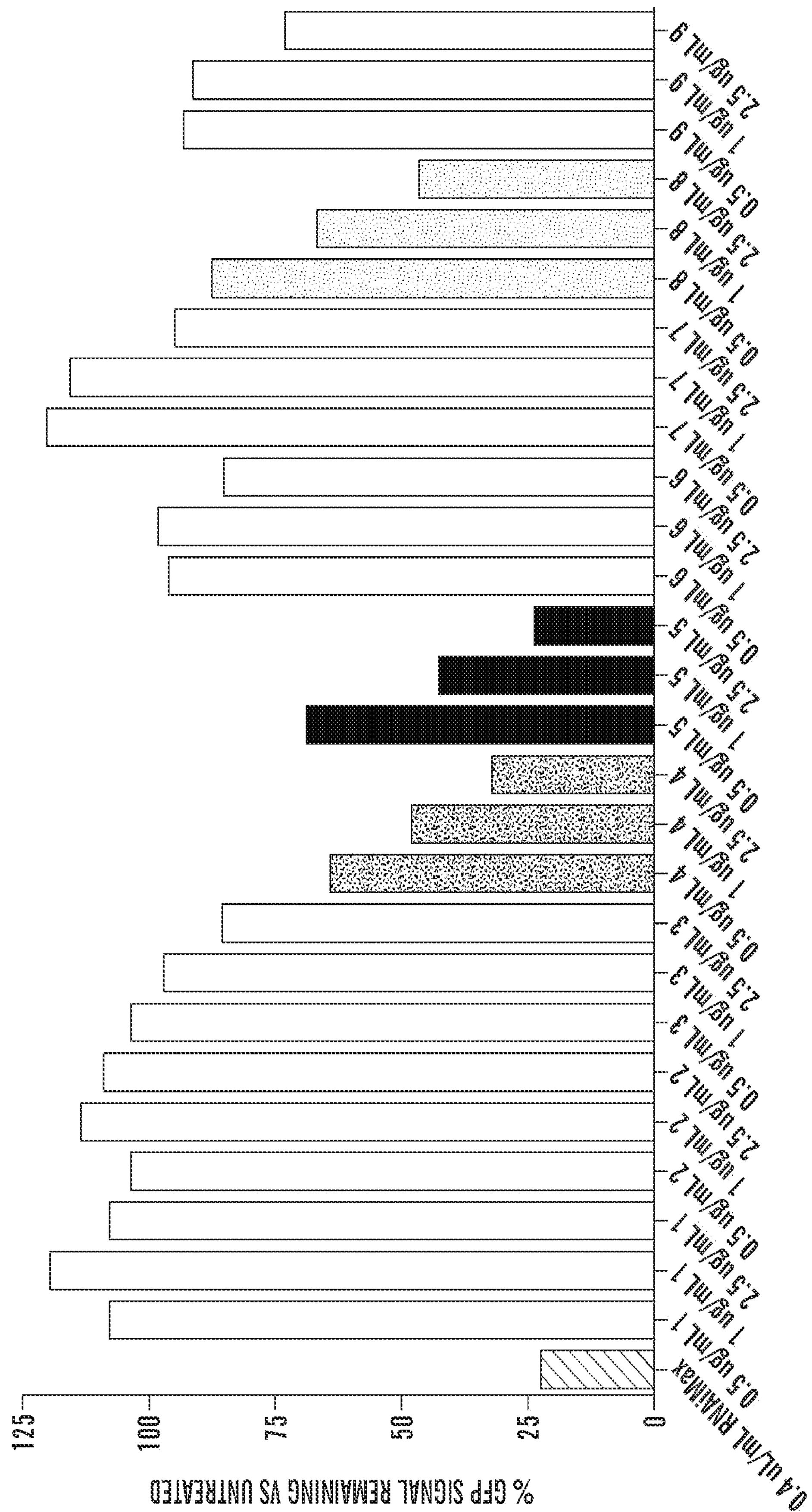


FIG. 23

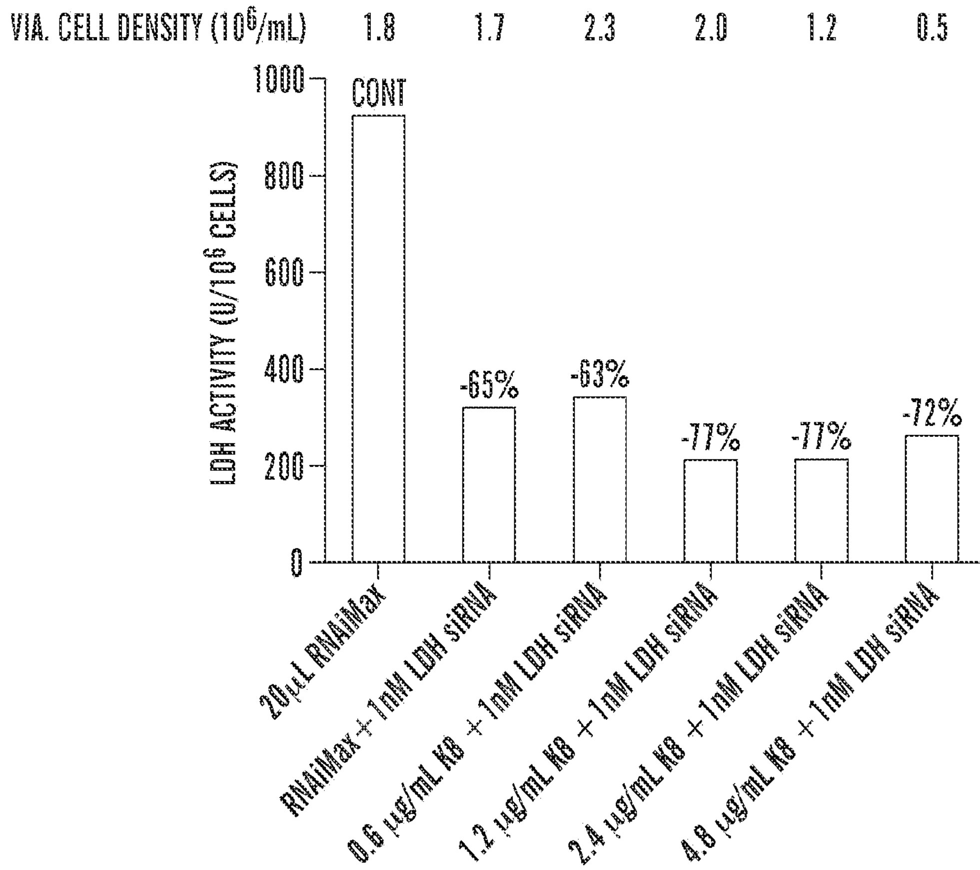


FIG. 24

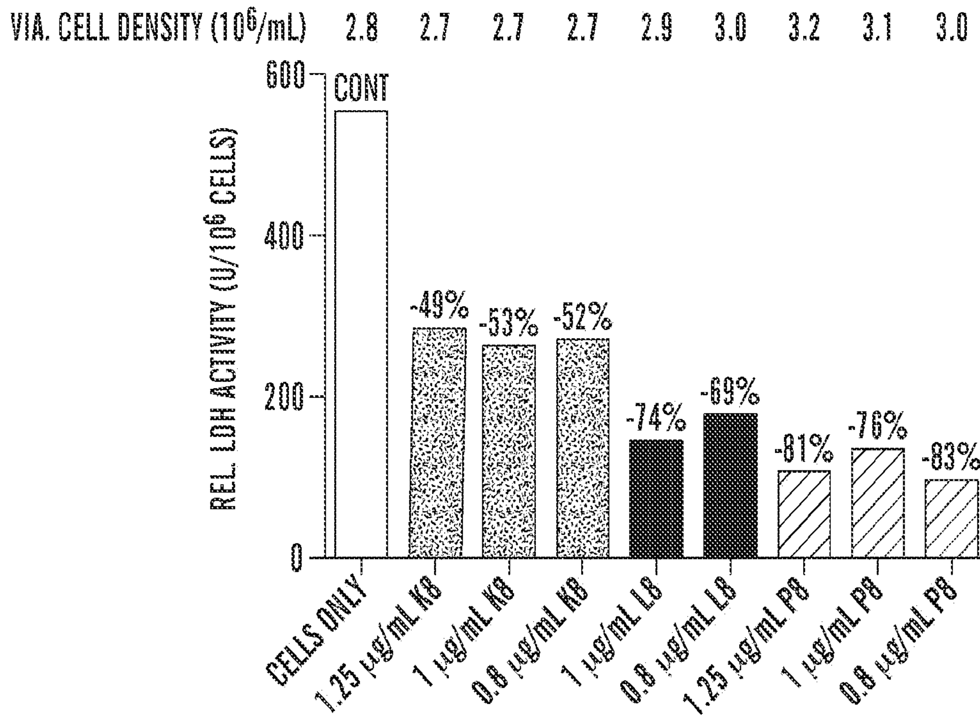


FIG. 25

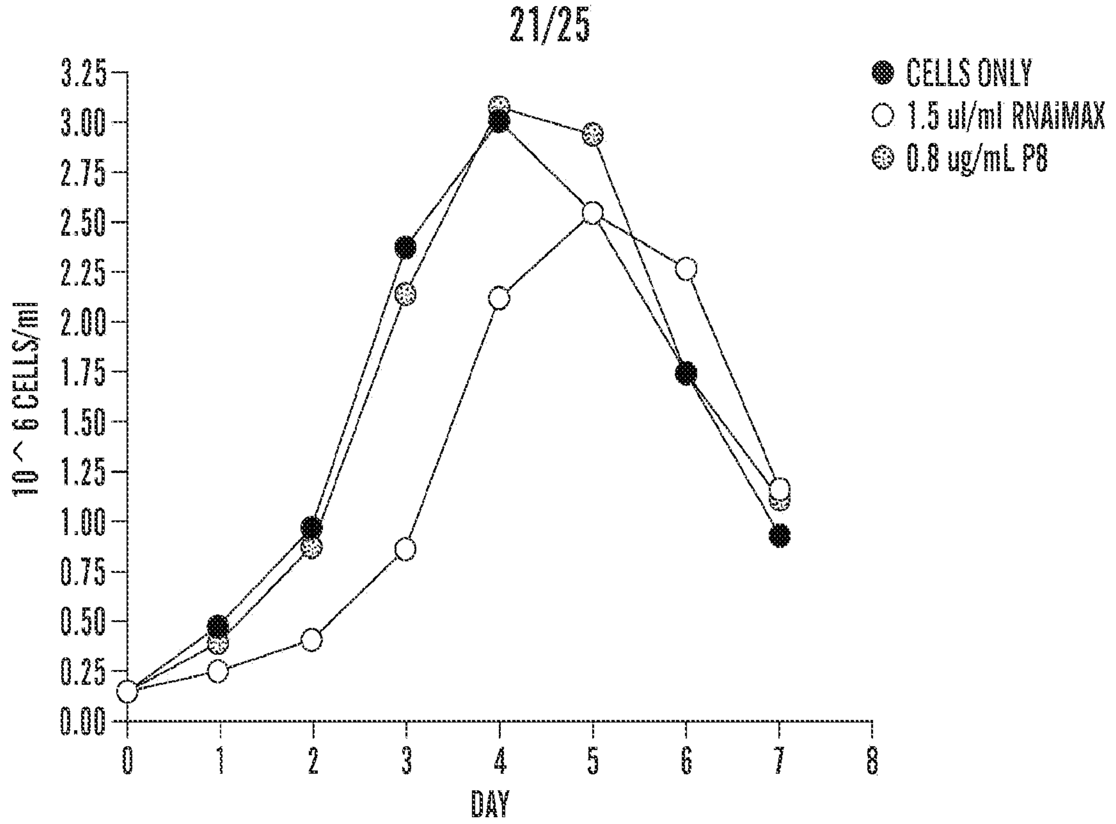


FIG. 26A

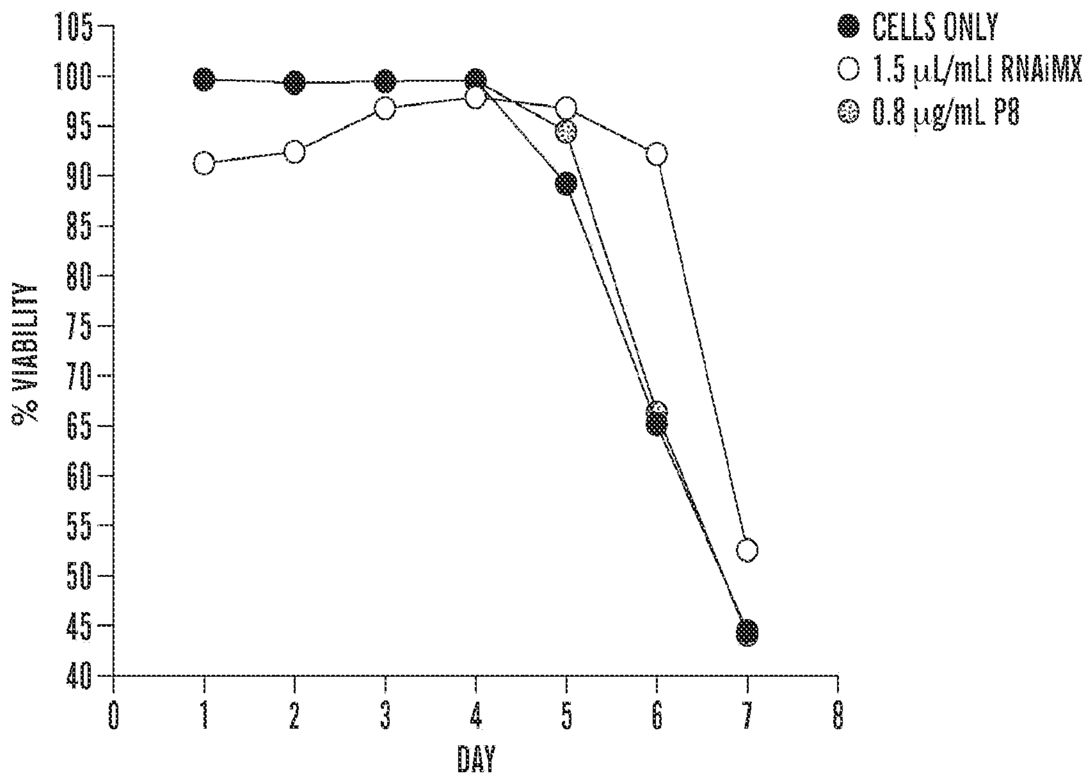


FIG. 26B

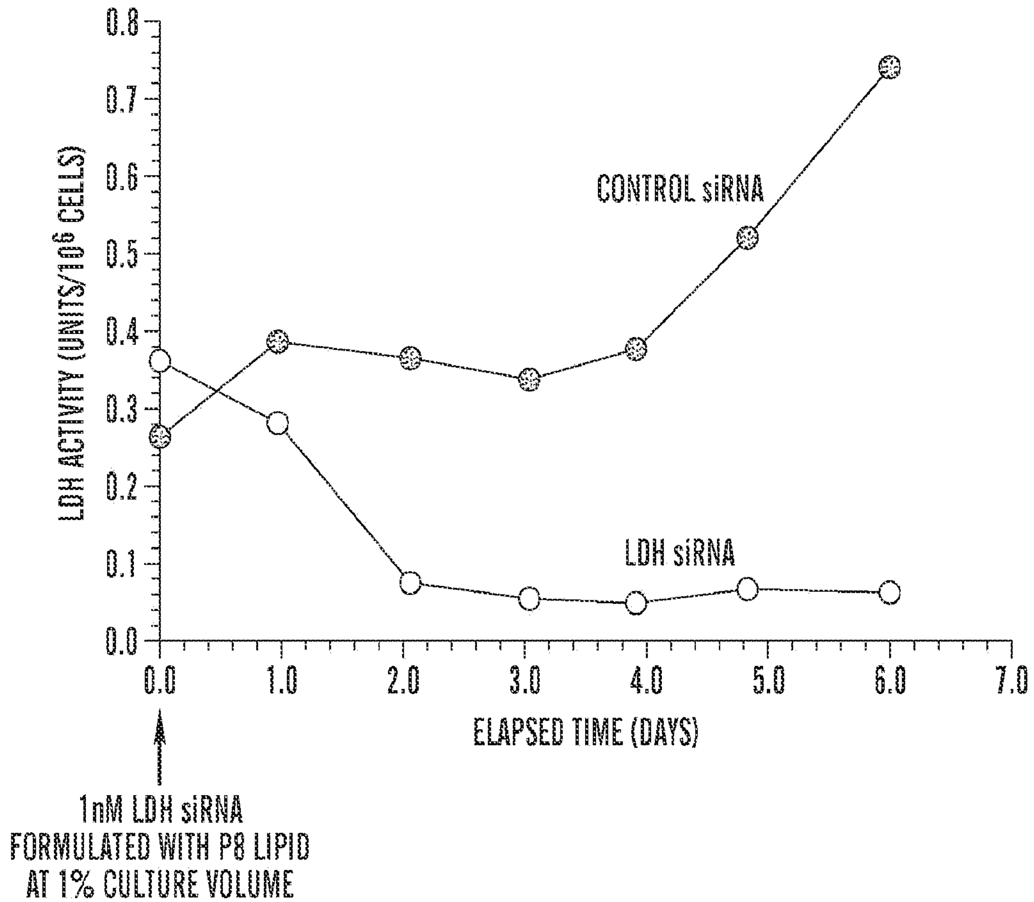


FIG. 27

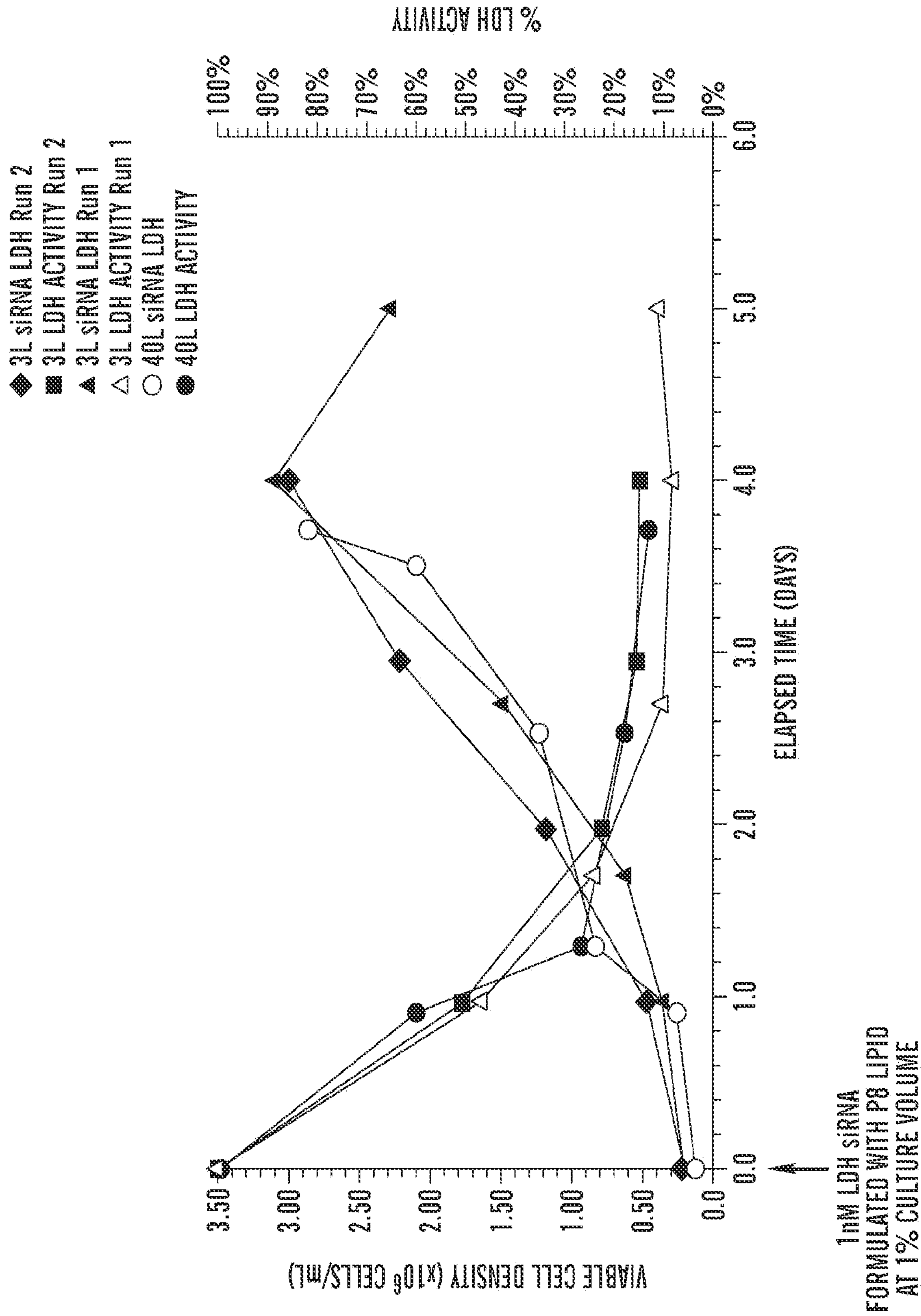


FIG. 28

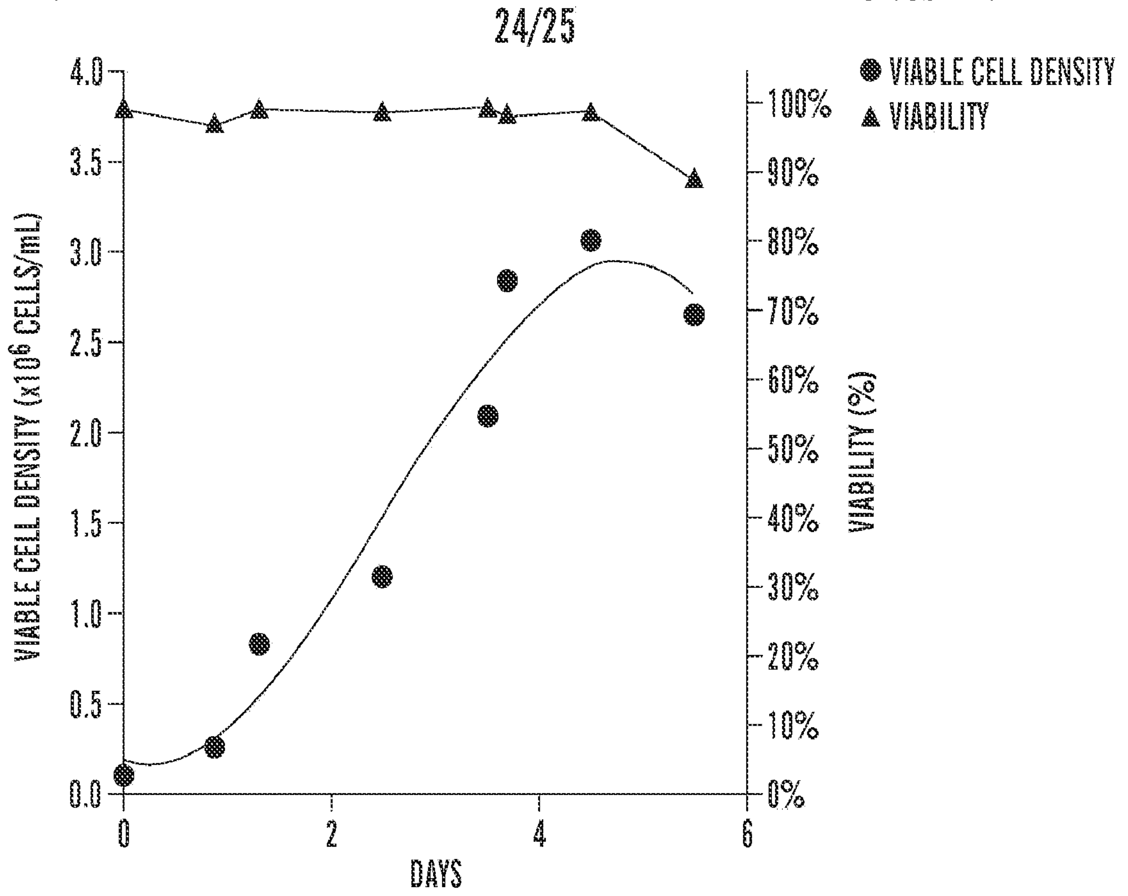


FIG. 29

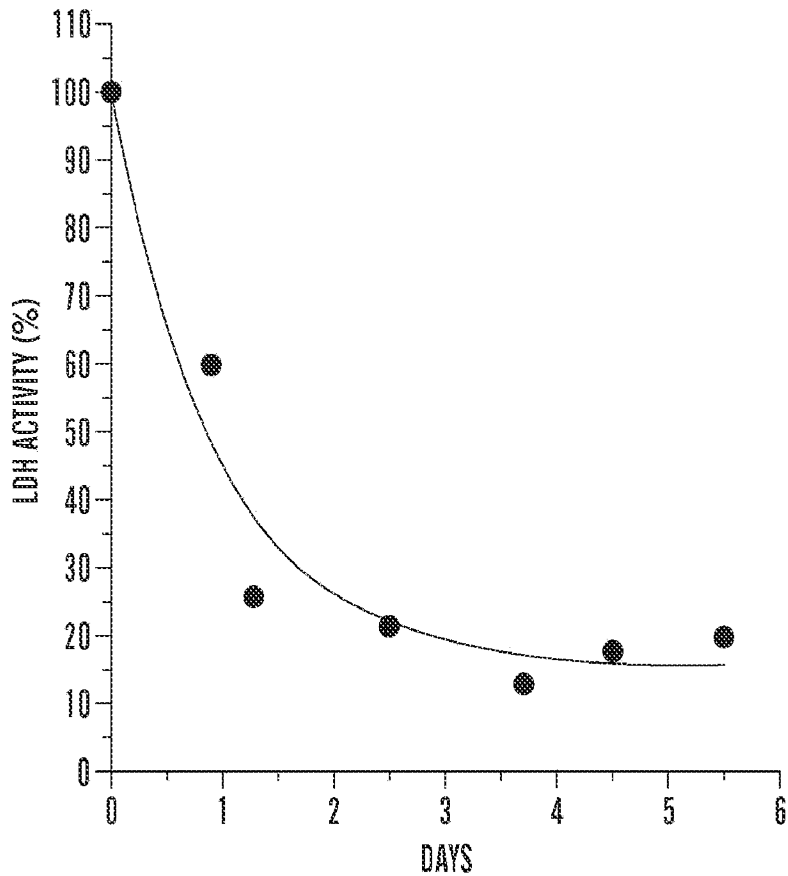


FIG. 30

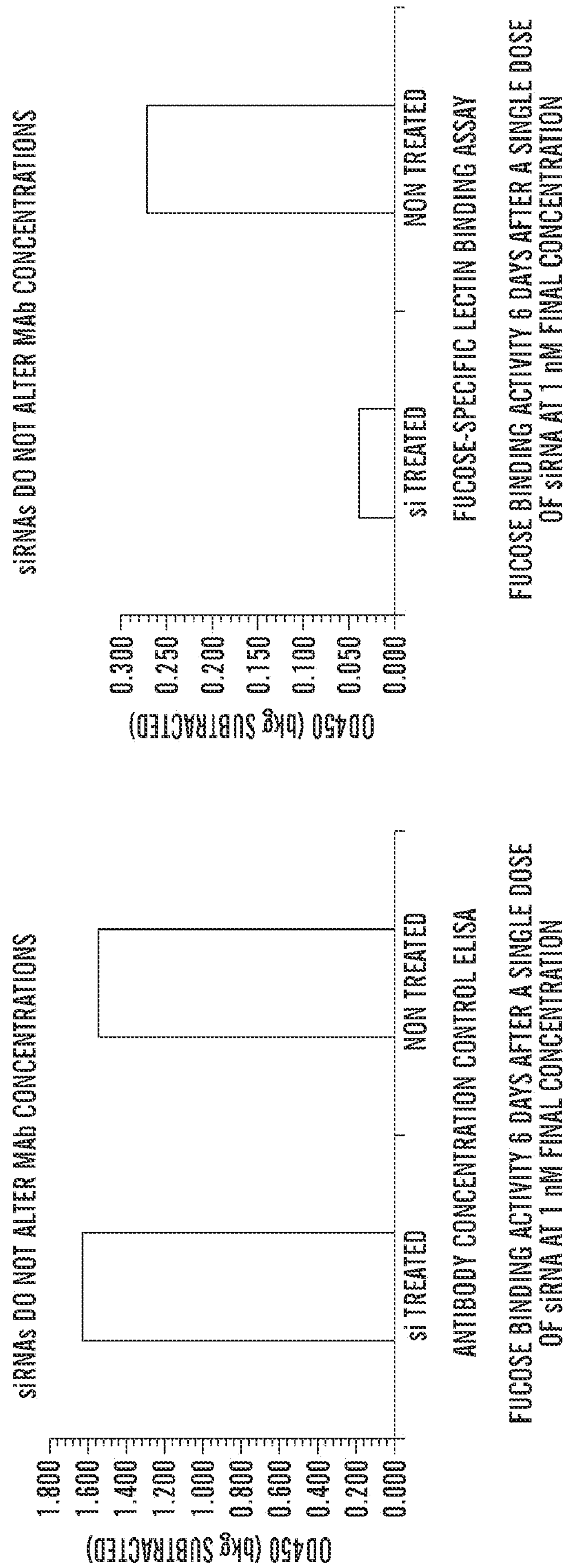


FIG. 31A

FIG. 31B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/41106

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00; C12N 15/11; C07H 21/02 (2010.01)
USPC - 424/184.1, 514/44A, 536/23.1
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)
IPC(8) - A61K 39/00; C12N 15/11; C07H 21/02 (2010.01)
USPC - 424/184.1, 514/44A, 536/23.1

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)
WEST - DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=YES; OP=ADJ; Google Scholar
search terms: immunog$, antibod$, protein, peptide, polypeptide, oligopeptide, produc$, generat$, make, making, made, develop$, manufact$, engineer$, cultivat$, create, creating, secret$, created, creation, improv$, enhanc$, optim$, better, efficien$, yield, promot$,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 2007/0130628 A1 (BROWN) 07 June 2007 (07.06.2007) abstract; claim 2; para [0045]-[0048]; [0061]; [0071]; [0109]; [0124].	2 ---- 1, 3-5, 65, 66, 110-119
Y	US 2009/0111143 A1 (GOLDENBERG et al.) 30 April 2009 (30.04.2009) abstract; para [0004]; [0009]; [0010]; [0030]; [0036]-[0048]; [0082]; [0145].	1, 3-5, 65, 66
Y	Lim et al. RNAi suppression of Bax and Bak enhances viability in fed-batch cultures of CHO cells. Metabolic Engineering 8 (2006) 509-522. abstract; Fig. 1; Table 1; Fig. 5B; p. 511, 1st-3rd para; p. 512, 1st para; p. 514, 2nd and 6th para; p. 516, 6th para; p. 518, 1st para; p. 519, 1st and 2nd para.	110-119, 125-141
Y	US 2005/0196755 A1 (ZAUDERER et al.) 08 September 2005 (08.09.2005) para [0033]; [0053].	125-141
Y	Fantin et al., Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell 9, 425-434, June 2006. p. 425, 2nd para; p. 426, 2nd-4th para; p. 432, 2nd para.	4, 5, 66, 112, 139
Y	US 2007/0173476 A1 (LEAKE et al.) 26 July 2007 (26.07.2007) abstract; para [0011]; [0093]; [0164]; [0253].	118, 141

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2010 (13.10.2010)	25 OCT 2010

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/41106

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-64, 67-109, 120-124, 142, 143
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.