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ICE-BASED LIPID NANOPARTICLE FORMULATIONS FOR DELIVERY OF MRNA

Abstract

The present invention provides, among other things, compositions and methods of formulating nucleic acid-containing nanoparticles comprising no more than three distinct lipids components, one distinct lipid component being a sterol-based cationic lipid. In some embodiments, the present invention provides compositions and methods in which the lipid nanoparticles further comprise helper lipids and PEG-modified lipids. The resulting formulation comprises a high encapsulation percentage for nucleic acids.

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Background/Summary

RELATED APPLICATIONS [0001] This application is a continuation of U.S. patent application Ser. No. 17/239,131, filed Apr. 23, 2021, which is a divisional of U.S. patent application Ser. No. 16/599,928, filed Oct. 11, 2019, now U.S. Pat. No. 11,013,812, which is a divisional of U.S. patent application Ser. No. 15/809,605, filed Nov. 10, 2017, now U.S. Pat. No. 10,471,153, which claims priority to U.S. Provisional Patent Application Ser. Nos. 62/420,421, filed Nov. 10, 2016, 62/420,428, filed Nov. 10, 2016, 62/421,021, filed Nov. 11, 2016, 62/421,007, filed Nov. 11, 2016, 62/464,327, filed Feb. 27, 2017, and 62/464,330, filed Feb. 27, 2017, the entire disclosures of which are hereby incorporated herein by reference.

SEQUENCE LISTING
[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML file, created on Jan. 15, 2025, is named 761236_SA9-871DIV3CON_ST26.xml and is 48,473 bytes in size.
BACKGROUND
[0003] Nucleic acid-based technologies are increasingly important for various therapeutic applications including, but not limited to, messenger RNA therapy. Efforts to deliver nucleic acids have included the creation of compositions formulated to protect nucleic acids from degradation when delivered in vivo. One type of delivery vehicle for nucleic acids has been lipid nanoparticles. Important parameters to consider for the successful use of lipid nanoparticles as a delivery vehicle include lipid nanoparticle formation, physical properties of lipid components, nucleic acid encapsulation efficiencies, in vivo nucleic acid release potential, and lipid nanoparticle toxicity.
SUMMARY OF THE INVENTION
[0004] This present invention offers a unique solution wherein sterol-based cationic lipid, helper lipid and PEG-modified lipid form a lipid nanoparticle formulation of RNA. This inventive three lipid component system upon formulation shows high RNA encapsulation efficiencies and successful efficacious delivery in vivo, particularly pulmonary delivery. Such formulation systems offer additional advantages of lower lipid load (compared to other conventional four lipid component systems) and higher tolerability/lower toxicity as the metabolized products of the cationic lipid (cholesterol derivative lipid) is cholesterol.
[0005] The invention is based, in part, on the surprising discovery that a three lipid component system based on sterol-based cationic lipids is unexpectedly effective in delivering mRNA and producing encoded protein or peptide in vivo, particularly in the lung. Indeed, prior to the present invention, cationic lipids have been extensively explored as an important component of liposomes

used to encapsulate nucleic acids, including mRNA, for in vivo delivery. Due to the uniquely fragile and long structure of mRNA and the complicated in vivo translation process, cationic lipids used in the liposomes typically play two roles. First, cationic lipids promote interaction with negatively charged mRNA during encapsulation, circulation and endocytosis, thereby capturing and protecting the mRNA. Then, once inside cytosol, cationic lipids need to be able to release the mRNA so that the mRNA can be translated to produce the encoded protein or peptide. Some cationic lipids, in particular, known as titratable cationic lipids, are particularly effective in delivering mRNA. Surprisingly, the present inventors found that liposomes comprising the sterol-based cationic lipids described herein can have an even higher encapsulation percentage for mRNA and can be even more effective in delivering various mRNA in vivo. Particularly, liposomes comprising the sterol-based cationic lipids described herein can be incredibly effective for pulmonary delivery of mRNA, and surprisingly successful for delivering mRNA via nebulization. Thus, the present inventors have demonstrated that the three lipid component system can be uniquely useful in delivering mRNA for highly efficient and sustained production of protein or peptide (e.g., therapeutic protein) in vivo, particularly in the pulmonary system. The present invention therefore permits an improved mRNA therapy that can significantly reduce the required amount of mRNA and associated lipids, administration frequency, and possible side effects, providing safer, more potent, and patient friendly mRNA therapy for various diseases.

[0006] In one aspect, the present invention provides methods of delivering nucleic acids in vivo comprising administering by pulmonary delivery to a subject in need of delivery a composition comprising nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid.

[0007] In another aspect, the present invention provides methods of delivering nucleic acids in vivo comprising administering by pulmonary delivery to a subject in need of delivery a composition comprising nucleic acids, wherein the nucleic acids encode a Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid.

[0008] In some embodiments, the pulmonary delivery comprises nebulization. In some embodiments, said subject is a subject in need of delivery.

[0009] In some embodiments, the lipid nanoparticles have an encapsulation percentage for nucleic acids of at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%).

[0010] In some embodiments, the three distinct lipid components comprise one or both of helper lipids and PEG-modified lipids.

[0011] In some embodiments, the sterol-based cationic lipid has a structure according to Formula (A), text missing or illegible when filed [0012] B-L.sup.1-S (Formula A), or a protonated form thereof, wherein B is a basic functional group wherein the protonated form has a pK.sub.a that is no more than about 8.0; L.sup.1 is an optionally substituted linker group that is a C.sub.1-C.sub.20 alkylene or a 2- to 20-membered heteroalkylene; and S is a sterol.

[0013] In some embodiments, B is an optionally substituted 5- or 6-membered nitrogen-containing heteroaryl. In some embodiments, B is a group selected from pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted.

[0014] In some embodiments, L.sup.1 is an optionally substituted linker group that is a C.sub.1-C.sub.20 alkylene. In some embodiments, L.sup.1 is an optionally substituted linker group that is a

2- to 20-membered heteroalkylene. In some embodiments, L.sup.1 is a 2- to 20-membered heteroalkylene that is non-peptidic. In some embodiments, L.sup.1 comprises a moiety that is an ester group, an amide group, a carbamate group, a carbonate group, or a urea group. In some embodiments, L.sup.1 comprises a moiety that is an amide group, a carbamate group, a carbonate group, or a urea group. In some embodiments, L.sup.1 comprises a moiety that is an amide group, a carbonate group, or a urea group. In some embodiments, L.sup.1 is —X.sup.1—C(X.sup.3)—X.sup.2, —(C.sub.1-C.sub.19 alkylene)-X.sup.1—C(X.sup.3)—X.sup.2, —X.sup.1—C(X.sup.3)—X.sup.2(C.sub.1-C.sub.19 alkylene)-, —(C.sub.1-C.sub.19 alkylene)-X.sup.1—, —X.sup.1—(C.sub.1-C.sub.19 alkylene)-, wherein each X.sup.1 and X.sup.2 is independently, a covalent bond, —O—, —S—, or —NH—; X.sup.3 is independently ═O, ═S, or ═NH; and wherein said C.sub.1-C.sub.19 alkylene is optionally substituted. In some embodiments, L.sup.1 does not comprise substituents having the structure —N(R′).sub.2, or a positively charged form thereof, wherein each R′ is independently hydrogen or optionally substituted C.sub.1-C.sub.20 alkyl.

[0015] In some embodiments, S is a zoosterol, or an oxidized or reduced form thereof. In some embodiments, S is a phytosterol, or an oxidized or reduced form thereof. In some embodiments, S is a synthetic sterol, or an oxidized or reduced form thereof. In some embodiments, S is a sterol selected from cholesterol, an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol. In some embodiments, S is a sterol selected from an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol. In some embodiments, S is a sterol selected from

##STR00001##

wherein R is optionally substituted C.sub.1-C.sub.20 alkyl. In some embodiments, S is a sterol selected from

##STR00002## ##STR00003##

wherein R is optionally substituted C.sub.1-C.sub.20 alkyl.

[0016] In some embodiments, the sterol-based cationic lipid comprises imidazole cholesterol ester (ICE). In some embodiments, the sterol-based cationic lipid does not comprise imidazole cholesterol ester (ICE).

[0017] In some embodiments, the nucleic acids are selected from DNA, siRNA, microRNA, and/or mRNA. In some embodiments, the nucleic acids are mRNA encoding a protein or a peptide. In some embodiments, the mRNA encoding a protein or a peptide is codon-optimized. In some embodiments, the mRNA comprises one or more modified nucleotides. In some embodiments, the mRNA comprises a modification of the 5′ untranslated region of said mRNA. In some embodiments, said modification of the 5′ untranslated region comprises the inclusion of a Cap1 structure. In some embodiments, the mRNA comprises a modification of the 3′ untranslated region of said mRNA. In some embodiments, said modification of the 3′ untranslated region comprises the inclusion of a poly A tail.

[0018] In some embodiments, the lipid nanoparticles have a size less than about 100 nm, 95 nm, 90 nm, 85 nm, 80 nm, 75 nm, 70 nm, 65 nm, 60 nm, 55 nm, 50 nm, 45 nm or 40 nm.

[0019] In another aspect, the present invention provides compositions formulated for nebulization comprising nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the lipid nanoparticles have an encapsulation percentage for nucleic acids of at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%).

[0020] In another aspect, the present invention provides compositions comprising nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the lipid nanoparticles have an encapsulation

percentage for nucleic acids of at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%).
[0021] In some embodiments, the lipid nanoparticles have an encapsulation percentage for nucleic acids of at least 85%. In some embodiments, the lipid nanoparticles have an encapsulation percentage for nucleic acids of at least 90%.
[0022] In another aspect, the present invention provides compositions comprising mRNA encoding a Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein; and lipid nanoparticles encapsulating the mRNA, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the lipid nanoparticles have an encapsulation percentage for mRNA of at least 80%.
[0023] In some embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 85% or at least 90%.
[0024] In another aspect, the present invention provides compositions comprising nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the sterol-based cationic lipid constitutes no more than 70% of the total lipids (e.g., no more than 68%, no more than 67%, no more than 66%, no more than 65%, no more than 60%, no more than 55%, no more than 50%, no more than 45%, or no more than 40%).
[0025] In another aspect, the present invention provides compositions comprising mRNA encoding a Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein; and lipid nanoparticles encapsulating the mRNA, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the sterol-based cationic lipid constitutes no more than 70% of the total lipids (e.g., no more than 68%, no more than 67%, no more than 66%, no more than 65%, no more than 60%, no more than 55%, no more than 50%, no more than 45%, or no more than 40%).
[0026] In some embodiments, the sterol-based cationic lipid constitutes no more than 65% of the total lipids. In some embodiments, the sterol-based cationic lipid constitutes no more than 60% of the total lipids. In some embodiments, the three distinct lipid components comprise one or both of helper lipids and PEG-modified lipids.
[0027] In some embodiments, the sterol-based cationic lipid has a structure according to Formula (A), text missing or illegible when filed [0028] B-L.sup.1-S (Formula A), or a protonated form thereof, wherein B is a basic functional group wherein the protonated form has a pK.sub.a that is no more than about 8.0; L.sup.1 is an optionally substituted linker group that is a C.sub.1-C.sub.20 alkylene or a 2- to 20-membered heteroalkylene; and S is a sterol.
[0029] In some embodiments, B is an optionally substituted 5- or 6-membered nitrogen-containing heteroaryl. In some embodiments, B is a group selected from pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted.
[0030] In some embodiments, L.sup.1 is an optionally substituted linker group that is a C.sub.1-C.sub.20 alkylene. In some embodiments, L.sup.1 is an optionally substituted linker group that is a 2- to 20-membered heteroalkylene. In some embodiments, L.sup.1 is a 2- to 20-membered heteroalkylene that is non-peptidic. In some embodiments, L.sup.1 comprises a moiety that is an ester group, an amide group, a carbamate group, a carbonate group, or a urea group. In some embodiments, L.sup.1 comprises a moiety that is an amide group, a carbamate group, a carbonate group, or a urea group. In some embodiments, L.sup.1 comprises a moiety that is an amide group, a

carbonate group, or a urea group. In some embodiments, L.sup.1 is —X.sup.1—C(X.sup.3)—X.sup.2, —(C.sub.1-C.sub.19 alkylene)-X.sup.1—C(X.sup.3)—X.sup.2, —X.sup.1—C(X.sup.3)—X.sup.2(C.sub.1-C.sub.19 alkylene)-, —(C.sub.1-C.sub.19 alkylene)-X.sup.1—, —X.sup.1—(C.sub.1-C.sub.19 alkylene)-, wherein each X.sup.1 and X.sup.2 is independently, a covalent bond, —O—, —S—, or —NH—; X.sup.3 is independently ═O, ═S, or ═NH; and wherein said C.sub.1-C.sub.19 alkylene is optionally substituted. In some embodiments, L.sup.1 does not comprise substituents having the structure —N(R′).sub.2, or a positively charged form thereof, wherein each R′ is independently hydrogen or optionally substituted C.sub.1-C.sub.20 alkyl.

[0031] In some embodiments, S is a zoosterol, or an oxidized or reduced form thereof. In some embodiments, S is a phytosterol, or an oxidized or reduced form thereof. In some embodiments, S is a synthetic sterol, or an oxidized or reduced form thereof. In some embodiments, S is a sterol selected from cholesterol, an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol. In some embodiments, S is a sterol selected from an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol. In some embodiments, S is a sterol selected from

##STR00004## ##STR00005##

wherein R is optionally substituted C.sub.1-C.sub.20 alkyl. In some embodiments, S is a sterol selected from

##STR00006##

wherein R is optionally substituted C.sub.1-C.sub.20 alkyl.

[0032] In some embodiments, the sterol-based cationic lipid comprises imidazole cholesterol ester (ICE). In some embodiments, the sterol-based cationic lipid does not comprise imidazole cholesterol ester (ICE).

[0033] In some embodiments, the nucleic acids are selected from DNA, siRNA, microRNA, and/or mRNA. In some embodiments, the nucleic acids are mRNA encoding a protein or a peptide. In some embodiments, the mRNA encoding a protein or a peptide is codon-optimized. In some embodiments, the mRNA comprises one or more modified nucleotides. In some embodiments, the mRNA comprises a modification of the 5′ untranslated region of said mRNA. In some embodiments, said modification of the 5′ untranslated region comprises the inclusion of a Cap1 structure. In some embodiments, the mRNA comprises a modification of the 3′ untranslated region of said mRNA. In some embodiments, said modification of the 3′ untranslated region comprises the inclusion of a poly A tail.

[0034] In some embodiments, the lipid nanoparticle has a size less than about 100 nm, 95 nm, 90 nm, 85 nm, 80 nm, 75 nm, 70 nm, 65 nm, 60 nm, 55 nm, 50 nm, 45 nm or 40 nm.

[0035] In some embodiments, the mRNA encoding a CFTR protein comprises SEQ ID NO: 1. In some embodiments, the mRNA encoding a CFTR protein comprises a polynucleotide sequence at least 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1.

[0036] In some embodiments, the lipid nanoparticle is formed by mixing an mRNA solution and a lipid solution into a 20% ethanol. In some embodiments, the lipid nanoparticles are further purified by Tangential Flow Filtration.

[0037] In another aspect, the present invention provides methods of delivering mRNA in vivo comprising administering a composition to a subject. In some embodiments, said subject is a subject in need of delivery. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is administered by pulmonary delivery. In some embodiments, the pulmonary delivery comprises nebulization.

[0038] In this application, the use of “or” means “and/or” unless stated otherwise. As used in this disclosure, the term “comprise” and variations of the term, such as “comprising” and “comprises,” are not intended to exclude other additives, components, integers or steps. As used in this application, the terms “about” and “approximately” are used as equivalents. Both terms are meant

to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.
[0039] Other features, objects, and advantages of the present invention are apparent in the detailed description, drawings and claims that follow. It should be understood, however, that the detailed description, the drawings, and the claims, while indicating embodiments of the present invention, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art.

Description

BRIEF DESCRIPTION OF THE DRAWING
[0040] The drawings are for illustration purposes only, not for limitation.
[0041] FIG. **1** depicts exemplary immunohistochemical detection of human Cystic Fibrosis Transmembrane Conductance Regulator (hCFTR) protein in mouse lung 24 hours after treatment with ICE-based LNPs encapsulating codon-optimized hCFTR mRNA, B=Untreated mouse lung at 10× & 20× magnification, respectively. C, D=CO-hCFTR mRNA ICE LNP-treated mouse lung at 10× & 20× magnification, respectively.
[0042] FIG. **2** depicts exemplary immunohistochemical detection of human CFTR protein in mouse lung 24 hours after treatment with ICE-based LNPs encapsulating codon-optimized hCFTR. A, B=Untreated mouse lung at 10× & 20× magnification, respectively. C, D=CO-hCFTR mRNA ICE LNP-treated mouse lung at 10× & 20× magnification, respectively.
[0043] FIG. **3** depicts exemplary immunohistochemical detection of human Cystic Fibrosis Transmembrane Conductance Regulator (hCFTR) protein in the lungs of rats 24 hours after treatment with nebulized ICE-based lipid nanoparticles (LNPs) (5% PEG) encapsulating codon-optimized hCFTR mRNA.
[0044] FIG. **4** depicts exemplary immunohistochemical detection of hCFTR protein in the lungs of rats 24 hours after treatment with ICE-based LNPs (5% PEG) encapsulating codon-optimized hCFTR mRNA via intratracheal administration.
[0045] FIG. **5** depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of rats 24 hours after treatment with nebulized ICE-based LNPs (3% PEG) encapsulating codon-optimized (CO) hCFTR mRNA.
[0046] FIG. **6** depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of rats 24 hours after treatment with ICE-based LNPs (3% PEG) encapsulating CO-hCFTR mRNA via intratracheal (Microsprayer) administration.
[0047] FIG. **7** depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of CFTR KO (knockout) mice 24 hours after treatment with nebulized ICE-based LNPs (N/P=2) encapsulating CO-hCFTR mRNA.
[0048] FIG. **8** depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of CFTR KO mice 24 hours after treatment with nebulized ICE-based LNPs (N/P=4) encapsulating CO-hCFTR mRNA.
[0049] FIG. **9** depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of wild-type mice 24 hours after treatment with nebulized ICE-based LNPs encapsulating various mRNA constructs including CFTR (CO-hCFTR mRNA), STOP (nonsense mutated CO-hCFTR mRNA unable to be translated into protein), and FFL (Firefly Luciferase mRNA).
[0050] FIG. **10** depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of wild-type mice at various time points after a single exposure to nebulized ICE-based LNPs encapsulating CO-hCFTR mRNA.
[0051] FIG. **11** depicts an exemplary graph of the ratio of copies of CO-hCFTR mRNA per micrograms of total RNA in frozen lung sections from rats treated with either controls (buffer, Empty ICE LNP) or CO-hCFTR mRNA loaded ICE LNPs.

[0052] FIG. **12** depicts an exemplary graph of fold-increase of copies of CO-hCFTR over endogenous levels of CFTR mRNA in frozen lung sections of rats treated with either controls (buffer, Empty ICE LNP) or CO-hCFTR mRNA loaded ICE LNPs.
[0053] FIG. **13** depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of rats after a single exposure to various doses of nebulized ICE-based LNPs encapsulating CO-hCFTR mRNA.
[0054] FIGS. **14**A and **14**B depict exemplary graphs of the effects of various dosages of hCFTR mRNA formulated with either branched PEI (FIG. **14**A) or ICE (FIG. **14**B) on the respiratory rate of rats relative to a control.
[0055] FIG. **15** depicts an exemplary graph showing a dose response of chloride-ion channel activity induced by hCFTR mRNA.

DEFINITIONS

[0056] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification. The publications and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.
[0057] Alkyl: As used herein, “alkyl” refers to a radical of a straight-chain or branched saturated hydrocarbon group having from 1 to 15 carbon atoms (“C.sub.1-15 alkyl”). In some embodiments, an alkyl group has 1 to 3 carbon atoms (“C.sub.1-3 alkyl”). Examples of C.sub.1-3 alkyl groups include methyl (C.sub.1), ethyl (C.sub.2), n-propyl (C.sub.3), and isopropyl (C.sub.3). In some embodiments, an alkyl group has 8 to 12 carbon atoms (“C.sub.8-12 alkyl”). Examples of C.sub.8-12 alkyl groups include, without limitation, n-octyl (C.sub.8), n-nonyl (C.sub.9), n-decyl (C.sub.10), n-undecyl (C.sub.11), n-dodecyl (C.sub.12) and the like. The prefix “n-” (normal) refers to unbranched alkyl groups. For example, n-C.sub.8 alkyl refers to —(CH.sub.2).sub.7CH.sub.3, n-C.sub.10 alkyl refers to —(CH.sub.2).sub.9CH.sub.3, etc.
[0058] Amino acid: As used herein, term “amino acid,” in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure H.sub.2N—C(H)(R)—COHO. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a d-amino acid; in some embodiments, an amino acid is an 1-amino acid. “Standard amino acid” refers to any of the twenty standard 1-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, “synthetic amino acid” encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxyl- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical groups that can change the peptide's circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as association with one or more chemical entities (e.g., methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, etc.). The term “amino acid” is used interchangeably with “amino acid residue,” and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.
[0059] Animal: As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In certain

embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0060] Approximately or about: As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0061] Delivery: As used herein, the term “delivery” encompasses both local and systemic delivery. For example, delivery of mRNA encompasses situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and retained within the target tissue (also referred to as “local distribution” or “local delivery”), and situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and secreted into patient's circulation system (e.g., serum) and systematically distributed and taken up by other tissues (also referred to as “systemic distribution” or “systemic delivery).

[0062] Encapsulation: As used herein, the term “encapsulation,” or grammatical equivalent, refers to the process of confining an mRNA molecule within a nanoparticle.

[0063] Expression: As used herein, “expression” of a nucleic acid sequence refers to translation of an mRNA into a peptide, polypeptide, or protein, assembly of multiple polypeptides (e.g., heavy chain or light chain of antibody) into an intact protein (e.g., antibody) and/or post-translational modification of a polypeptide or fully assembled protein (e.g., antibody). In this application, the terms “expression” and “production,” and grammatical equivalent, are used inter-changeably.

[0064] Functional: As used herein, a “functional” biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[0065] Half-life: As used herein, the term “half-life” is the time required for a quantity such as nucleic acid or protein concentration or activity to fall to half of its value as measured at the beginning of a time period.

[0066] Improve, increase, or reduce: As used herein, the terms “improve,” “increase” or “reduce,” or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control subject (or multiple control subject) in the absence of the treatment described herein. A “control subject” is a subject afflicted with the same form of disease as the subject being treated, who is about the same age as the subject being treated.

[0067] Impurities: As used herein, the term “impurities” refers to substances inside a confined amount of liquid, gas, or solid, which differ from the chemical composition of the target material or compound. Impurities are also referred to as contaminants.

[0068] In Vitro: As used herein, the term “in vitro” refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, etc., rather than within a multi-cellular organism.

[0069] In Vivo: As used herein, the term “in vivo” refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, in vitro systems).

[0070] Isolated: As used herein, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared,

and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. As used herein, calculation of percent purity of isolated substances and/or entities should not include excipients (e.g., buffer, solvent, water, etc.).

[0071] Local distribution or delivery: As used herein, the terms “local distribution,” “local delivery,” or grammatical equivalent, refer to tissue specific delivery or distribution. Typically, local distribution or delivery requires a protein or peptide encoded by mRNAs be translated and expressed intracellularly or with limited secretion that avoids entering the patient's circulation system.

[0072] messenger RNA (mRNA): As used herein, the term “messenger RNA (mRNA)” refers to a polynucleotide that encodes at least one peptide, polypeptide or protein. mRNA as used herein encompasses both modified and unmodified RNA. mRNA may contain one or more coding and non-coding regions. mRNA can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, etc. Where appropriate, e.g., in the case of chemically synthesized molecules, mRNA can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, etc. An mRNA sequence is presented in the 5′ to 3′ direction unless otherwise indicated. In some embodiments, an mRNA is or comprises natural nucleosides (e.g., adenosine, guanosine, cytidine, uridine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (e.g., 2′-fluororibose, ribose, 2′-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5′-N-phosphoramidite linkages).

[0073] In some embodiments, the mRNA comprises one or more nonstandard nucleotide residues. The nonstandard nucleotide residues may include, e.g., 5-methyl-cytidine (“5mC”), pseudouridine (“ψU”), and/or 2-thio-uridine (“2sU”). See, e.g., U.S. Pat. No. 8,278,036 or WO2011012316 for a discussion of such residues and their incorporation into mRNA. The mRNA may be RNA, which is defined as RNA in which 25% of U residues are 2-thio-uridine and 25% of C residues are 5-methylcytidine. Teachings for the use of RNA are disclosed US Patent Publication US 2012/0195936 and international publication WO 2011/012316, both of which are hereby incorporated by reference in their entirety. The presence of nonstandard nucleotide residues may render an mRNA more stable and/or less immunogenic than a control mRNA with the same sequence but containing only standard residues. In further embodiments, the mRNA may comprise one or more nonstandard nucleotide residues chosen from isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine and 2-chloro-6-aminopurine cytosine, as well as combinations of these modifications and other nucleobase modifications. Certain embodiments may further include additional modifications to the furanose ring or nucleobase. Additional modifications may include, for example, sugar modifications or substitutions (e.g., one or more of a 2′-O-alkyl modification, a locked nucleic acid (LNA)). In some embodiments, the RNAs may be complexed or hybridized with additional polynucleotides and/or peptide polynucleotides (PNA). In embodiments where the sugar modification is a 2′-O-alkyl modification, such modification may include, but are not limited to a

2′-deoxy-2′-fluoro modification, a 2′-O-methyl modification, a 2′-O-methoxyethyl modification and a 2′-deoxy modification. In certain embodiments, any of these modifications may be present in 0-100% of the nucleotides—for example, more than 0%, 1%, 10%, 25%, 50%, 75%, 85%, 90%, 95%, or 100% of the constituent nucleotides individually or in combination.

[0074] Nucleic acid: As used herein, the term "nucleic acid," in its broadest sense, refers to any compound and/or substance that is or can be incorporated into a polynucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into a polynucleotide chain via a phosphodiester linkage. In some embodiments, "nucleic acid" refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides). In some embodiments, "nucleic acid" refers to a polynucleotide chain comprising individual nucleic acid residues. In some embodiments, "nucleic acid" encompasses RNA as well as single and/or double-stranded DNA and/or cDNA. Furthermore, the terms "nucleic acid," "DNA," "RNA," and/or similar terms include nucleic acid analogs, i.e., analogs having other than a phosphodiester backbone. For example, the so-called "peptide nucleic acids," which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. The term "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and/or encode the same amino acid sequence. Nucleotide sequences that encode proteins and/or RNA may include introns. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, etc. Where appropriate, e.g., in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, etc. A nucleic acid sequence is presented in the 5′ to 3′ direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (e.g., 2′-fluororibose, ribose, 2′-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5′-N-phosphoramidite linkages). In some embodiments, the present invention is specifically directed to "unmodified nucleic acids," meaning nucleic acids (e.g., polynucleotides and residues, including nucleotides and/or nucleosides) that have not been chemically modified in order to facilitate or achieve delivery.

[0075] Patient: As used herein, the term "patient" or "subject" refers to any organism to which a provided composition may be administered, e.g., for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. A human includes pre and post-natal forms.

[0076] Pharmaceutically acceptable: The term "pharmaceutically acceptable" as used herein, refers to substances that, within the scope of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0077] Pharmaceutically acceptable salt: Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed

with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and N.sup.+(C.sub.1-4 alkyl).sub.4 salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium. quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, sulfonate and aryl sulfonate. Further pharmaceutically acceptable salts include salts formed from the quarternization of an amine using an appropriate electrophile, e.g., an alkyl halide, to form a quarternized alkylated amino salt.

[0078] Subject: As used herein, the term “subject” refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term “subject” is used herein interchangeably with “individual” or “patient.” A subject can be afflicted with or is susceptible to a disease or disorder (e.g., cystic fibrosis) but may or may not display symptoms of the disease or disorder.

[0079] Substantially: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0080] Systemic distribution or delivery: As used herein, the terms “systemic distribution,” “systemic delivery,” or grammatical equivalent, refer to a delivery or distribution mechanism or approach that affect the entire body or an entire organism. Typically, systemic distribution or delivery is accomplished via body's circulation system, e.g., blood stream. Compared to the definition of “local distribution or delivery.”

[0081] Target tissues: As used herein, the term “target tissues” refers to any tissue that is affected by a disease to be treated. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature.

[0082] Transfer vehicle: In some embodiments, the transfer vehicle is a liposomal vesicle, or other means to facilitate the transfer of a nucleic acid to target cells and tissues. Suitable transfer vehicles include, but are not limited to, liposomes, nanoliposomes, ceramide-containing nanoliposomes, proteoliposomes, nanoparticulates, calcium phosphor-silicate nanoparticulates, calcium phosphate nanoparticulates, silicon dioxide nanoparticulates, nanocrystalline particulates, semiconductor nanoparticulates, poly(D-arginine), nanodendrimers, starch-based delivery systems, micelles, emulsions, niosomes, plasmids, viruses, calcium phosphate nucleotides, aptamers, peptides and other vectorial tags. Also contemplated is the use of bionanocapsules and other viral capsid proteins assemblies as a suitable transfer vehicle. (Hum. Gene Ther. 2008 September; 19(9):887-95).

[0083] Therapeutically effective amount: As used herein, the term “therapeutically effective

amount" of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[0084] Treating: As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

[0085] Yield: As used herein, the term "yield" refers to the percentage of mRNA recovered after encapsulation as compared to the total mRNA as starting material. In some embodiments, the term "recovery" is used interchangeably with the term "yield".

DETAILED DESCRIPTION

[0086] The present invention provides, among other things, methods and compositions for delivering mRNA in vivo using improved liposomes incorporating a sterol-based cationic lipid, a helper lipid, and a PEG or PEG-modified lipid as described herein.

Lipid Nanoparticles

[0087] According to the present invention, suitable compositions described herein comprise nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein said lipid nanoparticles comprise distinct lipid components. In some embodiments, there are no more than three distinct lipid components and exemplary distinct lipid components include sterol-based cationic lipids, helper lipids (e.g., non-cationic lipids), and PEG-modified lipids.

[0088] In some embodiments, the lipid nanoparticles have an encapsulation percentage of nucleic acids that is at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%.

[0089] In some embodiments, the percentage of lipid nanoparticles in a composition that encapsulate a nucleic acid is at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%.

Sterol-Based Cationic Lipids

[0090] As used herein, the phrase "sterol-based lipid" or "sterol-based cationic lipid" refers to a cationic lipid comprising a steroid moiety. As used herein, the phrase "cationic lipid" refers to any of a number of lipid species that carry a net positive charge at a selected pH, such as physiological pH.

[0091] In embodiments, a sterol-based cationic lipid has a structure according to Formula (A), text missing or illegible when filed [0092] B-L.sup.1-S (Formula A), or a protonated form thereof. [0093] B is a basic functional group (e.g., wherein the protonated form has a pK.sub.a that is no more than about 8.0). [0094] L.sup.1 is an optionally substituted linker group (e.g., a C.sub.1-C.sub.20 alkylene or a 2- to 20-membered heteroalkylene). [0095] S is a sterol.

[0096] In some embodiments, B is a basic functional group wherein the protonated form has a pK.sub.a that is no more than about 9.0, about 8.5, about 8.0, about 7.5, or about 7.0. In embodiments, B is a basic functional group wherein the protonated form has a pK.sub.a that is no more than about 8.0 or about 7.5.

[0097] In some embodiments, B is a basic functional group wherein the protonated form has a pK.sub.a that is about 4.0 to about 9.0, about 4.0 to about 8.0, about 4.5 to about 8.0, about 5.0 to about 8.0, about 5.5 to about 8.0, or about 6.0 to about 8.0.

[0098] In some embodiments, B is an optionally substituted 5- or 6-membered nitrogen-containing heteroaryl.

[0099] In some embodiments, B is a group selected from pyrrolyl, imidazolyl, pyrazolyl, triazolyl,

tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. For any optionally substituted group described herein (e.g., a group that has 0, 1, 2, 3, or 4 independent substituent groups), exemplary substituent groups include but are not limited to: halogen (e.g., —F, —Cl, —Br, or —I), C.sub.1-20 alkyl (e.g., a C.sub.1-6 alkyl), C.sub.1-20 haloalkyl (e.g., a C.sub.1-6 haloalkyl), —CN, —OH, —O(C.sub.1-20 alkyl) (e.g., —O(C.sub.1-6 alkyl)), —O(C.sub.1-20 haloalkyl) (e.g., —O(C.sub.1-6 haloalkyl)), —NH.sub.2, —NH(C.sub.1-6 alkyl), and —NH(C.sub.1-6 alkyl).sub.2.

[0100] In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted.

[0101] In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted.

[0102] In some embodiments, L.sup.1 is a linker group that is optionally substituted C.sub.1-C.sub.20 alkylene (e.g., an optionally substituted C.sub.1-C.sub.10 alkylene or an optionally substituted C.sub.1-C.sub.5 alkylene). As used herein, the term “alkylene” represents a saturated divalent straight or branched chain hydrocarbon group and is exemplified by methylene, ethylene, isopropylene and the like.

[0103] In some embodiments, L.sup.1 is a linker group that is optionally substituted 2- to 20-membered heteroalkylene (e.g., an optionally substituted 2- to 12-membered heteroalkylene or an optionally substituted 2- to 6-membered heteroalkylene). As used herein, the term “heteroalkylene” refers to a divalent heteroalkyl group. The term “heteroalkyl group” refers to a stable straight or branched chain hydrocarbon radical, having the stated number of atoms, wherein the atoms are selected from carbon and from one to three heteroatoms selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms can optionally be oxidized and the nitrogen heteroatom can optionally be quaternized. The heteroatom(s) 0, N and S can be placed at any interior position of the heteroalkyl group.

[0104] In some embodiments, L.sup.1 is a linker group that is optionally substituted 2- to 20-membered heteroalkylene, and wherein L.sup.1 is non-peptidic (that is, a non-peptidic linker L.sup.1 is one that does not comprise amino acid residues).

[0105] In some embodiments, L.sup.1 is a linker group that comprises a moiety that is an ester group, an amide group, a carbamate group, a carbonate group, or a urea group.

[0106] In some embodiments, L.sup.1 is a linker group that comprises a moiety that is an amide group, a carbamate group, a carbonate group, or a urea group.

[0107] In some embodiments, L.sup.1 is a linker group that comprises a moiety that is an amide group, a carbonate group, or a urea group.

[0108] In some embodiments, L.sup.1 is a linker group represented by a formula that is: —X.sup.1—C(X.sup.3)—X.sup.2, —(C.sub.1-C.sub.19 alkylene)-X.sup.1—C(X.sup.3)—X.sup.2, —X.sup.1—C(X.sup.3)—X.sup.2(C.sub.1-C.sub.19 alkylene)-, —(C.sub.1-C.sub.19 alkylene)-X.sup.1—, —X.sup.1—(C.sub.1-C.sub.19 alkylene)-.

[0109] Each X.sup.1 and X.sup.2 is independently, a covalent bond, —O—, —S—, or —NH—.

[0110] X.sup.3 is independently ═O, ═S, or ═NH.

[0111] The C.sub.1-C.sub.19 alkylene group (e.g., a C.sub.1-C.sub.5 alkylene or a C.sub.1-C.sub.10 alkylene group) is independently optionally substituted.

[0112] In some embodiments, L.sup.1 is unsubstituted.

[0113] In some embodiments, L.sup.1 does not comprise substituents having the structure —N(R′).sub.2, or a positively charged form thereof, wherein each R′ is independently hydrogen or optionally substituted C.sub.1-C.sub.20 alkyl.

[0114] In some embodiments, S is a zoosterol, or an oxidized or reduced form thereof. In embodiments, S is a zoosterol. In embodiments, S is an oxidized form of a zoosterol. In embodiments, S is a reduced form of a zoosterol.

[0115] In some embodiments, S is a phytosterol, or an oxidized or reduced form thereof. In

embodiments, S is a phytosterol. In embodiments, S is an oxidized form of a phytosterol. In embodiments, S is a reduced form of a phytosterol.
[0116] In some embodiments, S is a synthetic sterol (e.g., non-naturally occurring), or an oxidized or reduced form thereof. In embodiments, S is a synthetic sterol. In embodiments, S is an oxidized form of a synthetic sterol. In embodiments, S is a reduced form of a synthetic sterol.
[0117] In some embodiments, S is an oxidized form of a sterol as described herein.
[0118] In some embodiments, an oxidized form of a sterol is one in which the parent sterol has been modified to include further oxygen-containing groups. In embodiments, an oxidized form of a sterol includes one or more (e.g., 1, 2, 3, or 4) additional hydroxyl groups and/or carbonyl-containing groups (e.g., a ketone, an aldehyde, a carboxylic acid, or a carboxylic ester moiety) as compared to the parent sterol.
[0119] In some embodiments, an oxidized form of a sterol is one in which the parent sterol has been modified to include unsaturated carbon-carbon bonds (e.g., carbon-carbon double bonds). In embodiments, an oxidized form of a sterol includes one or more (e.g., 1, 2, or 3) additional carbon-carbon double bonds as compared to the parent sterol.
[0120] In some embodiments, S is a reduced form of a sterol as described herein.
[0121] In some embodiments, a reduced form of a sterol is one in which the parent sterol has been modified to include fewer oxygen-containing groups. In embodiments, a reduced form of a sterol includes a reduced number (e.g., 1, 2, 3, or 4 fewer moieties) of hydroxyl groups and/or carbonyl-containing groups (e.g., a ketone, an aldehyde, a carboxylic acid, or a carboxylic ester moiety) as compared to the parent sterol.
[0122] In some embodiments, a reduced form of a sterol is one in which the parent sterol has been modified to include fewer unsaturated carbon-carbon bonds (e.g., carbon-carbon double bonds). In embodiments, a reduced form of a sterol includes a reduced number (e.g., 1, 2, or 3 fewer) of carbon-carbon double bonds as compared to the parent sterol.
[0123] In some embodiments, S is a sterol that is cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, or sitosterol, or any oxidized or reduced form thereof.
[0124] In some embodiments, S is a sterol selected from cholesterol, an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol.
[0125] In some embodiments, S is a sterol selected from an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol.
[0126] In some embodiments, S is a sterol that is cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, or sitosterol.
[0127] In some embodiments, S is a sterol that is an oxidized form of: cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, or sitosterol.
[0128] In some embodiments, S is a sterol that is a reduced form of: cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, or sitosterol.
[0129] In some embodiments, S is a sterol that is cholesterol, an oxidized form of cholesterol, or a reduced form of cholesterol.
[0130] In some embodiments, S is a sterol that is alkyl lithocholate, an oxidized form of alkyl lithocholate, or a reduced form of alkyl lithocholate.
[0131] In some embodiments, S is a sterol that is stigmasterol, an oxidized form of stigmasterol, or a reduced form of stigmasterol.
[0132] In some embodiments, S is a sterol that is stigmastanol, an oxidized form of stigmastanol, or a reduced form of stigmastanol.
[0133] In some embodiments, S is a sterol that is campesterol, an oxidized form of campesterol, or a reduced form of campesterol.
[0134] In some embodiments, S is a sterol that is ergosterol, an oxidized form of ergosterol, or a

reduced form of ergosterol.
[0135] In some embodiments, S is a sterol that is sitosterol, an oxidized form of sitosterol, or a reduced form of sitosterol.
[0136] In some embodiments, S is a sterol that is a bile acid or an alkyl ester thereof, or an oxidized form thereof, or a reduced form thereof.
[0137] In some embodiments, S is a sterol that is cholic acid or an alkyl ester thereof, or an oxidized form thereof, or a reduced form thereof.
[0138] In some embodiments, S is a sterol selected from
##STR00007##
wherein R is optionally substituted C.sub.1-C.sub.20 alkyl, and R′ is H or optionally substituted C.sub.1-C.sub.20 alkyl.
[0139] In some embodiments, S is a sterol that is
##STR00008##
[0140] In some embodiments, S is a sterol selected from
##STR00009## ##STR00010##
wherein R is optionally substituted C.sub.1-C.sub.20 alkyl, and R′ is H or optionally substituted C.sub.1-C.sub.20 alkyl.
[0141] In some embodiments, a sterol-based cationic lipid comprises imidazole cholesterol ester (ICE).
[0142] In some embodiments, a sterol-based cationic lipid does not comprise imidazole cholesterol ester (ICE).
[0143] In some embodiments, a sterol-based cationic lipid is a cholesterol-based cationic lipid. Such cholesterol-based cationic lipids can be used, either alone or in combination with other lipids as described herein. Suitable cholesterol-based cationic lipids include, for example, DC-Chol (N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, et al. Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf et al. BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335).
[0144] In embodiments, sterol-based cationic lipids are dialkylamino-, imidazole-, and guanidinium-containing sterol-based cationic lipids. For example, certain embodiments are directed to a composition comprising one or more sterol-based cationic lipids comprising an imidazole, for example, the imidazole cholesterol ester or “ICE” lipid (3S, 10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(1H-imidazol-4-yl)propanoate, as represented by structure (I) below. In certain embodiments, a lipid nanoparticle for delivery of RNA (e.g., mRNA) encoding a functional protein may comprise one or more imidazole-based cationic lipids, for example, the imidazole cholesterol ester or “ICE” lipid (3S, 10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(1H-imidazol-4-yl)propanoate, as represented by structure (I).
##STR00011##
[0145] An exemplary hydrolysis reaction of structure (I) is provided in Scheme 1.
##STR00012##
[0146] Without wishing to be bound by a particular theory, it is believed that the fusogenicity of the imidazole-based cationic lipid ICE is related to the endosomal disruption which is facilitated by the imidazole group, which has a lower pKa relative to traditional cationic lipids. The endosomal disruption in turn promotes osmotic swelling and the disruption of the liposomal membrane, followed by the transfection or intracellular release of the polynucleotide contents loaded or encapsulated therein into the target cell.
[0147] The imidazole-based cationic lipids are also characterized by their reduced toxicity relative to other cationic lipids. In some embodiments, one or more of the lipid nanoparticles which comprises the blended pharmaceutical composition comprise an imidazole-based cationic lipid

such as ICE, to reduce the relative concentration of other more toxic cationic lipids in such blended pharmaceutical composition. The imidazole-based cationic lipids (e.g., ICE) may be used as the sole cationic lipid in one or more of the lipid nanoparticles that comprise the blended formulations, or alternatively may be combined with traditional cationic lipids (e.g., DOPE, DC-Chol), non-cationic lipids, PEG-modified lipids and/or helper lipids. The cationic lipid may comprise a molar ratio of about 1% to about 90%, about 2% to about 90%, about 5% to about 90%, about 10% to about 90%, about 15% to about 90%, about 20% to about 90%, about 30% to about 90%, about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 70% to about 90%, about 80% to about 90%, about 1% to about 80%, about 1% to about 70%, about 1% to about 60%, about 1% to about 50%, about 1% to about 40%, about 1% to about 30%, about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 2% to about 70%, about 5% to about 50%, about 10% to about 40% of the total lipid present in the lipid nanoparticle, or preferably about 20% to about 70% of the total lipid present in the lipid nanoparticle.

Ratio of Distinct Lipid Components

[0148] In embodiments where a lipid nanoparticle comprises three and no more than three distinct lipid components, the ratio of total lipid content (i.e., the ratio of lipid component (1): lipid component (2): lipid component (3)) can be represented as x:y:z, wherein

[00001] $(y + z) = 100 - x$.

[0149] In some embodiments, each of “x,” “y,” and “z” represents molar percentages of the three distinct lipid components, and the ratio is a molar ratio.

[0150] In some embodiments, each of “x,” “y,” and “z” represents weight percentages of the three distinct lipid components, and the ratio is a weight ratio.

[0151] In some embodiments, lipid component (1), represented by variable “x,” is a sterol-based cationic lipid.

[0152] In some embodiments, lipid component (2), represented by variable “y,” is a helper lipid.

[0153] In some embodiments, lipid component (3), represented by variable “z”, is a PEG lipid.

[0154] In some embodiments, variable “x,” representing the molar percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is at least about 10%, about 20%, about 30%, about 40%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%.

[0155] In some embodiments, variable “x,” representing the molar percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is no more than about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 40%, about 30%, about 20%, or about 10%. In some embodiments, variable “x” is no more than about 65%, about 60%, about 55%, about 50%, or about 40%.

[0156] In some embodiments, variable “x,” representing the molar percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is: at least about 50% but less than about 95%; at least about 50% but less than about 90%; at least about 50% but less than about 85%; at least about 50% but less than about 80%; at least about 50% but less than about 75%; at least about 50% but less than about 70%; at least about 50% but less than about 65%; or at least about 50% but less than about 60%.

[0157] In some embodiments, variable “x,” representing the weight percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is at least about 10%, about 20%, about 30%, about 40%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%.

[0158] In some embodiments, variable “x,” representing the weight percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is no more than about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 40%, about 30%, about 20%, or about 10%.

[0159] In some embodiments, variable “x,” representing the weight percentage of lipid component

(1) (e.g., a sterol-based cationic lipid), is: at least about 50% but less than about 95%; at least about 50% but less than about 90%; at least about 50% but less than about 85%; at least about 50% but less than about 80%; at least about 50% but less than about 75%; at least about 50% but less than about 70%; at least about 50% but less than about 65%; or at least about 50% but less than about 60%.

[0160] In some embodiments, variable "z," representing the molar percentage of lipid component (3) (e.g., a PEG-modified lipid) is no more than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, or 25%. In some embodiments, variable "z," representing the molar percentage of lipid component (3) (e.g., a PEG-modified lipid) is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%. In embodiments, variable "z," representing the molar percentage of lipid component (3) (e.g., a PEG-modified lipid) is about 1% to about 10%, about 2% to about 10%, about 3% to about 10%, about 4% to about 10%, about 5% to about 10%, about 6% to about 10%, about 7% to about 10%, about 8% to about 10%, about 9% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7.5%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, about 1% to about 3%, about 1% to about 2%, about 2.5% to about 10%, about 2.5% to about 7.5%, about 2.5% to about 5%, about 5% to about 7.5%, or about 5% to about 10%.

[0161] In some embodiments, variable "z," representing the weight percentage of lipid component (3) (e.g., a PEG-modified lipid) is no more than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, or 25%. In some embodiments, variable "z," representing the weight percentage of lipid component (3) (e.g., a PEG lipid) is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%. In some embodiments, variable "z," representing the weight percentage of lipid component (3) (e.g., a PEG-modified lipid) is about 1% to about 10%, about 2% to about 10%, about 3% to about 10%, about 4% to about 10%, about 5% to about 10%, about 6% to about 10%, about 7% to about 10%, about 8% to about 10%, about 9% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7.5%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, about 1% to about 3%, about 1% to about 2%, about 2.5% to about 10%, about 2.5% to about 7.5%, about 2.5% to about 5%, about 5% to about 7.5%, or about 5% to about 10%.

[0162] For compositions having three and only three distinct lipid components, variables "x," "y," and "z" may be in any combination so long as the total of the three variables sums to 100% of the total lipid content.

Other Lipid Components

[0163] The use of polyethylene glycol (PEG)-modified phospholipids and derivatized lipids such as derivatized ceramides (PEG-CER), including N-Octanoyl-Sphingosine-1-[Succinyl(Methoxy Polyethylene Glycol)-2000](C8 PEG-2000 ceramide) is also contemplated by the present invention, either alone or preferably in combination with other lipid formulations together which comprise the transfer vehicle (e.g., a lipid nanoparticle). Contemplated PEG-modified lipids include, but are not limited to, a polyethylene glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C6-C20 length. The addition of such components may prevent complex aggregation and may also provide a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid composition to the target tissues, (Klibanov et al. (1990) FEBS Letters, 268 (1): 235-237), or they may be selected to rapidly exchange out of the formulation in vivo (see U.S. Pat. No. 5,885,613). Particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (e.g., C14 or C18).

[0164] The PEG-modified phospholipid and derivitized lipids of the present invention may comprise a molar ratio from about 0% to about 20%, about 0.5% to about 20%, about 1% to about 20%, about 2% to about 20%, about 3% to about 20%, about 4% to about 20%, about 5% to about 20%, about 10% to about 20%, about 15% to about 20%, about 0% to about 15%, about 0% to about 10%, about 0% to about 5%, about 0% to about 4%, about 0% to about 3%, about 0% to

about 220%, about 0% to about 1%, about 5% to about 15%, about 4% to about 10%, or about 2% of the total lipid present in the liposomal transfer vehicle.

[0165] The present invention also contemplates the use of non-cationic lipids. As used herein, the phrase “non-cationic lipid” refers to any neutral, zwitterionic or anionic lipid. As used herein, the phrase “anionic lipid” refers to any of a number of lipid species that carry a net negative charge at a selected pH, such as physiological pH. Non-cationic lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-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. Such non-cationic lipids may be used alone, but are preferably used in combination with other excipients, for example, cationic lipids.

[0166] In some embodiments, cationic lipids that are not sterol-based cationic lipids can be used in liposomal compositions of the invention. Cationic lipids include, but are not limited to, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 5-carboxyspermylglycinedioctadecylamide (DOGS), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium (DOSPA), 1,2-Dioleoyl-3-Dimethylammonium-Propane (DODAP), 1,2-Dioleoyl-3-Trimethylammonium-Propane (DOTAP), 1,2-distearyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 1,2-dioleyloxy-N,N-dimethyl-3-aminopropane (DODMA), 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane (DLenDMA), N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)propane (CLinDMA), 2-[5′-(cholest-5-en-3-beta-oxy)-3′-oxapentoxy)-3-dimethyl-1-(cis,cis-9′,1-2′-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleyloxybenzylamine (DMOBA), 1,2-N,N′-dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP), 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine (DLinDAP), 1,2-N,N′-Dilinoleylcarbamyl-3-dimethylaminopropane (DLincarbDAP), 1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane or (DLinCDAP), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-K-XTC2-DMA), or mixtures thereof.

[0167] Additional cationic lipids include 2-((2,3-Bis((9Z,12Z)-octadeca-9,12-dien-1-yloxy)propyl)disulfanyl)-N,N-dimethylethanamine (further described in U.S. Provisional Application No: PCT/US2012/041663, filed Jun. 8, 2012, the entire teachings of which are incorporated herein by reference in their entirety), cleavable lipids, such as, for example, one or more cationic lipids that comprise a cleavable disulfide (S—S) functional group, as further described in U.S. International Application No: PCT/US2012/041663. In addition, several reagents are commercially available to enhance transfection efficacy. Suitable examples include LIPOFECTIN (DOTMA:DOPE) (Invitrogen, Carlsbad, Calif.), LIPOFECTAMINE (DOSPA:DOPE) (Invitrogen), LIPOFECTAMINE2000 (Invitrogen), FUGENE, TRANSFECTAM (DOGS), and EFFECTENE.

Formation of Liposomes Encapsulating mRNA

[0168] The liposomal transfer vehicles for use in the present invention can be prepared by various techniques which are presently known in the art. Multi-lamellar vesicles (MLV) may be prepared conventional techniques, for example, by depositing a selected lipid on the inside wall of a suitable container or vessel by dissolving the lipid in an appropriate solvent, and then evaporating the

solvent to leave a thin film on the inside of the vessel or by spray drying. An aqueous phase may then be added to the vessel with a vortexing motion which results in the formation of MLVs. Uni-lamellar vesicles (ULV) can then be formed by homogenization, sonication or extrusion of the multi-lamellar vesicles. In addition, unilamellar vesicles can be formed by detergent removal techniques.

[0169] In certain embodiments of this invention, the compositions of the present invention comprise a transfer vehicle wherein the therapeutic RNA (e.g., mRNA encoding CFTR) is associated on both the surface of the transfer vehicle (e.g., a liposome) and encapsulated within the same transfer vehicle. For example, during preparation of the compositions of the present invention, cationic liposomal transfer vehicles may associate with the nucleic acids (e.g., mRNA) through electrostatic interactions with such therapeutic mRNA.

[0170] In some embodiments, the compositions and methods of the invention comprise mRNA encapsulated in a liposome. In some embodiments, the one or more mRNA species may be encapsulated in the same liposome. In some embodiments, the one or more mRNA species may be encapsulated in different liposomes. In some embodiments, the mRNA is encapsulated in one or more liposomes, which differ in their lipid composition, molar ratio of lipid components, size, charge (Zeta potential), targeting ligands and/or combinations thereof. In some embodiments, the one or more liposome may have a different composition of sterol-based cationic lipids, neutral lipid, PEG-modified lipid and/or combinations thereof. In some embodiments the one or more liposomes may have a different molar ratio of sterol-based cationic lipid, neutral lipid, and PEG-modified lipid used to create the liposome.

[0171] The process of incorporation of a desired mRNA into a liposome is often referred to as “loading”. Exemplary methods are described in Lasic, et al., FEBS Lett., 312: 255-258, 1992, which is incorporated herein by reference. The liposome-incorporated nucleic acids may be completely or partially located in the interior space of the liposome, within the bilayer membrane of the liposome, or associated with the exterior surface of the liposome membrane. The incorporation of a nucleic acid into liposomes is also referred to herein as “encapsulation” wherein the nucleic acid is entirely contained within the interior space of the liposome. The purpose of incorporating an mRNA into a transfer vehicle, such as a liposome, is often to protect the nucleic acid from an environment which may contain enzymes or chemicals that degrade nucleic acids and/or systems or receptors that cause the rapid excretion of the nucleic acids. Accordingly, in some embodiments, a suitable delivery vehicle is capable of enhancing the stability of the mRNA contained therein and/or facilitate the delivery of mRNA to the target cell or tissue.

[0172] Any desired lipids may be mixed at any ratios suitable for encapsulating mRNAs. In some embodiments, a suitable lipid solution contains a mixture of desired lipids including sterol-based cationic lipids, non-cationic lipids, and/or PEG-modified lipids. In some embodiments, a suitable lipid solution contain a mixture of desired lipids including one or more sterol-based cationic lipids, one or more helper lipids (e.g. non cationic lipids) and one or more PEG-modified lipids.

[0173] Exemplary combinations of sterol-based cationic lipids, non-cationic lipids, and PEG-modified lipids are described in the Examples section. For example, a suitable lipid solution may contain ICE, DOPE and DMG-PEG2K. The selection of sterol-based cationic lipids, non-cationic lipids and/or PEG-modified lipids which comprise the lipid mixture as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s) and the nature of the and the characteristics of the mRNA to be encapsulated. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipid(s). Thus the molar ratios may be adjusted accordingly.

[0174] In some embodiments, a process for encapsulating mRNA in lipid nanoparticles comprises mixing an mRNA solution and a lipid solution, wherein the mRNA solution and/or the lipid solution are heated to a pre-determined temperature greater than ambient temperature prior to

mixing to form lipid nanoparticles that encapsulate mRNA (see U.S. patent application Ser. No. 14/790,562 entitled “Encapsulation of messenger RNA”, filed Jul. 2, 2015 and its provisional U.S. patent application Ser. No. 62/020,163, filed Jul. 2, 2014, the disclosure of which are hereby incorporated in their entirety).

[0175] In some embodiments, a process for encapsulating mRNA in lipid nanoparticles comprises combining pre-formed lipid nanoparticles with mRNA (see U.S. Provisional Application Ser. No. 62/420,413, filed Nov. 10, 2016 and U.S. Provisional Application Ser. No. 62/580,155, filed Nov. 1, 2017, the disclosures of which are hereby incorporated by reference). In some embodiments, combining pre-formed lipid nanoparticles with mRNA results in lipid nanoparticles that show improved efficacy of intracellular delivery of the mRNA. In some embodiments, combining pre-formed lipid nanoparticles with mRNA results in very high encapsulation efficiencies of mRNA encapsulated in lipid nanoparticles (i.e., in the range of 90-95%). In some embodiments, combining pre-formed lipid nanoparticles with mRNA is achieved with pump systems which maintain the lipid/mRNA (N/P) ratio constant throughout the process and which also afford facile scale-up.

[0176] Suitable liposomes in accordance with the present invention may be made in various sizes. In some embodiments, provided liposomes may be made smaller than previously known mRNA encapsulating liposomes. In some embodiments, decreased size of liposomes is associated with more efficient delivery of mRNA. Selection of an appropriate liposome size may take into consideration the site of the target cell or tissue and to some extent the application for which the liposome is being made.

[0177] In some embodiments, an appropriate size of liposome is selected to facilitate systemic distribution of a protein or a peptide encoded by the mRNA. In some embodiments, it may be desirable to limit delivery of the mRNA to certain cells or tissues. For example, to target hepatocytes, a liposome may be sized such that its dimensions are smaller than the fenestrations of the endothelial layer lining hepatic sinusoids in the liver; in such cases the liposome could readily penetrate such endothelial fenestrations to reach the target hepatocytes.

[0178] Alternatively or additionally, a liposome may be sized such that the dimensions of the liposome are of a sufficient diameter to limit or expressly avoid distribution into certain cells or tissues. For example, a liposome may be sized such that its dimensions are larger than the fenestrations of the endothelial layer lining hepatic sinusoids to thereby limit distribution of the liposomes to hepatocytes.

[0179] In some embodiments, the size of a liposome is determined by the length of the largest diameter of the liposome particle. In some embodiments, a suitable liposome has a size no greater than about 250 nm (e.g., no greater than about 225 nm, 200 nm, 175 nm, 150 nm, 125 nm, 100 nm, 75 nm, or 50 nm). In some embodiments, a suitable liposome has a size ranging from about 10-250 nm (e.g., ranging from about 10-225 nm, 10-200 nm, 10-175 nm, 10-150 nm, 10-125 nm, 10-100 nm, 10-75 nm, or 10-50 nm). In some embodiments, a suitable liposome has a size ranging from about 100-250 nm (e.g., ranging from about 100-225 nm, 100-200 nm, 100-175 nm, 100-150 nm). In some embodiments, a suitable liposome has a size ranging from about 10-100 nm (e.g., ranging from about 10-90 nm, 10-80 nm, 10-70 nm, 10-60 nm, or 10-50 nm). In a particular embodiment, a suitable liposome has a size less than about 100 nm. In some embodiments, majority of nanoparticles in a composition, i.e., greater than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the purified nanoparticles, have a size less than about 100 nm (e.g., less than about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm, about 45 nm, or about 40 nm). In some embodiments, substantially all of the purified nanoparticles have a size less than 100 nm (e.g., less than about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm, about 45 nm, or about 40 nm).

[0180] A variety of alternative methods known in the art are available for sizing of a population of liposomes. One such sizing method is described in U.S. Pat. No. 4,737,323, incorporated herein by

reference. Sonicating a liposome suspension either by bath or probe sonication produces a progressive size reduction down to small ULV less than about 0.05 microns in diameter. Homogenization is another method that relies on shearing energy to fragment large liposomes into smaller ones. In a typical homogenization procedure, MLV are recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 microns, are observed. The size of the liposomes may be determined by quasi-electric light scattering (QELS) as described in Bloomfield, Ann. Rev. Biophys. Bioeng., 10:421-150 (1981), incorporated herein by reference. Average liposome diameter may be reduced by sonication of formed liposomes. Intermittent sonication cycles may be alternated with QELS assessment to guide efficient liposome synthesis.

Purification

[0181] Typically, subsequent to formulation the lipid nanoparticles containing mRNA are purified and/or concentrated. Various purification methods may be used. In some embodiments, lipid nanoparticles are purified using Tangential Flow Filtration. Tangential flow filtration (TFF), also referred to as cross-flow filtration, is a type of filtration wherein the material to be filtered is passed tangentially across a filter rather than through it. In TFF, undesired permeate passes through the filter, while the desired retentate passes along the filter and is collected downstream. It is important to note that the desired material is typically contained in the retentate in TFF, which is the opposite of what one normally encounters in traditional-dead end filtration.

[0182] Depending upon the material to be filtered, TFF is usually used for either microfiltration or ultrafiltration. Microfiltration is typically defined as instances where the filter has a pore size of between 0.05 μm and 1.0 μm, inclusive, while ultrafiltration typically involves filters with a pore size of less than 0.05 μm. Pore size also determines the nominal molecular weight limits (NMWL), also referred to as the molecular weight cut off (MWCO) for a particular filter, with microfiltration membranes typically having NMWLs of greater than 1,000 kilodaltons (kDa) and ultrafiltration filters having NMWLs of between 1 kDa and 1,000 kDa.

[0183] A principal advantage of tangential flow filtration is that non-permeable particles that may aggregate in and block the filter (sometimes referred to as “filter cake”) during traditional “dead-end” filtration are instead carried along the surface of the filter. This advantage allows tangential flow filtration to be widely used in industrial processes requiring continuous operation since down time is significantly reduced because filters do not generally need to be removed and cleaned.

[0184] Tangential flow filtration can be used for several purposes including concentration and diafiltration, among others. Concentration is a process whereby solvent is removed from a solution while solute molecules are retained. In order to effectively concentrate a sample, a membrane having a NMWL or MWCO that is substantially lower than the molecular weight of the solute molecules to be retained is used. Generally, one of skill may select a filter having a NMWL or MWCO of three to six times below the molecular weight of the target molecule(s).

[0185] Diafiltration is a fractionation process whereby small undesired particles are passed through a filter while larger desired nanoparticles are maintained in the retentate without changing the concentration of those nanoparticles in solution. Diafiltration is often used to remove salts or reaction buffers from a solution. Diafiltration may be either continuous or discontinuous. In continuous diafiltration, a diafiltration solution is added to the sample feed at the same rate that filtrate is generated. In discontinuous diafiltration, the solution is first diluted and then concentrated back to the starting concentration. Discontinuous diafiltration may be repeated until a desired concentration of nanoparticles is reached.

[0186] Purified and/or concentrated lipid nanoparticles may be formulated in a desired buffer such as, for example, PBS.

[0187] Thus, the present invention provides a composition comprising purified nanoparticles described herein. In some embodiments, majority of purified nanoparticles in a composition, i.e., greater than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or

99% of the purified nanoparticles, have a size less than about 100 nm (e.g., less than about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm, about 45 nm, or about 40 nm). In some embodiments, substantially all of the purified nanoparticles have a size less than 100 nm (e.g., less than about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm, about 45 nm, or about 40 nm).

[0188] In some embodiments, greater than about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% of the purified nanoparticles in a composition provided by the present invention have a size ranging from about 40-90 nm (e.g., about 40-85 nm, about 40-80 nm, about 40-75 nm, about 40-70 nm, about 40-65 nm, or about 40-60 nm). In some embodiments, substantially all of the purified nanoparticles have a size ranging from about 40-90 nm (e.g., about 40-85 nm, about 40-80 nm, about 40-75 nm, about 40-70 nm, about 40-65 nm, or about 40-60 nm).

[0189] In some embodiments, the dispersity, or measure of heterogeneity in size of molecules (PDI), of nanoparticles in a composition provided by the present invention is less than about 0.16 (e.g., less than about 0.15, 0.14, 0.13, 0.12, 0.11, 0.10, 0.09, or 0.08).

[0190] In some embodiments, greater than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the purified lipid nanoparticles in a composition provided by the present invention encapsulate an mRNA within each individual particle. In some embodiments, substantially all of the purified lipid nanoparticles in a composition encapsulate an mRNA within each individual particle.

[0191] In some embodiments, a composition according to the present invention contains at least about 1 mg, 5 mg, 10 mg, 100 mg, 500 mg, or 1000 mg of encapsulated mRNA. In some embodiments, a process according to the present invention results in greater than about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% recovery of mRNA.

Nucleic Acids and mRNA

[0192] The present invention can be used to deliver any type of nucleic acid. A nucleic acid may be selected from the group comprising (but not limited to) DNA, messenger RNA (mRNA), small nuclear RNA (snRNA), guide RNA (gRNA), CRISPR RNA (crRNA), long noncoding RNA (lncRNA), micro RNA (miRNA), small interfering RNA (siRNA), and short hairpin RNA (shRNA).

[0193] The present invention can be used to deliver any mRNA. mRNA is typically thought of as the type of RNA that carries information from DNA to the ribosome. Typically, in eukaryotic organisms, mRNA processing comprises the addition of a “cap” on the N-terminal (5′) end, and a “tail” on the C-terminal (3′) end. A typical cap is a 7-methylguanosine cap, which is a guanosine that is linked through a 5′-5′-triphosphate bond to the first transcribed nucleotide. The presence of the cap is important in providing resistance to nucleases found in most eukaryotic cells. The tail is typically a polyadenylation event whereby a polyadenylyl moiety is added to the 3′ end of the mRNA molecule. The presence of this “tail” serves to protect the mRNA from exonuclease degradation. Messenger RNA typically is translated by the ribosomes into a series of amino acids that make up a protein or a peptide.

[0194] Any mRNA capable of being translated into one or more peptides (e.g., antigens) or peptide fragments is contemplated as within the scope of the present invention. In some embodiments, an mRNA encodes one or more naturally occurring peptides. In some embodiments, an mRNA encodes one or more modified or non-natural peptides.

[0195] In some embodiments an mRNA encodes an intracellular protein or peptide. In some embodiments, an mRNA encodes a cytosolic protein or peptide. In some embodiments, an mRNA encodes a protein or peptide associated with the actin cytoskeleton. In some embodiments, an mRNA encodes a protein or peptide associated with the plasma membrane. In some specific embodiments, an mRNA encodes a transmembrane protein or peptide. In some specific embodiments an mRNA encodes an ion channel protein or peptide. In some embodiments, an

mRNA encodes a perinuclear protein or peptide. In some embodiments, an mRNA encodes a nuclear protein or peptide. In some specific embodiments, an mRNA encodes a transcription factor. In some embodiments, an mRNA encodes a chaperone protein or peptide. In some embodiments, an mRNA encodes an intracellular enzyme (e.g., mRNA encoding an enzyme associated with urea cycle or lysosomal storage metabolic disorders). In some embodiments, an mRNA encodes a protein or peptide involved in cellular metabolism, DNA repair, transcription and/or translation. In some embodiments, an mRNA encodes an extracellular protein or peptide. In some embodiments, an mRNA encodes a protein or peptide associated with the extracellular matrix. In some embodiments an mRNA encodes a secreted protein or peptide. In specific embodiments, an mRNA used in the composition and methods of the invention may be used to express functional proteins or enzymes that are excreted or secreted by one or more target cells into the surrounding extracellular fluid (e.g., mRNA encoding hormones and/or neurotransmitters).

[0196] In some embodiments, the compositions and methods of the invention provide for delivery of mRNA encoding a secreted protein. In some embodiments, the compositions and methods of the invention provide for delivery of mRNA encoding one or more secreted proteins listed in Table 1; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 1 (or a homolog thereof) along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein listed in Table 1 (or a homolog thereof) along with other components set out herein.

TABLE-US-00001 TABLE 1 Secreted Proteins Uniprot ID Protein Name Gene Name A1E959 Odontogenic ameloblast-associated protein ODAM A1KZ92 Peroxidasin-like protein PXDNL A1L453 Serine protease 38 PRSS38 A1L4H1 Soluble scavenger receptor cysteine-rich domain-containing SSC5D protein SSC5D A2RUU4 Colipase-like protein 1 CLPSL1 A2VDF0 Fucose mutarotase FUOM A2VEC9 SCO-spondin SSPO A3KMH1 von Willebrand factor A domain-containing protein 8 VWA8 A4D0S4 Laminin subunit beta-4 LAMB4 A4D1T9 Probable inactive serine protease 37 PRSS37 A5D8T8 C-type lectin domain family 18 member A CLEC18A A6NC86 phospholipase A2 inhibitor and Ly6/PLAUR PINLYP domain-containing protein A6NCI4 von Willebrand factor A domain-containing protein 3A VWA3A A6ND01 Probable folate receptor delta FOLR4 A6NDD2 Beta-defensin 108B-like A6NE02 BTB/POZ domain-containing protein 17 BTBD17 A6NEF6 Growth hormone 1 GH1 A6NF02 NPIP-like protein LOC730153 A6NFB4 HCG1749481, isoform CRA_k CSH1 A6NFZ4 Protein FAM24A FAM24A A6NG13 Glycosyltransferase 54 domain-containing protein A6NGN9 IgLON family member 5 IGLON5 A6NHN0 Otolin-1 OTOL1 A6NHN6 Nuclear pore complex-interacting protein-like 2 NPIPL2 A6NI73 Leukocyte immunoglobulin-like receptor LILRA5 subfamily A member 5 A6NIT4 Chorionic somatomammotropin hormone 2 isoform 2 CSH2 A6NJ69 IgA-inducing protein homolog IGIP A6NKQ9 Choriogonadotropin subunit beta variant 1 CGB1 A6NMZ7 Collagen alpha-6(VI) chain COL6A6 A6NNS2 Dehydrogenase/reductase SDR family member 7C DHRS7C A6XGL2 Insulin A chain INS A8K0G1 Protein Wnt WNT7B A8K2U0 Alpha-2-macroglobulin-like protein 1 A2ML1 A8K7I4 Calcium-activated chloride channel regulator 1 CLCA1 A8MTL9 Serpin-like protein HMSD HMSD A8MV23 Serpin E3 SERPINE3 A8MZH6 Oocyte-secreted protein 1 homolog OOSP1 A8TX70 Collagen alpha-5(VI) chain COL6A5 B0ZBE8 Natriuretic peptide NPPA B1A4G9 Somatotropin GH1 B1A4H2 HCG1749481, isoform CRA_d CSH1 B1A4H9 Chorionic somatomammotropin hormone CSH2 B1AJZ6 Protein Wnt WNT4 B1AKI9 Isthmin-1 ISM1 B2RNN3 Complement C1q and tumor necrosis factor-related C1QTNF9B protein 9B B2RUY7 von Willebrand factor C domain-containing protein 2-like VWC2L B3GLJ2 Prostate and testis expressed protein 3 PATE3 B4DI03 SEC11-like 3 (*S. cerevisiae*), isoform CRA_a SEC11L3 B4DJF9 Protein Wnt WNT4 B4DUL4 SEC11-like 1 (*S. cerevisiae*), isoform CRA_d SEC11L1 B5MCC8 Protein Wnt WNT10B B8A595 Protein Wnt WNT7B B8A597 Protein Wnt WNT7B B8A598 Protein Wnt WNT7B B9A064 Immunoglobulin lambda-like polypeptide 5 IGLL5 C9J3H3 Protein Wnt WNT10B C9J8I8 Protein Wnt WNT5A C9JAF2 Insulin-like growth

factor II Ala-25 Del IGF2 C9JCI2 Protein Wnt WNT10B C9JL84 HERV-H LTR-associating protein 1 HHLA1 C9JNR5 Insulin A chain INS C9JUI2 Protein Wnt WNT2 D6RF47 Protein Wnt WNT8A D6RF94 Protein Wnt WNT8A E2RYF7 Protein PBMUCL2 HCG22 E5RFR1 PENK(114-133) PENK E7EML9 Serine protease 44 PRSS44 E7EPC3 Protein Wnt WNT9B E7EVP0 Nociceptin PNOC E9PD02 Insulin-like growth factor I IGF1 E9PH60 Protein Wnt WNT16 E9PJL6 Protein Wnt WNT11 F5GYM2 Protein Wnt WNT5B F5H034 Protein Wnt WNT5B F5H364 Protein Wnt WNT5B F5H7Q6 Protein Wnt WNT5B F8WCM5 Protein INS-IGF2 INS-IGF2 F8WDR1 Protein Wnt WNT2 H0Y663 Protein Wnt WNT4 H0YK72 Signal peptidase complex catalytic subunit SEC11A SEC11A H0YK83 Signal peptidase complex catalytic subunit SEC11A SEC11A H0YM39 Chorionic somatomammotropin hormone CSH2 H0YMT7 Chorionic somatomammotropin hormone CSH1 H0YN17 Chorionic somatomammotropin hormone CSH2 H0YNA5 Signal peptidase complex catalytic subunit SEC11A SEC11A H0YNG3 Signal peptidase complex catalytic subunit SEC11A SEC11A H0YNX5 Signal peptidase complex catalytic subunit SEC11A SEC11A H7BZB8 Protein Wnt WNT10A H9KV56 Choriogonadotropin subunit beta variant 2 CGB2 I3L0L8 Protein Wnt WNT9B J3KNZ1 Choriogonadotropin subunit beta variant 1 CGB1 J3KP00 Choriogonadotropin subunit beta CGB7 J3QT02 Choriogonadotropin subunit beta variant 1 CGB1 O00175 C—C motif chemokine 24 CCL24 O00182 Galectin-9 LGALS9 O00187 Mannan-binding lectin serine protease 2 MASP2 O00230 Cortistatin CORT O00253 Agouti-related protein AGRP O00270 12-(S)-hydroxy-5,8,10,14-eicosatetraenoic acid receptor GPR31 O00292 Left-right determination factor 2 LEFTY2 O00294 Tubby-related protein 1 TULP1 O00295 Tubby-related protein 2 TULP2 O00300 Tumor necrosis factor receptor superfamily member 11B TNFRSF11B O00339 Matrilin-2 MATN2 O00391 Sulfhydryl oxidase 1 QSOX1 O00468 Agrin AGRN O00515 Ladinin-1 LAD1 O00533 Processed neural cell adhesion molecule L1-like protein CHL1 O00584 Ribonuclease T2 RNASET2 O00585 C—C motif chemokine 21 CCL21 O00602 Ficolin-1 FCN1 O00622 Protein CYR61 CYR61 O00626 MDC(5-69) CCL22 O00634 Netrin-3 NTN3 O00744 Protein Wnt-10b WNT10B O00755 Protein Wnt-7a WNT7A O14498 Immunoglobulin superfamily containing leucine-rich ISLR repeat protein O14511 Pro-neuregulin-2, membrane-bound isoform NRG2 O14594 Neurocan core protein NCAN O14625 C—X—C motif chemokine 11 CXCL11 O14638 Ectonucleotide pyrophosphatase/ ENPP3 phosphodiesterase family member 3 O14656 Torsin-1A TOR1A O14657 Torsin-1B TOR1B O14786 Neuropilin-1 NRP1 O14788 Tumor necrosis factor ligand superfamily member 11, TNFSF11 membrane form O14791 Apolipoprotein L1 APOL1 O14793 Growth/differentiation factor 8 MSTN O14904 Protein Wnt-9a WNT9A O14905 Protein Wnt-9b WNT9B O14944 Proepiregulin EREG O14960 Leukocyte cell-derived chemotaxin-2 LECT2 O15018 Processed PDZ domain-containing protein 2 PDZD2 O15041 Semaphorin-3E SEMA3E O15072 A disintegrin and metalloproteinase with thrombospondin ADAMTS3 motifs 3 O15123 Angiopoietin-2 ANGPT2 O15130 Neuropeptide FF NPFF O15197 Ephrin type-B receptor 6 EPHB6 O15204 ADAM DEC1 ADAMDEC1 O15230 Laminin subunit alpha-5 LAMA5 O15232 Matrilin-3 MATN3 O15240 Neuroendocrine regulatory peptide-1 VGF O15263 Beta-defensin 4A DEFB4A O15335 Chondroadherin CHAD O15393 Transmembrane protease serine 2 catalytic chain TMPRSS2 O15444 C—C motif chemokine 25 CCL25 O15467 C—C motif chemokine 16 CCL16 O15496 Group 10 secretory phospholipase A2 PLA2G10 O15520 Fibroblast growth factor 10 FGF10 O15537 Retinoschisin RS1 O43157 Plexin-B1 PLXNB1 O43184 Disintegrin and metalloproteinase domain-containing ADAM12 protein 12 O43240 Kallikrein-10 KLK10 O43278 Kunitz-type protease inhibitor 1 SPINT1 O43320 Fibroblast growth factor 16 FGF16 O43323 Desert hedgehog protein C-product DHH O43405 Cochlin COCH O43508 Tumor necrosis factor ligand superfamily member 12, TNFSF12 membrane form O43555 Progonadoliberin-2 GNRH2 O43557 Tumor necrosis factor ligand superfamily member 14, TNFSF14 soluble form O43692 Peptidase inhibitor 15 PI15 O43699 Sialic acid-binding Ig-like lectin 6 SIGLEC6 O43820 Hyaluronidase-3 HYAL3 O43827 Angiopoietin-related protein 7 ANGPTL7 O43852 Calumenin CALU O43854 EGF-like repeat and

discoidin I-like domain-containing EDIL3 protein 3 O43866 CD5 antigen-like CD5L O43897 Tolloid-like protein 1 TLL1 O43915 Vascular endothelial growth factor D FIGF O43927 C—X—C motif chemokine 13 CXCL13 O60218 Aldo-keto reductase family 1 member B10 AKR1B10 O60235 Transmembrane protease serine 11D TMPRSS11D O60258 Fibroblast growth factor 17 FGF17 O60259 Kallikrein-8 KLK8 O60383 Growth/differentiation factor 9 GDF9 O60469 Down syndrome cell adhesion molecule DSCAM O60542 Persephin PSPN O60565 Gremlin-1 GREM1 O60575 Serine protease inhibitor Kazal-type 4 SPINK4 O60676 Cystatin-8 CST8 O60687 Sushi repeat-containing protein SRPX2 SRPX2 O60844 Zymogen granule membrane protein 16 ZG16 O60882 Matrix metalloproteinase-20 MMP20 O60938 Keratocan KERA O75015 Low affinity immunoglobulin gamma Fc region FCGR3B receptor III-B O75077 Disintegrin and metalloproteinase domain-containing ADAM23 protein 23 O75093 Slit homolog 1 protein SLIT1 O75094 Slit homolog 3 protein SLIT3 O75095 Multiple epidermal growth factor-like domains protein 6 MEGF6 O75173 A disintegrin and metalloproteinase with thrombospondin ADAMTS4 motifs 4 O75200 Nuclear pore complex-interacting protein-like 1 NPIPL1 O75339 Cartilage intermediate layer protein 1 C1 CILP O75354 Ectonucleoside triphosphate diphosphohydrolase 6 ENTPD6 O75386 Tubby-related protein 3 TULP3 O75398 Deformed epidermal autoregulatory factor 1 homolog DEAF1 O75443 Alpha-tectorin TECTA O75445 Usherin USH2A O75462 Cytokine receptor-like factor 1 CRLF1 O75487 Glypican-4 GPC4 O75493 Carbonic anhydrase-related protein 11 CA11 O75594 Peptidoglycan recognition protein 1 PGLYRP1 O75596 C-type lectin domain family 3 member A CLEC3A O75610 Left-right determination factor 1 LEFTY1 O75629 Protein CREG1 CREG1 O75636 Ficolin-3 FCN3 O75711 Scrapie-responsive protein 1 SCRG1 O75715 Epididymal secretory glutathione peroxidase GPX5 O75718 Cartilage-associated protein CRTAP O75829 Chondrosurfactant protein LECT1 O75830 Serpin I2 SERPINI2 O75882 Attractin ATRN O75888 Tumor necrosis factor ligand superfamily member 13 TNFSF13 O75900 Matrix metalloproteinase-23 MMP23A O75951 Lysozyme-like protein 6 LYZL6 O75973 C1q-related factor C1QL1 O76038 Secretagogin SCGN O76061 Stanniocalcin-2 STC2 O76076 WNT1-inducible-signaling pathway protein 2 WISP2 O76093 Fibroblast growth factor 18 FGF18 O76096 Cystatin-F CST7 O94769 Extracellular matrix protein 2 ECM2 O94813 Slit homolog 2 protein C-product SLIT2 O94907 Dickkopf-related protein 1 DKK1 O94919 Endonuclease domain-containing 1 protein ENDOD1 O94964 N-terminal form SOGA1 O95025 Semaphorin-3D SEMA3D O95084 Serine protease 23 PRSS23 O95150 Tumor necrosis factor ligand superfamily member 15 TNFSF15 O95156 Neurexophilin-2 NXPH2 O95157 Neurexophilin-3 NXPH3 O95158 Neurexophilin-4 NXPH4 O95388 WNT1-inducible-signaling pathway protein 1 WISP1 O95389 WNT1-inducible-signaling pathway protein 3 WISP3 O95390 Growth/differentiation factor 11 GDF11 O95393 Bone morphogenetic protein 10 BMP 10 O95399 Urotensin-2 UTS2 O95407 Tumor necrosis factor receptor superfamily member 6B TNFRSF6B O95428 Papilin PAPLN O95445 Apolipoprotein M APOM O95450 A disintegrin and metalloproteinase with thrombospondin ADAMTS2 motifs 2 O95460 Matrilin-4 MATN4 O95467 LHAL tetrapeptide GNAS O95631 Netrin-1 NTN1 O95633 Follistatin-related protein 3 FSTL3 O95711 Lymphocyte antigen 86 LY86 O95715 C—X—C motif chemokine 14 CXCL14 O95750 Fibroblast growth factor 19 FGF19 O95760 Interleukin-33 IL33 O95813 Cerberus CER1 O95841 Angiopoietin-related protein 1 ANGPTL1 O95897 Noelin-2 OLFM2 O95925 Eppin EPPIN O95965 Integrin beta-like protein 1 ITGBL1 O95967 EGF-containing fibulin-like extracellular matrix protein 2 EFEMP2 O95968 Secretoglobin family 1D member 1 SCGB1D1 O95969 Secretoglobin family 1D member 2 SCGB1D2 O95970 Leucine-rich glioma-inactivated protein 1 LGI1 O95972 Bone morphogenetic protein 15 BMP15 O95994 Anterior gradient protein 2 homolog AGR2 O95998 Interleukin-18-binding protein IL18BP O96009 Napsin-A NAPSA O96014 Protein Wnt-11 WNT11 P00450 Ceruloplasmin CP P00451 Factor VIIIa light chain F8 P00488 Coagulation factor XIII A chain F13A1 P00533 Epidermal growth factor receptor EGFR P00709 Alpha-lactalbumin LALBA P00734 Prothrombin F2 P00738 Haptoglobin beta chain HP P00739 Haptoglobin-related protein

HPR P00740 Coagulation factor IXa heavy chain F9 P00742 Factor X heavy chain F10 P00746 Complement factor D CFD P00747 Plasmin light chain B PLG P00748 Coagulation factor XIIa light chain F12 P00749 Urokinase-type plasminogen activator long chain A PLAU P00750 Tissue-type plasminogen activator PLAT P00751 Complement factor B Ba fragment CFB P00797 Renin REN P00973 2′-5′-oligoadenylate synthase 1 OAS1 P00995 Pancreatic secretory trypsin inhibitor SPINK1 P01008 Antithrombin-III SERPINC1 P01009 Alpha-1-antitrypsin SERPINA1 P01011 Alpha-1-antichymotrypsin His-Pro-less SERPINA3 P01019 Angiotensin-1 AGT P01023 Alpha-2-macroglobulin A2M P01024 Acylation stimulating protein C3 P01031 Complement C5 beta chain C5 P01033 Metalloproteinase inhibitor 1 TIMP1 P01034 Cystatin-C CST3 P01036 Cystatin-S CST4 P01037 Cystatin-SN CST1 P01042 Kininogen-1 light chain KNG1 P01127 Platelet-derived growth factor subunit B PDGFB P01135 Transforming growth factor alpha TGFA P01137 Transforming growth factor beta-1 TGFB1 P01138 Beta-nerve growth factor NGF P01148 Gonadoliberin-1 GNRH1 P01160 Atrial natriuretic factor NPPA P01178 Oxytocin OXT P01185 Vasopressin-neurophysin 2-copeptin AVP P01189 Corticotropin POMC P01210 PENK(237-258) PENK P01213 Alpha-neoendorphin PDYN P01215 Glycoprotein hormones alpha chain CGA P01222 Thyrotropin subunit beta TSHB P01225 Follitropin subunit beta FSHB P01229 Lutropin subunit beta LHB P01233 Choriogonadotropin subunit beta CGB8 P01236 Prolactin PRL P01241 Somatotropin GH1 P01242 Growth hormone variant GH2 P01243 Chorionic somatomammotropin hormone CSH2 P01258 Katacalcin CALCA P01266 Thyroglobulin TG P01270 Parathyroid hormone PTH P01275 Glucagon GCG P01282 Intestinal peptide PHM-27 VIP P01286 Somatoliberin GHRH P01298 Pancreatic prohormone PPY P01303 C-flanking peptide of NPY NPY P01308 Insulin INS P01344 Insulin-like growth factor II IGF2 P01350 Big gastrin GAST P01374 Lymphotoxin-alpha LTA P01375 C-domain 1 TNF P01562 Interferon alpha-1/13 IFNA1 P01563 Interferon alpha-2 IFNA2 P01566 Interferon alpha-10 IFNA10 P01567 Interferon alpha-7 IFNA7 P01568 Interferon alpha-21 IFNA21 P01569 Interferon alpha-5 IFNA5 P01570 Interferon alpha-14 IFNA14 P01571 Interferon alpha-17 IFNA17 P01574 Interferon beta IFNB1 P01579 Interferon gamma IFNG P01583 Interleukin-1 alpha IL1A P01584 Interleukin-1 beta IL1B P01588 Erythropoietin EPO P01591 Immunoglobulin J chain IGJ P01732 T-cell surface glycoprotein CD8 alpha chain CD8A P01833 Polymeric immunoglobulin receptor PIGR P01857 Ig gamma-1 chain C region IGHG1 P01859 Ig gamma-2 chain C region IGHG2 P01860 Ig gamma-3 chain C region IGHG3 P01861 Ig gamma-4 chain C region IGHG4 P01871 Ig mu chain C region IGHM P01880 Ig delta chain C region IGHD P02452 Collagen alpha-1(I) chain COL1A1 P02458 Chondrocalcin COL2A1 P02461 Collagen alpha-1(III) chain COL3A1 P02462 Collagen alpha-1(IV) chain COL4A1 P02647 Apolipoprotein A-I APOA1 P02649 Apolipoprotein E APOE P02652 Apolipoprotein A-II APOA2 P02654 Apolipoprotein C-I APOC1 P02655 Apolipoprotein C-II APOC2 P02656 Apolipoprotein C-III APOC3 P02671 Fibrinogen alpha chain FGA P02675 Fibrinopeptide B FGB P02679 Fibrinogen gamma chain FGG P02741 C-reactive protein CRP P02743 Serum amyloid P-component(1-203) APCS P02745 Complement C1q subcomponent subunit A C1QA P02746 Complement C1q subcomponent subunit B C1QB P02747 Complement C1q subcomponent subunit C C1QC P02748 Complement component C9b C9 P02749 Beta-2-glycoprotein 1 APOH P02750 Leucine-rich alpha-2-glycoprotein LRG1 P02751 Ugl-Y2 FN1 P02753 Retinol-binding protein 4 RBP4 P02760 Trypstatin AMBP P02763 Alpha-1-acid glycoprotein 1 ORM1 P02765 Alpha-2-HS-glycoprotein chain A AHSG P02766 Transthyretin TTR P02768 Serum albumin ALB P02771 Alpha-fetoprotein AFP P02774 Vitamin D-binding protein GC P02775 Connective tissue-activating peptide III PPBP P02776 Platelet factor 4 PF4 P02778 CXCL10(1-73) CXCL10 P02786 Transferrin receptor protein 1 TFRC P02787 Serotransferrin TF P02788 Lactoferroxin-C LTF P02790 Hemopexin HPX P02808 Statherin STATH P02810 Salivary acidic proline-rich phosphoprotein 1/2 PRH2 P02812 Basic salivary proline-rich protein 2 PRB2 P02814 Peptide D1A SMR3B P02818 Osteocalcin BGLAP P03950 Angiogenin ANG P03951 Coagulation factor XIa heavy chain F11 P03952 Plasma kallikrein KLKB1 P03956 27 kDa

interstitial collagenase MMP1 P03971 Muellerian-inhibiting factor AMH P03973 Antileukoproteinase SLPI P04003 C4b-binding protein alpha chain C4BPA P04004 Somatomedin-B VTN P04054 Phospholipase A2 PLA2G1B P04085 Platelet-derived growth factor subunit A PDGFA P04090 Relaxin A chain RLN2 P04114 Apolipoprotein B-100 APOB P04118 Colipase CLPS P04141 Granulocyte-macrophage colony-stimulating factor CSF2 P04155 Trefoil factor 1 TFF1 P04180 Phosphatidylcholine-sterol acyltransferase LCAT P04196 Histidine-rich glycoprotein HRG P04217 Alpha-1B-glycoprotein A1BG P04275 von Willebrand antigen 2 VWF P04278 Sex hormone-binding globulin SHBG P04279 Alpha-inhibin-31 SEMG1 P04280 Basic salivary proline-rich protein 1 PRB1 P04628 Proto-oncogene Wnt-1 WNT1 P04745 Alpha-amylase 1 AMY1A P04746 Pancreatic alpha-amylase AMY2A P04808 Prorelaxin H1 RLN1 P05000 Interferon omega-1 IFNW1 P05013 Interferon alpha-6 IFNA6 P05014 Interferon alpha-4 IFNA4 P05015 Interferon alpha-16 IFNA16 P05019 Insulin-like growth factor I IGF1 P05060 GAWK peptide CHGB P05090 Apolipoprotein D APOD P05109 Protein S100-A8 S100A8 P05111 Inhibin alpha chain INHA P05112 Interleukin-4 IL4 P05113 Interleukin-5 IL5 P05120 Plasminogen activator inhibitor 2 SERPINB2 P05121 Plasminogen activator inhibitor 1 SERPINE1 P05154 Plasma serine protease inhibitor SERPINA5 P05155 Plasma protease C1 inhibitor SERPING1 P05156 Complement factor I heavy chain CFI P05160 Coagulation factor XIII B chain F13B P05161 Ubiquitin-like protein ISG15 ISG15 P05230 Fibroblast growth factor 1 FGF1 P05231 Interleukin-6 IL6 P05305 Big endothelin-1 EDN1 P05408 C-terminal peptide SCG5 P05451 Lithostathine-1-alpha REG1A P05452 Tetranectin CLEC3B P05543 Thyroxine-binding globulin SERPINA7 P05814 Beta-casein CSN2 P05997 Collagen alpha-2(V) chain COL5A2 P06276 Cholinesterase BCHE P06307 Cholecystokinin-12 CCK P06396 Gelsolin GSN P06681 Complement C2 C2 P06702 Protein S100-A9 S100A9 P06727 Apolipoprotein A-IV APOA4 P06734 Low affinity immunoglobulin epsilon Fc receptor FCER2 soluble form P06744 Glucose-6-phosphate isomerase GPI P06850 Corticoliberin CRH P06858 Lipoprotein lipase LPL P06881 Calcitonin gene-related peptide 1 CALCA P07093 Glia-derived nexin SERPINE2 P07098 Gastric triacylglycerol lipase LIPF P07225 Vitamin K-dependent protein S PROS1 P07237 Protein disulfide-isomerase P4HB P07288 Prostate-specific antigen KLK3 P07306 Asialoglycoprotein receptor 1 ASGR1 P07355 Annexin A2 ANXA2 P07357 Complement component C8 alpha chain C8A P07358 Complement component C8 beta chain C8B P07360 Complement component C8 gamma chain C8G P07477 Alpha-trypsin chain 2 PRSS1 P07478 Trypsin-2 PRSS2 P07492 Neuromedin-C GRP P07498 Kappa-casein CSN3 P07585 Decorin DCN P07911 Uromodulin UMOD P07942 Laminin subunit beta-1 LAMB1 P07988 Pulmonary surfactant-associated protein B SFTPB P07998 Ribonuclease pancreatic RNASE1 P08118 Beta-microseminoprotein MSMB P08123 Collagen alpha-2(I) chain COL1A2 P08185 Corticosteroid-binding globulin SERPINA6 P08217 Chymotrypsin-like elastase family member 2A CELA2A P08218 Chymotrypsin-like elastase family member 2B CELA2B P08253 72 kDa type IV collagenase MMP2 P08254 Stromelysin-1 MMP3 P08294 Extracellular superoxide dismutase [Cu—Zn] SOD3 P08476 Inhibin beta A chain INHBA P08493 Matrix Gla protein MGP P08572 Collagen alpha-2(IV) chain COL4A2 P08581 Hepatocyte growth factor receptor MET P08603 Complement factor H CFH P08620 Fibroblast growth factor 4 FGF4 P08637 Low affinity immunoglobulin gamma Fc region FCGR3A receptor III-A P08697 Alpha-2-antiplasmin SERPINF2 P08700 Interleukin-3 IL3 P08709 Coagulation factor VII F7 P08833 Insulin-like growth factor-binding protein 1 IGFBP1 P08887 Interleukin-6 receptor subunit alpha IL6R P08949 Neuromedin-B-32 NMB P08F94 Fibrocystin PKHD1 P09038 Fibroblast growth factor 2 FGF2 P09228 Cystatin-SA CST2 P09237 Matrilysin MMP7 P09238 Stromelysin-2 MMP10 P09341 Growth-regulated alpha protein CXCL1 P09382 Galectin-1 LGALS1 P09466 Glycodelin PAEP P09486 SPARC SPARC P09529 Inhibin beta B chain INHBB P09544 Protein Wnt-2 WNT2 P09603 Processed macrophage colony-stimulating factor 1 CSF1 P09681 Gastric inhibitory polypeptide GIP P09683 Secretin SCT P09919 Granulocyte colony-stimulating factor CSF3 P0C091 FRAS1-related extracellular matrix protein 3

FREM3 P0C0L4 C4d-A C4A P0C0L5 Complement C4-B alpha chain C4B P0C0P6 Neuropeptide S NPS P0C7L1 Serine protease inhibitor Kazal-type 8 SPINK8 P0C862 Complement C1q and tumor necrosis factor- C1QTNF9 related protein 9A P0C8F1 Prostate and testis expressed protein 4 PATE4 P0CG01 Gastrokine-3 GKN3P P0CG36 Cryptic family protein 1B CFC1B P0CG37 Cryptic protein CFC1 P0CJ68 Humanin-like protein 1 MTRNR2L1 P0CJ69 Humanin-like protein 2 MTRNR2L2 P0CJ70 Humanin-like protein 3 MTRNR2L3 P0CJ71 Humanin-like protein 4 MTRNR2L4 P0CJ72 Humanin-like protein 5 MTRNR2L5 P0CJ73 Humanin-like protein 6 MTRNR2L6 P0CJ74 Humanin-like protein 7 MTRNR2L7 P0CJ75 Humanin-like protein 8 MTRNR2L8 P0CJ76 Humanin-like protein 9 MTRNR2L9 P0CJ77 Humanin-like protein 10 MTRNR2L10 P0DJD7 Pepsin A-4 PGA4 P0DJD8 Pepsin A-3 PGA3 P0DJD9 Pepsin A-5 PGA5 P0DJI8 Amyloid protein A SAA1 P0DJI9 Serum amyloid A-2 protein SAA2 P10082 Peptide YY(3-36) PYY P10092 Calcitonin gene-related peptide 2 CALCB P10124 Serglycin SRGN P10145 MDNCF-a IL8 P10147 MIP-1-alpha(4-69) CCL3 P10163 Peptide P-D PRB4 P10451 Osteopontin SPP1 P10599 Thioredoxin TXN P10600 Transforming growth factor beta-3 TGFB3 P10643 Complement component C7 C7 P10645 Vasostatin-2 CHGA P10646 Tissue factor pathway inhibitor TFPI P10720 Platelet factor 4 variant(4-74) PF4V1 P10745 Retinol-binding protein 3 RBP3 P10767 Fibroblast growth factor 6 FGF6 P10909 Clusterin alpha chain CLU P10912 Growth hormone receptor GHR P10915 Hyaluronan and proteoglycan link protein 1 HAPLN1 P10966 T-cell surface glycoprotein CD8 beta chain CD8B P10997 Islet amyloid polypeptide IAPP P11047 Laminin subunit gamma-1 LAMC1 P11150 Hepatic triacylglycerol lipase LIPC P11226 Mannose-binding protein C MBL2 P11464 Pregnancy-specific beta-1-glycoprotein 1 PSG1 P11465 Pregnancy-specific beta-1-glycoprotein 2 PSG2 P11487 Fibroblast growth factor 3 FGF3 P11597 Cholesteryl ester transfer protein CETP P11684 Uteroglobin SCGB1A1 P11686 Pulmonary surfactant-associated protein C SFTPC P12034 Fibroblast growth factor 5 FGF5 P12107 Collagen alpha-1(XI) chain COL11A1 P12109 Collagen alpha-1(VI) chain COL6A1 P12110 Collagen alpha-2(VI) chain COL6A2 P12111 Collagen alpha-3(VI) chain COL6A3 P12259 Coagulation factor V F5 P12272 PTHrP[1-36] PTHLH P12273 Prolactin-inducible protein PIP P12544 Granzyme A GZMA P12643 Bone morphogenetic protein 2 BMP2 P12644 Bone morphogenetic protein 4 BMP4 P12645 Bone morphogenetic protein 3 BMP3 P12724 Eosinophil cationic protein RNASE3 P12821 Angiotensin-converting enzyme, soluble form ACE P12838 Neutrophil defensin 4 DEFA4 P12872 Motilin MLN P13232 Interleukin-7 IL7 P13236 C—C motif chemokine 4 CCL4 P13284 Gamma-interferon-inducible lysosomal thiol IFI30 reductase P13500 C—C motif chemokine 2 CCL2 P13501 C—C motif chemokine 5 CCL5 P13521 Secretogranin-2 SCG2 P13591 Neural cell adhesion molecule 1 NCAM1 P13611 Versican core protein VCAN P13671 Complement component C6 C6 P13688 Carcinoembryonic antigen-related cell adhesion CEACAM1 molecule 1 P13725 Oncostatin-M OSM P13726 Tissue factor F3 P13727 Eosinophil granule major basic protein PRG2 P13942 Collagen alpha-2(XI) chain COL11A2 P13987 CD59 glycoprotein CD59 P14138 Endothelin-3 EDN3 P14174 Macrophage migration inhibitory factor MIF P14207 Folate receptor beta FOLR2 P14222 Perforin-1 PRF1 P14543 Nidogen-1 NID1 P14555 Phospholipase A2, membrane associated PLA2G2A P14625 Endoplasmin HSP90B1 P14735 Insulin-degrading enzyme IDE P14778 Interleukin-1 receptor type 1, soluble form IL1R1 P14780 82 kDa matrix metalloproteinase-9 MMP9 P15018 Leukemia inhibitory factor LIF P15085 Carboxypeptidase A1 CPA1 P15086 Carboxypeptidase B CPB1 P15151 Poliovirus receptor PVR P15169 Carboxypeptidase N catalytic chain CPN1 P15248 Interleukin-9 IL9 P15291 N-acetyllactosamine synthase B4GALT1 P15309 PAPG9 ACPP P15328 Folate receptor alpha FOLR1 P15374 Ubiquitin carboxyl-terminal hydrolase isozyme L3 UCHL3 P15502 Elastin ELN P15509 Granulocyte-macrophage colony-stimulating CSF2RA factor receptor subunit alpha P15515 Histatin-1 HTN1 P15516 His3-(31-51)-peptide HTN3 P15692 Vascular endothelial growth factor A VEGFA P15814 Immunoglobulin lambda-like polypeptide 1 IGLL1 P15907 Beta-galactoside alpha-2,6-sialyltransferase 1 ST6GAL1 P15941 Mucin-1 subunit beta MUC1 P16035

Metalloproteinase inhibitor 2 TIMP2 P16112 Aggrecan core protein 2 ACAN P16233 Pancreatic triacylglycerol lipase PNLIP P16442 Histo-blood group ABO system transferase ABO P16471 Prolactin receptor PRLR P16562 Cysteine-rich secretory protein 2 CRISP2 P16619 C—C motif chemokine 3-like 1 CCL3L1 P16860 BNP(3-29) NPPB P16870 Carboxypeptidase E CPE P16871 Interleukin-7 receptor subunit alpha IL7R P17213 Bactericidal permeability-increasing protein BPI P17538 Chymotrypsinogen B CTRB1 P17931 Galectin-3 LGALS3 P17936 Insulin-like growth factor-binding protein 3 IGFBP3 P17948 Vascular endothelial growth factor receptor 1 FLT1 P18065 Insulin-like growth factor-binding protein 2 IGFBP2 P18075 Bone morphogenetic protein 7 BMP7 P18428 Lipopolysaccharide-binding protein LBP P18509 PACAP-related peptide ADCYAP1 P18510 Interleukin-1 receptor antagonist protein IL1RN P18827 Syndecan-1 SDC1 P19021 Peptidylglycine alpha-hydroxylating monooxygenase PAM P19235 Erythropoietin receptor EPOR P19438 Tumor necrosis factor-binding protein 1 TNFRSF1A P19652 Alpha-1-acid glycoprotein 2 ORM2 P19801 Amiloride-sensitive amine oxidase [copper-containing] ABP1 P19823 Inter-alpha-trypsin inhibitor heavy chain H2 ITIH2 P19827 Inter-alpha-trypsin inhibitor heavy chain H1 ITIH1 P19835 Bile salt-activated lipase CEL P19875 C—X—C motif chemokine 2 CXCL2 P19876 C—X—C motif chemokine 3 CXCL3 P19883 Follistatin FST P19957 Elafin PI3 P19961 Alpha-amylase 2B AMY2B P20061 Transcobalamin-1 TCN1 P20062 Transcobalamin-2 TCN2 P20142 Gastricsin PGC P20155 Serine protease inhibitor Kazal-type 2 SPINK2 P20231 Tryptase beta-2 TPSB2 P20333 Tumor necrosis factor receptor superfamily member 1B TNFRSF1B P20366 Substance P TAC1 P20382 Melanin-concentrating hormone PMCH P20396 Thyroliberin TRH P20742 Pregnancy zone protein PZP P20774 Mimecan OGN P20783 Neurotrophin-3 NTF3 P20800 Endothelin-2 EDN2 P20809 Interleukin-11 IL11 P20827 Ephrin-A1 EFNA1 P20849 Collagen alpha-1(IX) chain COL9A1 P20851 C4b-binding protein beta chain C4BPB P20908 Collagen alpha-1(V) chain COL5A1 P21128 Poly(U)-specific endoribonuclease ENDOU P21246 Pleiotrophin PTN P21583 Kit ligand KITLG P21741 Midkine MDK P21754 Zona pellucida sperm-binding protein 3 ZP3 P21781 Fibroblast growth factor 7 FGF7 P21802 Fibroblast growth factor receptor 2 FGFR2 P21810 Biglycan BGN P21815 Bone sialoprotein 2 IBSP P21860 Receptor tyrosine-protein kinase erbB-3 ERBB3 P21941 Cartilage matrix protein MATN1 P22003 Bone morphogenetic protein 5 BMP5 P22004 Bone morphogenetic protein 6 BMP6 P22079 Lactoperoxidase LPO P22105 Tenascin-X TNXB P22301 Interleukin-10 IL10 P22303 Acetylcholinesterase ACHE P22352 Glutathione peroxidase 3 GPX3 P22362 C—C motif chemokine 1 CCL1 P22455 Fibroblast growth factor receptor 4 FGFR4 P22466 Galanin message-associated peptide GAL P22692 Insulin-like growth factor-binding protein 4 IGFBP4 P22749 Granulysin GNLY P22792 Carboxypeptidase N subunit 2 CPN2 P22891 Vitamin K-dependent protein Z PROZ P22894 Neutrophil collagenase MMP8 P23142 Fibulin-1 FBLN1 P23280 Carbonic anhydrase 6 CA6 P23352 Anosmin-1 KAL1 P23435 Cerebellin-1 CBLN1 P23560 Brain-derived neurotrophic factor BDNF P23582 C-type natriuretic peptide NPPC P23946 Chymase CMA1 P24043 Laminin subunit alpha-2 LAMA2 P24071 Immunoglobulin alpha Fc receptor FCAR P24347 Stromelysin-3 MMP11 P24387 Corticotropin-releasing factor-binding protein CRHBP P24592 Insulin-like growth factor-binding protein 6 IGFBP6 P24593 Insulin-like growth factor-binding protein 5 IGFBP5 P24821 Tenascin TNC P24855 Deoxyribonuclease-1 DNASE1 P25067 Collagen alpha-2(VIII) chain COL8A2 P25311 Zinc-alpha-2-glycoprotein AZGP1 P25391 Laminin subunit alpha-1 LAMA1 P25445 Tumor necrosis factor receptor superfamily member 6 FAS P25940 Collagen alpha-3(V) chain COL5A3 P25942 Tumor necrosis factor receptor superfamily member 5 CD40 P26022 Pentraxin-related protein PTX3 PTX3 P26927 Hepatocyte growth factor-like protein beta chain MST1 P27169 Serum paraoxonase/arylesterase 1 PON1 P27352 Gastric intrinsic factor GIF P27487 Dipeptidyl peptidase 4 membrane form DPP4 P27539 Embryonic growth/differentiation factor 1 GDF1 P27658 Vastatin COL8A1 P27797 Calreticulin CALR P27918 Properdin CFP P28039 Acyloxyacyl hydrolase AOAH P28300 Protein-lysine 6-oxidase LOX P28325 Cystatin-D CST5 P28799 Granulin-1 GRN P29122 Proprotein convertase

subtilisin/kexin type 6 PCSK6 P29279 Connective tissue growth factor CTGF P29320 Ephrin type-A receptor 3 EPHA3 P29400 Collagen alpha-5(IV) chain COL4A5 P29459 Interleukin-12 subunit alpha IL12A P29460 Interleukin-12 subunit beta IL12B P29508 Serpin B3 SERPINB3 P29622 Kallistatin SERPINA4 P29965 CD40 ligand, soluble form CD40LG P30990 Neurotensin/neuromedin N NTS P31025 Lipocalin-1 LCN1 P31151 Protein S100-A7 S100A7 P31371 Fibroblast growth factor 9 FGF9 P31431 Syndecan-4 SDC4 P31947 14-3-3 protein sigma SFN P32455 Interferon-induced guanylate-binding protein 1 GBP1 P32881 Interferon alpha-8 IFNA8 P34096 Ribonuclease 4 RNASE4 P34130 Neurotrophin-4 NTF4 P34820 Bone morphogenetic protein 8B BMP8B P35030 Trypsin-3 PRSS3 P35052 Secreted glypican-1 GPC1 P35070 Betacellulin BTC P35225 Interleukin-13 IL13 P35247 Pulmonary surfactant-associated protein D SFTPD P35318 ADM ADM P35542 Serum amyloid A-4 protein SAA4 P35555 Fibrillin-1 FBN1 P35556 Fibrillin-2 FBN2 P35625 Metalloproteinase inhibitor 3 TIMP3 P35858 Insulin-like growth factor-binding protein complex IGFALS acid labile subunit P35916 Vascular endothelial growth factor receptor 3 FLT4 P35968 Vascular endothelial growth factor receptor 2 KDR P36222 Chitinase-3-like protein 1 CHI3L1 P36952 Serpin B5 SERPINB5 P36955 Pigment epithelium-derived factor SERPINF1 P36980 Complement factor H-related protein 2 CFHR2 P39059 Collagen alpha-1(XV) chain COL15A1 P39060 Collagen alpha-1(XVIII) chain COL18A1 P39877 Calcium-dependent phospholipase A2 PLA2G5 P39900 Macrophage metalloelastase MMP12 P39905 Glial cell line-derived neurotrophic factor GDNF P40225 Thrombopoietin THPO P40967 M-alpha PMEL P41159 Leptin LEP P41221 Protein Wnt-5a WNT5A P41222 Prostaglandin-H2 D-isomerase PTGDS P41271 Neuroblastoma suppressor of tumorigenicity 1 NBL1 P41439 Folate receptor gamma FOLR3 P42127 Agouti-signaling protein ASIP P42702 Leukemia inhibitory factor receptor LIFR P42830 ENA-78(9-78) CXCL5 P43026 Growth/differentiation factor 5 GDF5 P43251 Biotinidase BTD P43652 Afamin AFM P45452 Collagenase 3 MMP13 P47710 Casoxin-D CSN1S1 P47929 Galectin-7 LGALS7B P47972 Neuronal pentraxin-2 NPTX2 P47989 Xanthine oxidase XDH P47992 Lymphotactin XCL1 P48023 Tumor necrosis factor ligand superfamily member 6, FASLG membrane form P48052 Carboxypeptidase A2 CPA2 P48061 Stromal cell-derived factor 1 CXCL12 P48304 Lithostathine-1-beta REG1B P48307 Tissue factor pathway inhibitor 2 TFPI2 P48357 Leptin receptor LEPR P48594 Serpin B4 SERPINB4 P48645 Neuromedin-U-25 NMU P48740 Mannan-binding lectin serine protease 1 MASP1 P48745 Protein NOV homolog NOV P48960 CD97 antigen subunit beta CD97 P49223 Kunitz-type protease inhibitor 3 SPINT3 P49747 Cartilage oligomeric matrix protein COMP P49763 Placenta growth factor PGF P49765 Vascular endothelial growth factor B VEGFB P49767 Vascular endothelial growth factor C VEGFC P49771 Fms-related tyrosine kinase 3 ligand FLT3LG P49862 Kallikrein-7 KLK7 P49863 Granzyme K GZMK P49908 Selenoprotein P SEPP1 P49913 Antibacterial protein FALL-39 CAMP P50607 Tubby protein homolog TUB P51124 Granzyme M GZMM P51512 Matrix metalloproteinase-16 MMP16 P51654 Glypican-3 GPC3 P51671 Eotaxin CCL11 P51884 Lumican LUM P51888 Prolargin PRELP P52798 Ephrin-A4 EFNA4 P52823 Stanniocalcin-1 STC1 P53420 Collagen alpha-4(IV) chain COL4A4 P53621 Coatomer subunit alpha COPA P54108 Cysteine-rich secretory protein 3 CRISP3 P54315 Pancreatic lipase-related protein 1 PNLIPRP1 P54317 Pancreatic lipase-related protein 2 PNLIPRP2 P54793 Arylsulfatase F ARSF P55000 Secreted Ly-6/uPAR-related protein 1 SLURP1 P55001 Microfibrillar-associated protein 2 MFAP2 P55056 Apolipoprotein C-IV APOC4 P55058 Phospholipid transfer protein PLTP P55075 Fibroblast growth factor 8 FGF8 P55081 Microfibrillar-associated protein 1 MFAP1 P55083 Microfibril-associated glycoprotein 4 MFAP4 P55107 Bone morphogenetic protein 3B GDF10 P55145 Mesencephalic astrocyte-derived neurotrophic factor MANF P55259 Pancreatic secretory granule membrane major GP2 glycoprotein GP2 P55268 Laminin subunit beta-2 LAMB2 P55773 CCL23(30-99) CCL23 P55774 C—C motif chemokine 18 CCL18 P55789 FAD-linked sulfhydryl oxidase ALR GFER P56703 Proto-oncogene Wnt-3 WNT3 P56704 Protein Wnt-3a WNT3A P56705 Protein Wnt-4 WNT4

P56706 Protein Wnt-7b WNT7B P56730 Neurotrypsin PRSS12 P56851 Epididymal secretory protein E3-beta EDDM3B P56975 Neuregulin-3 NRG3 P58062 Serine protease inhibitor Kazal-type 7 SPINK7 P58215 Lysyl oxidase homolog 3 LOXL3 P58294 Prokineticin-1 PROK1 P58335 Anthrax toxin receptor 2 ANTXR2 P58397 A disintegrin and metalloproteinase with thrombospondin ADAMTS12 motifs 12 P58417 Neurexophilin-1 NXPH1 P58499 Protein FAM3B FAM3B P59510 A disintegrin and metalloproteinase with thrombospondin ADAMTS20 motifs 20 P59665 Neutrophil defensin 1 DEFA1B P59666 Neutrophil defensin 3 DEFA3 P59796 Glutathione peroxidase 6 GPX6 P59826 BPI fold-containing family B member 3 BPIFB3 P59827 BPI fold-containing family B member 4 BPIFB4 P59861 Beta-defensin 131 DEFB131 P60022 Beta-defensin 1 DEFB1 P60153 Inactive ribonuclease-like protein 9 RNASE9 P60827 Complement C1q tumor necrosis factor-related protein 8 C1QTNF8 P60852 Zona pellucida sperm-binding protein 1 ZP1 P60985 Keratinocyte differentiation-associated protein KRTDAP P61109 Kidney androgen-regulated protein KAP P61278 Somatostatin-14 SST P61366 Osteocrin OSTN P61626 Lysozyme C LYZ P61769 Beta-2-microglobulin B2M P61812 Transforming growth factor beta-2 TGFB2 P61916 Epididymal secretory protein E1 NPC2 P62502 Epididymal-specific lipocalin-6 LCN6 P62937 Peptidyl-prolyl cis-trans isomerase A PPIA P67809 Nuclease-sensitive element-binding protein 1 YBX1 P67812 Signal peptidase complex catalytic subunit SEC11A SEC11A P78310 Coxsackievirus and adenovirus receptor CXADR P78333 Secreted glypican-5 GPC5 P78380 Oxidized low-density lipoprotein receptor 1 OLR1 P78423 Processed fractalkine CX3CL1 P78509 Reelin RELN P78556 CCL20(2-70) CCL20 P80075 MCP-2(6-76) CCL8 P80098 C—C motif chemokine 7 CCL7 P80108 Phosphatidylinositol-glycan-specific phospholipase D GPLD1 P80162 C—X—C motif chemokine 6 CXCL6 P80188 Neutrophil gelatinase-associated lipocalin LCN2 P80303 Nucleobindin-2 NUCB2 P80511 Calcitermin S100A12 P81172 Hepcidin-25 HAMP P81277 Prolactin-releasing peptide PRLH P81534 Beta-defensin 103 DEFB103A P81605 Dermcidin DCD P82279 Protein crumbs homolog 1 CRB1 P82987 ADAMTS-like protein 3 ADAMTSL3 P83105 Serine protease HTRA4 HTRA4 P83110 Serine protease HTRA3 HTRA3 P83859 Orexigenic neuropeptide QRFP QRFP P98088 Mucin-5AC MUC5AC P98095 Fibulin-2 FBLN2 P98160 Basement membrane-specific heparan sulfate proteoglycan core protein HSPG2 P98173 Protein FAM3A FAM3A Q00604 Norrin NDP Q00796 Sorbitol dehydrogenase SORD Q00887 Pregnancy-specific beta-1-glycoprotein 9 PSG9 Q00888 Pregnancy-specific beta-1-glycoprotein 4 PSG4 Q00889 Pregnancy-specific beta-1-glycoprotein 6 PSG6 Q01523 HD5(56-94) DEFA5 Q01524 Defensin-6 DEFA6 Q01955 Collagen alpha-3(IV) chain COL4A3 Q02297 Pro-neuregulin-1, membrane-bound isoform NRG1 Q02325 Plasminogen-like protein B PLGLB1 Q02383 Semenogelin-2 SEMG2 Q02388 Collagen alpha-1(VII) chain COL7A1 Q02505 Mucin-3A MUC3A Q02509 Otoconin-90 OC90 Q02747 Guanylin GUCA2A Q02763 Angiopoietin-1 receptor TEK Q02817 Mucin-2 MUC2 Q02985 Complement factor H-related protein 3 CFHR3 Q03167 Transforming growth factor beta receptor type 3 TGFBR3 Q03403 Trefoil factor 2 TFF2 Q03405 Urokinase plasminogen activator surface receptor PLAUR Q03591 Complement factor H-related protein 1 CFHR1 Q03692 Collagen alpha-1(X) chain COL10A1 Q04118 Basic salivary proline-rich protein 3 PRB3 Q04756 Hepatocyte growth factor activator short chain HGFAC Q04900 Sialomucin core protein 24 CD164 Q05315 Eosinophil lysophospholipase CLC Q05707 Collagen alpha-1(XIV) chain COL14A1 Q05996 Processed zona pellucida sperm-binding protein 2 ZP2 Q06033 Inter-alpha-trypsin inhibitor heavy chain H3 ITIH3 Q06141 Regenerating islet-derived protein 3-alpha REG3A Q06828 Fibromodulin FMOD Q07092 Collagen alpha-1(XVI) chain COL16A1 Q07325 C—X—C motif chemokine 9 CXCL9 Q07507 Dermatopontin DPT Q075Z2 Binder of sperm protein homolog 1 BSPH1 Q07654 Trefoil factor 3 TFF3 Q07699 Sodium channel subunit beta-1 SCN1B Q08345 Epithelial discoidin domain-containing receptor 1 DDR1 Q08380 Galectin-3-binding protein LGALS3BP Q08397 Lysyl oxidase homolog 1 LOXL1 Q08431 Lactadherin MFGE8 Q08629 Testican-1 SPOCK1 Q08648 Sperm-associated antigen 11B SPAG11B Q08830 Fibrinogen-like protein 1 FGL1 Q10471 Polypeptide N-

acetylgalactosaminyltransferase 2 GALNT2 Q10472 Polypeptide N-acetylgalactosaminyltransferase 1 GALNT1 Q11201 CMP-N-acetylneuraminate-beta-galactosamide- ST3GAL1 alpha-2,3-sialyltransferase 1 Q11203 CMP-N-acetylneuraminate-beta-1,4-galactoside ST3GAL3 alpha-2,3-sialyltransferase Q11206 CMP-N-acetylneuraminate-beta-galactosamide- ST3GAL4 alpha-2,3-sialyltransferase 4 Q12794 Hyaluronidase-1 HYAL1 Q12805 EGF-containing fibulin-like extracellular matrix EFEMP1 protein 1 Q12836 Zona pellucida sperm-binding protein 4 ZP4 Q12841 Follistatin-related protein 1 FSTL1 Q12904 Aminoacyl tRNA synthase complex-interacting AIMP1 multifunctional protein 1 Q13018 Soluble secretory phospholipase A2 receptor PLA2R1 Q13072 B melanoma antigen 1 BAGE Q13093 Platelet-activating factor acetylhydrolase PLA2G7 Q13103 Secreted phosphoprotein 24 SPP2 Q13162 Peroxiredoxin-4 PRDX4 Q13201 Platelet glycoprotein Ia* MMRN1 Q13214 Semaphorin-3B SEMA3B Q13219 Pappalysin-1 PAPPA Q13231 Chitotriosidase-1 CHIT1 Q13253 Noggin NOG Q13261 Interleukin-15 receptor subunit alpha IL15RA Q13275 Semaphorin-3F SEMA3F Q13291 Signaling lymphocytic activation molecule SLAMF1 Q13316 Dentin matrix acidic phosphoprotein 1 DMP1 Q13361 Microfibrillar-associated protein 5 MFAP5 Q13410 Butyrophilin subfamily 1 member A1 BTN1A1 Q13421 Mesothelin, cleaved form MSLN Q13429 Insulin-like growth factor I IGF-I Q13443 Disintegrin and metalloproteinase domain-containing ADAM9 protein 9 Q13519 Neuropeptide 1 PNOC Q13751 Laminin subunit beta-3 LAMB3 Q13753 Laminin subunit gamma-2 LAMC2 Q13790 Apolipoprotein F APOF Q13822 Ectonucleotide pyrophosphatase/phosphodiesterase family ENPP2 member 2 Q14031 Collagen alpha-6(IV) chain COL4A6 Q14050 Collagen alpha-3(IX) chain COL9A3 Q14055 Collagen alpha-2(IX) chain COL9A2 Q14112 Nidogen-2 NID2 Q14114 Low-density lipoprotein receptor-related protein 8 LRP8 Q14118 Dystroglycan DAG1 Q14314 Fibroleukin FGL2 Q14393 Growth arrest-specific protein 6 GAS6 Q14406 Chorionic somatomammotropin hormone-like 1 CSHL1 Q14507 Epididymal secretory protein E3-alpha EDDM3A Q14508 WAP four-disulfide core domain protein 2 WFDC2 Q14512 Fibroblast growth factor-binding protein 1 FGFBP1 Q14515 SPARC-like protein 1 SPARCL1 Q14520 Hyaluronan-binding protein 2 27 kDa light chain HABP2 Q14563 Semaphorin-3A SEMA3A Q14623 Indian hedgehog protein IHH Q14624 Inter-alpha-trypsin inhibitor heavy chain H4 ITIH4 Q14667 UPF0378 protein KIAA0100 KIAA0100 Q14703 Membrane-bound transcription factor site-1 protease MBTPS1 Q14766 Latent-transforming growth factor beta-binding protein 1 LTBP1 Q14767 Latent-transforming growth factor beta-binding protein 2 LTBP2 Q14773 Intercellular adhesion molecule 4 ICAM4 Q14993 Collagen alpha-1(XIX) chain COL19A1 Q14CN2 Calcium-activated chloride channel regulator 4, CLCA4 110 kDa form Q15046 Lysine--tRNA ligase KARS Q15063 Periostin POSTN Q15109 Advanced glycosylation end product-specific AGER receptor Q15113 Procollagen C-endopeptidase enhancer 1 PCOLCE Q15166 Serum paraoxonase/lactonase 3 PON3 Q15195 Plasminogen-like protein A PLGLA Q15198 Platelet-derived growth factor receptor-like protein PDGFRL Q15223 Poliovirus receptor-related protein 1 PVRL1 Q15238 Pregnancy-specific beta-1-glycoprotein 5 PSG5 Q15363 Transmembrane emp24 domain-containing protein 2 TMED2 Q15375 Ephrin type-A receptor 7 EPHA7 Q15389 Angiopoietin-1 ANGPT1 Q15465 Sonic hedgehog protein SHH Q15485 Ficolin-2 FCN2 Q15517 Corneodesmosin CDSN Q15582 Transforming growth factor-beta-induced protein ig-h3 TGFBI Q15661 Tryptase alpha/beta-1 TPSAB1 Q15726 Metastin KISS1 Q15782 Chitinase-3-like protein 2 CHI3L2 Q15828 Cystatin-M CST6 Q15846 Clusterin-like protein 1 CLUL1 Q15848 Adiponectin ADIPOQ Q16206 Protein disulfide-thiol oxidoreductase ENOX2 Q16270 Insulin-like growth factor-binding protein 7 IGFBP7 Q16363 Laminin subunit alpha-4 LAMA4 Q16378 Proline-rich protein 4 PRR4 Q16557 Pregnancy-specific beta-1-glycoprotein 3 PSG3 Q16568 CART(42-89) CARTPT Q16610 Extracellular matrix protein 1 ECM1 Q16619 Cardiotrophin-1 CTF1 Q16623 Syntaxin-1A STX1A Q16627 HCC-1(9-74) CCL14 Q16651 Prostasin light chain PRSS8 Q16661 Guanylate cyclase C-activating peptide 2 GUCA2B Q16663 CCL15(29-92) CCL15 Q16674 Melanoma-derived growth regulatory protein MIA Q16769

Glutaminyl-peptide cyclotransferase QPCT Q16787 Laminin subunit alpha-3 LAMA3 Q16842 CMP-N-acetylneuraminate-beta-galactosamide- ST3GAL2 alpha-2,3-sialyltransferase 2 Q17RR3 Pancreatic lipase-related protein 3 PNLIPRP3 Q17RW2 Collagen alpha-1(XXIV) chain COL24A1 Q17RY6 Lymphocyte antigen 6K LY6K Q1L6U9 Prostate-associated microseminoprotein MSMP Q1W4C9 Serine protease inhibitor Kazal-type 13 SPINK13 Q1ZYL8 Izumo sperm-egg fusion protein 4 IZUMO4 Q29960 HLA class I histocompatibility antigen, Cw-16 HLA-C alpha chain Q2I0M5 R-spondin-4 RSPO4 Q2L4Q9 Serine protease 53 PRSS53 Q2MKA7 R-spondin-1 RSPO1 Q2MV58 Tectonic-1 TCTN1 Q2TAL6 Brorin VWC2 Q2UY09 Collagen alpha-1(XXVIII) chain COL28A1 Q2VPA4 Complement component receptor 1-like protein CR1L Q2WEN9 Carcinoembryonic antigen-related cell adhesion CEACAM16 molecule 16 Q30KP8 Beta-defensin 136 DEFB136 Q30KP9 Beta-defensin 135 DEFB135 Q30KQ1 Beta-defensin 133 DEFB133 Q30KQ2 Beta-defensin 130 DEFB130 Q30KQ4 Beta-defensin 116 DEFB116 Q30KQ5 Beta-defensin 115 DEFB115 Q30KQ6 Beta-defensin 114 DEFB114 Q30KQ7 Beta-defensin 113 DEFB113 Q30KQ8 Beta-defensin 112 DEFB112 Q30KQ9 Beta-defensin 110 DEFB110 Q30KR1 Beta-defensin 109 DEFB109P1 Q32P28 Prolyl 3-hydroxylase 1 LEPRE1 Q3B7J2 Glucose-fructose oxidoreductase domain-containing protein 2 GFOD2 Q3SY79 Protein Wnt WNT3A Q3T906 N-acetylglucosamine-1-phosphotransferase subunits GNPTAB alpha/beta Q495T6 Membrane metallo-endopeptidase-like 1 MMEL1 Q49AH0 Cerebral dopamine neurotrophic factor CDNF Q4G0G5 Secretoglobin family 2B member 2 SCGB2B2 Q4G0M1 Protein FAM132B FAM132B Q4LDE5 Sushi, von Willebrand factor type A, EGF and pentraxin SVEP1 domain-containing protein 1 Q4QY38 Beta-defensin 134 DEFB134 Q4VAJ4 Protein Wnt WNT10B Q4W5P6 Protein TMEM155 TMEM155 Q4ZHG4 Fibronectin type III domain-containing protein 1 FNDC1 Q53H76 Phospholipase A1 member A PLA1A Q53RD9 Fibulin-7 FBLN7 Q53S33 BolA-like protein 3 BOLA3 Q5BLP8 Neuropeptide-like protein C4orf48 C4orf48 Q5DT21 Serine protease inhibitor Kazal-type 9 SPINK9 Q5EBL8 PDZ domain-containing protein 11 PDZD11 Q5FYB0 Arylsulfatase J ARSJ Q5FYB1 Arylsulfatase I ARSI Q5GAN3 Ribonuclease-like protein 13 RNASE13 Q5GAN4 Ribonuclease-like protein 12 RNASE12 Q5GAN6 Ribonuclease-like protein 10 RNASE10 Q5GFL6 von Willebrand factor A domain-containing protein 2 VWA2 Q5H8A3 Neuromedin-S NMS Q5H8C1 FRAS1-related extracellular matrix protein 1 FREM1 Q5IJ48 Protein crumbs homolog 2 CRB2 Q5J5C9 Beta-defensin 121 DEFB121 Q5JS37 NHL repeat-containing protein 3 NHLRC3 Q5JTB6 Placenta-specific protein 9 PLAC9 Q5JU69 Torsin-2A TOR2A Q5JXM2 Methyltransferase-like protein 24 METTL24 Q5JZY3 Ephrin type-A receptor 10 EPHA10 Q5K4E3 Polyserase-2 PRSS36 Q5SRR4 Lymphocyte antigen 6 complex locus protein G5c LY6G5C Q5T1H1 Protein eyes shut homolog EYS Q5T4F7 Secreted frizzled-related protein 5 SFRP5 Q5T4W7 Artemin ARTN Q5T7M4 Protein FAM132A FAM132A Q5TEH8 Protein Wnt WNT2B Q5TIE3 von Willebrand factor A domain-containing protein 5B1 VWA5B1 Q5UCC4 ER membrane protein complex subunit 10 EMC10 Q5VST6 Abhydrolase domain-containing protein FAM108B1 FAM108B1 Q5VTL7 Fibronectin type III domain-containing protein 7 FNDC7 Q5VUM1 UPF0369 protein C6orf57 C6orf57 Q5VV43 Dyslexia-associated protein KIAA0319 KIAA0319 Q5VWW1 Complement C1q-like protein 3 C1QL3 Q5VXI9 Lipase member N LIPN Q5VXJ0 Lipase member K LIPK Q5VXM1 CUB domain-containing protein 2 CDCP2 Q5VYX0 Renalase RNLS Q5VYY2 Lipase member M LIPM Q5W186 Cystatin-9 CST9 Q5W5W9 Regulated endocrine-specific protein 18 RESP18 Q5XG92 Carboxylesterase 4A CES4A Q63HQ2 Pikachurin EGFLAM Q641Q3 Meteorin-like protein METRNL Q66K79 Carboxypeptidase Z CPZ Q685J3 Mucin-17 MUC17 Q68BL7 Olfactomedin-like protein 2A OLFML2A Q68BL8 Olfactomedin-like protein 2B OLFML2B Q68DV7 E3 ubiquitin-protein ligase RNF43 RNF43 Q6B9Z1 Insulin growth factor-like family member 4 IGFL4 Q6BAA4 Fc receptor-like B FCRLB Q6E0U4 Dermokine DMKN Q6EMK4 Vasorin VASN Q6FHJ7 Secreted frizzled-related protein 4 SFRP4 Q6GPI1 Chymotrypsin B2 chain B CTRB2 Q6GTS8 Probable Carboxypeptidase PM20D1 PM20D1 Q6H9L7 Isthmin-2 ISM2 Q6IE36 Ovostatin homolog 2 OVOS2 Q6IE37 Ovostatin

homolog 1 OVOS1 Q6IE38 Serine protease inhibitor Kazal-type 14 SPINK14 Q6ISS4 Leukocyte-associated immunoglobulin-like receptor 2 LAIR2 Q6JVE5 Epididymal-specific lipocalin-12 LCN12 Q6JVE6 Epididymal-specific lipocalin-10 LCN10 Q6JVE9 Epididymal-specific lipocalin-8 LCN8 Q6KF10 Growth/differentiation factor 6 GDF6 Q6MZW2 Follistatin-related protein 4 FSTL4 Q6NSX1 Coiled-coil domain-containing protein 70 CCDC70 Q6NT32 Carboxylesterase 5A CES5A Q6NT52 Choriogonadotropin subunit beta variant 2 CGB2 Q6NUI6 Chondroactherin-like protein CHADL Q6NUJ1 Saposin A-like PSAPL1 Q6P093 Arylacetamide deacetylase-like 2 AADACL2 Q6P4A8 Phospholipase B-like 1 PLBD1 Q6P5S2 UPF0762 protein C6orf58 C6orf58 Q6P988 Protein notum homolog NOTUM Q6PCB0 von Willebrand factor A domain-containing protein 1 VWA1 Q6PDA7 Sperm-associated antigen 11A SPAG11A Q6PEW0 Inactive serine protease 54 PRSS54 Q6PEZ8 Podocan-like protein 1 PODNL1 Q6PKH6 Dehydrogenase/reductase SDR family member 4-like 2 DHRS4L2 Q6Q788 Apolipoprotein A-V APOA5 Q6SPF0 Atherin SAMD1 Q6UDR6 Kunitz-type protease inhibitor 4 SPINT4 Q6URK8 Testis, prostate and placenta-expressed protein TEPP Q6UW01 Cerebellin-3 CBLN3 Q6UW10 Surfactant-associated protein 2 SFTA2 Q6UW15 Regenerating islet-derived protein 3-gamma REG3G Q6UW32 Insulin growth factor-like family member 1 IGFL1 Q6UW78 UPF0723 protein C11orf83 C11orf83 Q6UW88 Epigen EPGN Q6UWE3 Colipase-like protein 2 CLPSL2 Q6UWF7 NXPE family member 4 NXPE4 Q6UWF9 Protein FAM180A FAM180A Q6UWM5 GLIPR1-like protein 1 GLIPR1L1 Q6UWN8 Serine protease inhibitor Kazal-type 6 SPINK6 Q6UWP2 Dehydrogenase/reductase SDR family member 11 DHRS11 Q6UWP8 Suprabasin SBSN Q6UWQ5 Lysozyme-like protein 1 LYZL1 Q6UWQ7 Insulin growth factor-like family member 2 IGFL2 Q6UWR7 Ectonucleotide pyrophosphatase/phosphodiesterase ENPP6 family member 6 soluble form Q6UWT2 Adropin ENHO Q6UWU2 Beta-galactosidase-1-like protein GLB1L Q6UWW0 Lipocalin-15 LCN15 Q6UWX4 HHIP-like protein 2 HHIPL2 Q6UWY0 Arylsulfatase K ARSK Q6UWY2 Serine protease 57 PRSS57 Q6UWY5 Olfactomedin-like protein 1 OLFML1 Q6UX06 Olfactomedin-4 OLFM4 Q6UX07 Dehydrogenase/reductase SDR family member 13 DHRS13 Q6UX39 Amelotin AMTN Q6UX46 Protein FAM150B FAM150B Q6UX73 UPF0764 protein C16orf89 C16orf89 Q6UXB0 Protein FAM131A FAM131A Q6UXB1 Insulin growth factor-like family member 3 IGFL3 Q6UXB2 VEGF co-regulated chemokine 1 CXCL17 Q6UXF7 C-type lectin domain family 18 member B CLEC18B Q6UXH0 Hepatocellular carcinoma-associated protein TD26 C19orf80 Q6UXH1 Cysteine-rich with EGF-like domain protein 2 CRELD2 Q6UXH8 Collagen and calcium-binding EGF domain-containing CCBE1 protein 1 Q6UXH9 Inactive serine protease PAMR1 PAMR1 Q6UXI7 Vitrin VIT Q6UXI9 Nephronectin NPNT Q6UXN2 Trem-like transcript 4 protein TREML4 Q6UXS0 C-type lectin domain family 19 member A CLEC19A Q6UXT8 Protein FAM150A FAM150A Q6UXT9 Abhydrolase domain-containing protein 15 ABHD15 Q6UXV4 Apolipoprotein O-like APOOL Q6UXX5 Inter-alpha-trypsin inhibitor heavy chain H6 ITIH6 Q6UXX9 R-spondin-2 RSPO2 Q6UY14 ADAMTS-like protein 4 ADAMTSL4 Q6UY27 Prostate and testis expressed protein 2 PATE2 Q6W4X9 Mucin-6 MUC6 Q6WN34 Chordin-like protein 2 CHRDL2 Q6WRI0 Immunoglobulin superfamily member 10 IGSF10 Q6X4U4 Sclerostin domain-containing protein 1 SOSTDC1 Q6X784 Zona pellucida-binding protein 2 ZPBP2 Q6XE38 Secretoglobin family 1D member 4 SCGB1D4 Q6XPR3 Repetin RPTN Q6XZB0 Lipase member I LIPI Q6ZMM2 ADAMTS-like protein 5 ADAMTSL5 Q6ZMP0 Thrombospondin type-1 domain-containing THSD4 protein 4 Q6ZNF0 Iron/zinc purple acid phosphatase-like protein PAPL Q6ZRI0 Otogelin OTOG Q6ZRP7 Sulfhydryl oxidase 2 QSOX2 Q6ZWJ8 Kielin/chordin-like protein KCP Q75N90 Fibrillin-3 FBN3 Q765I0 Urotensin-2B UTS2D Q76B58 Protein FAM5C FAM5C Q76LX8 A disintegrin and metalloproteinase with ADAMTS13 thrombospondin motifs 13 Q76M96 Coiled-coil domain-containing protein 80 CCDC80 Q7L1S5 Carbohydrate sulfotransferase 9 CHST9 Q7L513 Fc receptor-like A FCRLA Q7L8A9 Vasohibin-1 VASH1 Q7RTM1 Otopetrin-1 OTOP1 Q7RTW8 Otoancorin OTOA Q7RTY5 Serine protease 48 PRSS48 Q7RTY7 Ovochymase-1 OVCH1 Q7RTZ1 Ovochymase-2

OVCH2 Q7Z304 MAM domain-containing protein 2 MAMDC2 Q7Z3S9 Notch homolog 2 N-terminal-like protein NOTCH2NL Q7Z4H4 Intermedin-short ADM2 Q7Z4P5 Growth/differentiation factor 7 GDF7 Q7Z4R8 UPF0669 protein C6orf120 C6orf120 Q7Z4W2 Lysozyme-like protein 2 LYZL2 Q7Z5A4 Serine protease 42 PRSS42 Q7Z5A7 Protein FAM19A5 FAM19A5 Q7Z5A8 Protein FAM19A3 FAM19A3 Q7Z5A9 Protein FAM19A1 FAM19A1 Q7Z5J1 Hydroxysteroid 11-beta-dehydrogenase 1-like protein HSD11B1L Q7Z5L0 Vitelline membrane outer layer protein 1 homolog VMO1 Q7Z5L3 Complement C1q-like protein 2 C1QL2 Q7Z5L7 Podocan PODN Q7Z5P4 17-beta-hydroxysteroid dehydrogenase 13 HSD17B13 Q7Z5P9 Mucin-19 MUC19 Q7Z5Y6 Bone morphogenetic protein 8A BMP8A Q7Z7B7 Beta-defensin 132 DEFB132 Q7Z7B8 Beta-defensin 128 DEFB128 Q7Z7C8 Transcription initiation factor TFIID subunit 8 TAF8 Q7Z7H5 Transmembrane emp24 domain-containing protein 4 TMED4 Q86SG7 Lysozyme g-like protein 2 LYG2 Q86SI9 Protein CEI C5orf38 Q86TE4 Leucine zipper protein 2 LUZP2 Q86TH1 ADAMTS-like protein 2 ADAMTSL2 Q86U17 Serpin A11 SERPINA11 Q86UU9 Endokinin-A TAC4 Q86UW8 Hyaluronan and proteoglycan link protein 4 HAPLN4 Q86UX2 Inter-alpha-trypsin inhibitor heavy chain H5 ITIH5 Q86V24 Adiponectin receptor protein 2 ADIPOR2 Q86VB7 Soluble CD163 CD163 Q86VR8 Four-jointed box protein 1 FJX1 Q86WD7 Serpin A9 SERPINA9 Q86WN2 Interferon epsilon IFNE Q86WS3 Placenta-specific 1-like protein PLAC1L Q86X52 Chondroitin sulfate synthase 1 CHSY1 Q86XP6 Gastrokine-2 GKN2 Q86XS5 Angiopoietin-related protein 5 ANGPTL5 Q86Y27 B melanoma antigen 5 BAGE5 Q86Y28 B melanoma antigen 4 BAGE4 Q86Y29 B melanoma antigen 3 BAGE3 Q86Y30 B melanoma antigen 2 BAGE2 Q86Y38 Xylosyltransferase 1 XYLT1 Q86Y78 Ly6/PLAUR domain-containing protein 6 LYPD6 Q86YD3 Transmembrane protein 25 TMEM25 Q86YJ6 Threonine synthase-like 2 THNSL2 Q86YW7 Glycoprotein hormone beta-5 GPHB5 Q86Z23 Complement C1q-like protein 4 C1QL4 Q8IU57 Interleukin-28 receptor subunit alpha IL28RA Q8IUA0 WAP four-disulfide core domain protein 8 WFDC8 Q8IUB2 WAP four-disulfide core domain protein 3 WFDC3 Q8IUB3 Protein WFDC10B WFDC10B Q8IUB5 WAP four-disulfide core domain protein 13 WFDC13 Q8IUH2 Protein CREG2 CREG2 Q8IUK5 Plexin domain-containing protein 1 PLXDC1 Q8IUL8 Cartilage intermediate layer protein 2 C2 CILP2 Q8IUX7 Adipocyte enhancer-binding protein 1 AEBP1 Q8IUX8 Epidermal growth factor-like protein 6 EGFL6 Q8IVL8 Carboxypeptidase O CPO Q8IVN8 Somatomedin-B and thrombospondin type-1 SBSPON domain-containing protein Q8IVW8 Protein spinster homolog 2 SPNS2 Q8IW75 Serpin A12 SERPINA12 Q8IW92 Beta-galactosidase-1-like protein 2 GLB1L2 Q8IWL1 Pulmonary surfactant-associated protein A2 SFTPA2 Q8IWL2 Pulmonary surfactant-associated protein A1 SFTPA1 Q8IWV2 Contactin-4 CNTN4 Q8IWY4 Signal peptide, CUB and EGF-like domain-containing protein 1 SCUBE1 Q8IX30 Signal peptide, CUB and EGF-like domain-containing protein 3 SCUBE3 Q8IXA5 Sperm acrosome membrane-associated protein 3, SPACA3 membrane form Q8IXB1 DnaJ homolog subfamily C member 10 DNAJC10 Q8IXL6 Extracellular serine/threonine protein kinase Fam20C FAM20C Q8IYD9 Lung adenoma susceptibility protein 2 LAS2 Q8IYP2 Serine protease 58 PRSS58 Q8IYS5 Osteoclast-associated immunoglobulin-like receptor OSCAR Q8IZC6 Collagen alpha-1(XXVII) chain COL27A1 Q8IZJ3 C3 and PZP-like alpha-2-macroglobulin domain-containing CPAMD8 protein 8 Q8IZN7 Beta-defensin 107 DEFB107B Q8N0V4 Leucine-rich repeat LGI family member 2 LGI2 Q8N104 Beta-defensin 106 DEFB106B Q8N119 Matrix metalloproteinase-21 MMP21 Q8N129 Protein canopy homolog 4 CNPY4 Q8N135 Leucine-rich repeat LGI family member 4 LGI4 Q8N145 Leucine-rich repeat LGI family member 3 LGI3 Q8N158 Glypican-2 GPC2 Q8N1E2 Lysozyme g-like protein 1 LYG1 Q8N2E2 von Willebrand factor D and EGF domain-containing VWDE protein Q8N2E6 Prosalusin TOR2A Q8N2S1 Latent-transforming growth factor beta-binding LTBP4 protein 4 Q8N302 Angiogenic factor with G patch and FHA domains 1 AGGF1 Q8N307 Mucin-20 MUC20 Q8N323 NXPE family member 1 NXPE1 Q8N387 Mucin-15 MUC15 Q8N3Z0 Inactive serine protease 35 PRSS35 Q8N436 Inactive carboxypeptidase-like protein X2 CPXM2 Q8N474 Secreted frizzled-related protein 1 SFRP1

Q8N475 Follistatin-related protein 5 FSTL5 Q8N4F0 BPI fold-containing family B member 2 BPIFB2 Q8N4T0 Carboxypeptidase A6 CPA6 Q8N5W8 Protein FAM24B FAM24B Q8N687 Beta-defensin 125 DEFB125 Q8N688 Beta-defensin 123 DEFB123 Q8N690 Beta-defensin 119 DEFB119 Q8N6C5 Immunoglobulin superfamily member 1 IGSF1 Q8N6C8 Leukocyte immunoglobulin-like receptor subfamily A LILRA3 member 3 Q8N6G6 ADAMTS-like protein 1 ADAMTSL1 Q8N6Y2 Leucine-rich repeat-containing protein 17 LRRC17 Q8N729 Neuropeptide W-23 NPW Q8N8U9 BMP-binding endothelial regulator protein BMPER Q8N907 DAN domain family member 5 DAND5 Q8NAT1 Glycosyltransferase-like domain-containing protein 2 GTDC2 Q8NAU1 Fibronectin type III domain-containing protein 5 FNDC5 Q8NB37 Parkinson disease 7 domain-containing protein 1 PDDC1 Q8NBI3 Draxin DRAXIN Q8NBM8 Prenylcysteine oxidase-like PCYOX1L Q8NBP7 Proprotein convertase subtilisin/kexin type 9 PCSK9 Q8NBQ5 Estradiol 17-beta-dehydrogenase 11 HSD17B11 Q8NBV8 Synaptotagmin-8 SYT8 Q8NCC3 Group XV phospholipase A2 PLA2G15 Q8NCF0 C-type lectin domain family 18 member C CLEC18C Q8NCW5 NAD(P)H-hydrate epimerase APOA1BP Q8NDA2 Hemicentin-2 HMCN2 Q8NDX9 Lymphocyte antigen 6 complex locus protein G5b LY6G5B Q8NDZ4 Deleted in autism protein 1 C3orf58 Q8NEB7 Acrosin-binding protein ACRBP Q8NES8 Beta-defensin 124 DEFB124 Q8NET1 Beta-defensin 108B DEFB108B Q8NEX5 Protein WFDC9 WFDC9 Q8NEX6 Protein WFDC11 WFDC11 Q8NF86 Serine protease 33 PRSS33 Q8NFM7 Interleukin-17 receptor D IL17RD Q8NFQ5 BPI fold-containing family B member 6 BPIFB6 Q8NFQ6 BPI fold-containing family C protein BPIFC Q8NFU4 Follicular dendritic cell secreted peptide FDCSP Q8NFW1 Collagen alpha-1(XXII) chain COL22A1 Q8NG35 Beta-defensin 105 DEFB105B Q8NG41 Neuropeptide B-23 NPB Q8NHW6 Otospiralin OTOS Q8NI99 Angiopoietin-related protein 6 ANGPTL6 Q8TAA1 Probable ribonuclease 11 RNASE11 Q8TAG5 V-set and transmembrane domain-containing protein 2A VSTM2A Q8TAL6 Fin bud initiation factor homolog FIBIN Q8TAT2 Fibroblast growth factor-binding protein 3 FGFBP3 Q8TAX7 Mucin-7 MUC7 Q8TB22 Spermatogenesis-associated protein 20 SPATA20 Q8TB73 Protein NDNF NDNF Q8TB96 T-cell immunomodulatory protein ITFG1 Q8TC92 Protein disulfide-thiol oxidoreductase ENOX1 Q8TCV5 WAP four-disulfide core domain protein 5 WFDC5 Q8TD06 Anterior gradient protein 3 homolog AGR3 Q8TD33 Secretoglobin family 1C member 1 SCGB1C1 Q8TD46 Cell surface glycoprotein CD200 receptor 1 CD200R1 Q8TDE3 Ribonuclease 8 RNASE8 Q8TDF5 Neuropilin and tolloid-like protein 1 NETO1 Q8TDL5 BPI fold-containing family B member 1 BPIFB1 Q8TE56 A disintegrin and metalloproteinase with ADAMTS17 thrombospondin motifs 17 Q8TE57 A disintegrin and metalloproteinase with ADAMTS16 thrombospondin motifs 16 Q8TE58 A disintegrin and metalloproteinase with ADAMTS15 thrombospondin motifs 15 Q8TE59 A disintegrin and metalloproteinase with ADAMTS19 thrombospondin motifs 19 Q8TE60 A disintegrin and metalloproteinase with ADAMTS18 thrombospondin motifs 18 Q8TE99 Acid phosphatase-like protein 2 ACPL2 Q8TER0 Sushi, nidogen and EGF-like domain-containing SNED1 protein 1 Q8TEU8 WAP, kazal, immunoglobulin, kunitz and NTR WFIKKN2 domain-containing protein 2 Q8WTQ1 Beta-defensin 104 DEFB104B Q8WTR8 Netrin-5 NTN5 Q8WTU2 Scavenger receptor cysteine-rich domain- SRCRB4D containing group B protein Q8WU66 Protein TSPEAR TSPEAR Q8WUA8 Tsukushin TSKU Q8WUF8 Protein FAM172A FAM172A Q8WUJ1 Neuferricin CYB5D2 Q8WUY1 UPF0670 protein THEM6 THEM6 Q8WVN6 Secreted and transmembrane protein 1 SECTM1 Q8WVQ1 Soluble calcium-activated nucleotidase 1 CANT1 Q8WWA0 Intelectin-1 ITLN1 Q8WWG1 Neuregulin-4 NRG4 Q8WWQ2 Inactive heparanase-2 HPSE2 Q8WWU7 Intelectin-2 ITLN2 Q8WWY7 WAP four-disulfide core domain protein 12 WFDC12 Q8WWY8 Lipase member H LIPH Q8WWZ8 Oncoprotein-induced transcript 3 protein OIT3 Q8WX39 Epididymal-specific lipocalin-9 LCN9 Q8WXA2 Prostate and testis expressed protein 1 PATE1 Q8WXD2 Secretogranin-3 SCG3 Q8WXF3 Relaxin-3 A chain RLN3 Q8WXI7 Mucin-16 MUC16 Q8WXQ8 Carboxypeptidase A5 CPA5 Q8WXS8 A disintegrin and metalloproteinase with ADAMTS14 thrombospondin motifs 14 Q92484 Acid sphingomyelinase-

like phosphodiesterase 3a SMPDL3A Q92485 Acid sphingomyelinase-like phosphodiesterase 3b SMPDL3B Q92496 Complement factor H-related protein 4 CFHR4 Q92520 Protein FAM3C FAM3C Q92563 Testican-2 SPOCK2 Q92583 C—C motif chemokine 17 CCL17 Q92626 Peroxidasin homolog PXDN Q92743 Serine protease HTRA1 HTRA1 Q92752 Tenascin-R TNR Q92765 Secreted frizzled-related protein 3 FRZB Q92819 Hyaluronan synthase 2 HAS2 Q92820 Gamma-glutamyl hydrolase GGH Q92824 Proprotein convertase subtilisin/kexin type 5 PCSK5 Q92832 Protein kinase C-binding protein NELL1 NELL1 Q92838 Ectodysplasin-A, membrane form EDA Q92874 Deoxyribonuclease-1-like 2 DNASE1L2 Q92876 Kallikrein-6 KLK6 Q92913 Fibroblast growth factor 13 FGF13 Q92954 Proteoglycan 4 C-terminal part PRG4 Q93038 Tumor necrosis factor receptor superfamily TNFRSF25 member 25 Q93091 Ribonuclease K6 RNASE6 Q93097 Protein Wnt-2b WNT2B Q93098 Protein Wnt-8b WNT8B Q95460 Major histocompatibility complex class I-related MR1 gene protein Q969D9 Thymic stromal lymphopoietin TSLP Q969E1 Liver-expressed antimicrobial peptide 2 LEAP2 Q969H8 UPF0556 protein C19orf10 C19orf10 Q969Y0 NXPE family member 3 NXPE3 Q96A54 Adiponectin receptor protein 1 ADIPOR1 Q96A83 Collagen alpha-1(XXVI) chain EMID2 Q96A84 EMI domain-containing protein 1 EMID1 Q96A98 Tuberoinfundibular peptide of 39 residues PTH2 Q96A99 Pentraxin-4 PTX4 Q96BH3 Epididymal sperm-binding protein 1 ELSPBP1 Q96BQ1 Protein FAM3D FAM3D Q96CG8 Collagen triple helix repeat-containing protein 1 CTHRC1 Q96DA0 Zymogen granule protein 16 homolog B ZG16B Q96DN2 von Willebrand factor C and EGF domain-containing VWCE protein Q96DR5 BPI fold-containing family A member 2 BPIFA2 Q96DR8 Mucin-like protein 1 MUCL1 Q96DX4 RING finger and SPRY domain-containing protein 1 RSPRY1 Q96EE4 Coiled-coil domain-containing protein 126 CCDC126 Q96GS6 Abhydrolase domain-containing protein FAM108A1 FAM108A1 Q96GW7 Brevican core protein BCAN Q96HF1 Secreted frizzled-related protein 2 SFRP2 Q96I82 Kazal-type serine protease inhibitor domain-containing KAZALD1 protein 1 Q96ID5 Immunoglobulin superfamily member 21 IGSF21 Q96II8 Leucine-rich repeat and calponin homology LRCH3 domain-containing protein 3 Q96IY4 Carboxypeptidase B2 CPB2 Q96JB6 Lysyl oxidase homolog 4 LOXL4 Q96JK4 HHIP-like protein 1 HHIPL1 Q96KN2 Beta-Ala-His dipeptidase CNDP1 Q96KW9 Protein SPACA7 SPACA7 Q96KX0 Lysozyme-like protein 4 LYZL4 Q96L15 Ecto-ADP-ribosyltransferase 5 ART5 Q96LB8 Peptidoglycan recognition protein 4 PGLYRP4 Q96LB9 Peptidoglycan recognition protein 3 PGLYRP3 Q96LC7 Sialic acid-binding Ig-like lectin 10 SIGLEC10 Q96LR4 Protein FAM19A4 FAM19A4 Q96MK3 Protein FAM20A FAM20A Q96MS3 Glycosyltransferase 1 domain-containing protein 1 GLT1D1 Q96NY8 Processed poliovirus receptor-related protein 4 PVRL4 Q96NZ8 WAP, kazal, immunoglobulin, kunitz and NTR WFIKKN1 domain-containing protein 1 Q96NZ9 Proline-rich acidic protein 1 PRAP1 Q96P44 Collagen alpha-1(XXI) chain COL21A1 Q96PB7 Noelin-3 OLFM3 Q96PC5 Melanoma inhibitory activity protein 2 MIA2 Q96PD5 N-acetylmuramoyl-L-alanine amidase PGLYRP2 Q96PH6 Beta-defensin 118 DEFB118 Q96PL1 Secretoglobin family 3A member 2 SCGB3A2 Q96PL2 Beta-tectorin TECTB Q96QH8 Sperm acrosome-associated protein 5 SPACA5 Q96QR1 Secretoglobin family 3A member 1 SCGB3A1 Q96QU1 Protocadherin-15 PCDH15 Q96QV1 Hedgehog-interacting protein HHIP Q96RW7 Hemicentin-1 HMCN1 Q96S42 Nodal homolog NODAL Q96S86 Hyaluronan and proteoglycan link protein 3 HAPLN3 Q96SL4 Glutathione peroxidase 7 GPX7 Q96SM3 Probable carboxypeptidase X1 CPXM1 Q96T91 Glycoprotein hormone alpha-2 GPHA2 Q99062 Granulocyte colony-stimulating factor receptor CSF3R Q99102 Mucin-4 alpha chain MUC4 Q99217 Amelogenin, X isoform AMELX Q99218 Amelogenin, Y isoform AMELY Q99435 Protein kinase C-binding protein NELL2 NELL2 Q99470 Stromal cell-derived factor 2 SDF2 Q99542 Matrix metalloproteinase-19 MMP19 Q99574 Neuroserpin SERPINI1 Q99584 Protein S100-A13 S100A13 Q99616 C—C motif chemokine 13 CCL13 Q99645 Epiphycan EPYC Q99674 Cell growth regulator with EF hand domain protein 1 CGREF1 Q99715 Collagen alpha-1(XII) chain COL12A1 Q99727 Metalloproteinase inhibitor 4 TIMP4 Q99731 C—C motif

chemokine 19 CCL19 Q99748 Neurturin NRTN Q99935 Proline-rich protein 1 PROL1 Q99942 E3 ubiquitin-protein ligase RNF5 RNF5 Q99944 Epidermal growth factor-like protein 8 EGFL8 Q99954 Submaxillary gland androgen-regulated protein 3A SMR3A Q99969 Retinoic acid receptor responder protein 2 RARRES2 Q99972 Myocilin MYOC Q99983 Osteomodulin OMD Q99985 Semaphorin-3C SEMA3C Q99988 Growth/differentiation factor 15 GDF15 Q9BPW4 Apolipoprotein L4 APOL4 Q9BQ08 Resistin-like beta RETNLB Q9BQ16 Testican-3 SPOCK3 Q9BQ51 Programmed cell death 1 ligand 2 PDCD1LG2 Q9BQB4 Sclerostin SOST Q9BQI4 Coiled-coil domain-containing protein 3 CCDC3 Q9BQP9 BPI fold-containing family A member 3 BPIFA3 Q9BQR3 Serine protease 27 PRSS27 Q9BQY6 WAP four-disulfide core domain protein 6 WFDC6 Q9BRR6 ADP-dependent glucokinase ADPGK Q9BS86 Zona pellucida-binding protein 1 ZPBP Q9BSG0 Protease-associated domain-containing protein 1 PRADC1 Q9BSG5 Retbindin RTBDN Q9BT30 Probable alpha-ketoglutarate-dependent ALKBH7 dioxygenase ABH7 Q9BT56 Spexin C12orG9 Q9BT67 NEDD4 family-interacting protein 1 NDFIP1 Q9BTY2 Plasma alpha-L-fucosidase FUCA2 Q9BU40 Chordin-like protein 1 CHRDL1 Q9BUD6 Spondin-2 SPON2 Q9BUN1 Protein MENT MENT Q9BUR5 Apolipoprotein O APOO Q9BV94 ER degradation-enhancing alpha-mannosidase-like 2 EDEM2 Q9BWP8 Collectin-11 COLEC11 Q9BWS9 Chitinase domain-containing protein 1 CHID1 Q9BX67 Junctional adhesion molecule C JAM3 Q9BX93 Group XIIB secretory phospholipase A2-like protein PLA2G12B Q9BXI9 Complement C1q tumor necrosis factor-related protein 6 C1QTNF6 Q9BXJ0 Complement C1q tumor necrosis factor-related protein 5 C1QTNF5 Q9BXJ1 Complement C1q tumor necrosis factor-related protein 1 C1QTNF1 Q9BXJ2 Complement C1q tumor necrosis factor-related protein 7 C1QTNF7 Q9BXJ3 Complement C1q tumor necrosis factor-related protein 4 C1QTNF4 Q9BXJ4 Complement C1q tumor necrosis factor-related protein 3 C1QTNF3 Q9BXJ5 Complement C1q tumor necrosis factor-related protein 2 C1QTNF2 Q9BXN1 Asporin ASPN Q9BXP8 Pappalysin-2 PAPPA2 Q9BXR6 Complement factor H-related protein 5 CFHR5 Q9BXS0 Collagen alpha-1(XXV) chain COL25A1 Q9BXX0 EMILIN-2 EMILIN2 Q9BXY4 R-spondin-3 RSPO3 Q9BY15 EGF-like module-containing mucin-like hormone EMR3 receptor-like 3 subunit beta Q9BY50 Signal peptidase complex catalytic subunit SEC11C SEC11C Q9BY76 Angiopoietin-related protein 4 ANGPTL4 Q9BYF1 Processed angiotensin-converting enzyme 2 ACE2 Q9BYJ0 Fibroblast growth factor-binding protein 2 FGFBP2 Q9BYW3 Beta-defensin 126 DEFB126 Q9BYX4 Interferon-induced helicase C domain-containing IFIH1 protein 1 Q9BYZ8 Regenerating islet-derived protein 4 REG4 Q9BZ76 Contactin-associated protein-like 3 CNTNAP3 Q9BZG9 Ly-6/neurotoxin-like protein 1 LYNX1 Q9BZJ3 Tryptase delta TPSD1 Q9BZM1 Group XIIA secretory phospholipase A2 PLA2G12A Q9BZM2 Group IIF secretory phospholipase A2 PLA2G2F Q9BZM5 NKG2D ligand 2 ULBP2 Q9BZP6 Acidic mammalian chitinase CHIA Q9BZZ2 Sialoadhesin SIGLEC1 Q9C0B6 Protein FAM5B FAM5B Q9GZM7 Tubulointerstitial nephritis antigen-like TINAGL1 Q9GZN4 Brain-specific serine protease 4 PRSS22 Q9GZP0 Platelet-derived growth factor D, receptor-binding form PDGFD Q9GZT5 Protein Wnt-10a WNT10A Q9GZU5 Nyctalopin NYX Q9GZV7 Hyaluronan and proteoglycan link protein 2 HAPLN2 Q9GZV9 Fibroblast growth factor 23 FGF23 Q9GZX9 Twisted gastrulation protein homolog 1 TWSG1 Q9GZZ7 GDNF family receptor alpha-4 GFRA4 Q9GZZ8 Extracellular glycoprotein lacritin LACRT Q9H0B8 Cysteine-rich secretory protein LCCL domain-containing 2 CRISPLD2 Q9H106 Signal-regulatory protein delta SIRPD Q9H114 Cystatin-like 1 CSTL1 Q9H173 Nucleotide exchange factor SIL1 SIL1 Q9H1E1 Ribonuclease 7 RNASE7 Q9H1F0 WAP four-disulfide core domain protein 10A WFDC10A Q9H1J5 Protein Wnt-8a WNT8A Q9H1J7 Protein Wnt-5b WNT5B Q9H1M3 Beta-defensin 129 DEFB129 Q9H1M4 Beta-defensin 127 DEFB127 Q9H1Z8 Augurin C2orf40 Q9H239 Matrix metalloproteinase-28 MMP28 Q9H2A7 C—X—C motif chemokine 16 CXCL16 Q9H2A9 Carbohydrate sulfotransferase 8 CHST8 Q9H2R5 Kallikrein-15 KLK15 Q9H2X0 Chordin CHRD Q9H2X3 C-type lectin domain family 4 member M CLEC4M Q9H306 Matrix metalloproteinase-27 MMP27 Q9H324 A disintegrin and

metalloproteinase with ADAMTS10 thrombospondin motifs 10 Q9H336 Cysteine-rich secretory protein LCCL domain-containing 1 CRISPLD1 Q9H3E2 Sorting nexin-25 SNX25 Q9H3R2 Mucin-13 MUC13 Q9H3U7 SPARC-related modular calcium-binding protein 2 SMOC2 Q9H3Y0 Peptidase inhibitor R3HDML R3HDML Q9H4A4 Aminopeptidase B RNPEP Q9H4F8 SPARC-related modular calcium-binding protein 1 SMOC1 Q9H4G1 Cystatin-9-like CST9L Q9H5V8 CUB domain-containing protein 1 CDCP1 Q9H6B9 Epoxide hydrolase 3 EPHX3 Q9H6E4 Coiled-coil domain-containing protein 134 CCDC134 Q9H741 UPF0454 protein C12orf49 C12orf49 Q9H772 Gremlin-2 GREM2 Q9H7Y0 Deleted in autism-related protein 1 CXorf36 Q9H8L6 Multimerin-2 MMRN2 Q9H9S5 Fukutin-related protein FKRP Q9HAT2 Sialate O-acetylesterase SIAE Q9HB40 Retinoid-inducible serine carboxypeptidase SCPEP1 Q9HB63 Netrin-4 NTN4 Q9HBJ0 Placenta-specific protein 1 PLAC1 Q9HC23 Prokineticin-2 PROK2 Q9HC57 WAP four-disulfide core domain protein 1 WFDC1 Q9HC73 Cytokine receptor-like factor 2 CRLF2 Q9HC84 Mucin-5B MUC5B Q9HCB6 Spondin-1 SPON1 Q9HCQ7 Neuropeptide NPSF NPVF Q9HCT0 Fibroblast growth factor 22 FGF22 Q9HD89 Resistin RETN Q9NNX1 Tuftelin TUFT1 Q9NNX6 CD209 antigen CD209 Q9NP55 BPI fold-containing family A member 1 BPIFA1 Q9NP70 Ameloblastin AMBN Q9NP95 Fibroblast growth factor 20 FGF20 Q9NP99 Triggering receptor expressed on myeloid cells 1 TREM1 Q9NPA2 Matrix metalloproteinase-25 MMP25 Q9NPE2 Neugrin NGRN Q9NPH0 Lysophosphatidic acid phosphatase type 6 ACP6 Q9NPH6 Odorant-binding protein 2b OBP2B Q9NQ30 Endothelial cell-specific molecule 1 ESM1 Q9NQ36 Signal peptide, CUB and EGF-like domain-containing SCUBE2 protein 2 Q9NQ38 Serine protease inhibitor Kazal-type 5 SPINK5 Q9NQ76 Matrix extracellular phosphoglycoprotein MEPE Q9NQ79 Cartilage acidic protein 1 CRTAC1 Q9NR16 Scavenger receptor cysteine-rich type 1 protein M160 CD163L1 Q9NR23 Growth/differentiation factor 3 GDF3 Q9NR71 Neutral ceramidase ASAH2 Q9NR99 Matrix-remodeling-associated protein 5 MXRA5 Q9NRA1 Platelet-derived growth factor C PDGFC Q9NRC9 Otoraplin OTOR Q9NRE1 Matrix metalloproteinase-26 MMP26 Q9NRJ3 C—C motif chemokine 28 CCL28 Q9NRM1 Enamelin ENAM Q9NRN5 Olfactomedin-like protein 3 OLFML3 Q9NRR1 Cytokine-like protein 1 CYTL1 Q9NS15 Latent-transforming growth factor beta-binding protein 3 LTBP3 Q9NS62 Thrombospondin type-1 domain-containing protein 1 THSD1 Q9NS71 Gastrokine-1 GKN1 Q9NS98 Semaphorin-3G SEMA3G Q9NSA1 Fibroblast growth factor 21 FGF21 Q9NT22 EMILIN-3 EMILIN3 Q9NTU7 Cerebellin-4 CBLN4 Q9NVR0 Kelch-like protein 11 KLHL11 Q9NWH7 Spermatogenesis-associated protein 6 SPATA6 Q9NXC2 Glucose-fructose oxidoreductase domain-containing protein 1 GFOD1 Q9NY56 Odorant-binding protein 2a OBP2A Q9NY84 Vascular non-inflammatory molecule 3 VNN3 Q9NZ20 Group 3 secretory phospholipase A2 PLA2G3 Q9NZC2 Triggering receptor expressed on myeloid cells 2 TREM2 Q9NZK5 Adenosine deaminase CECR1 CECR1 Q9NZK7 Group IIE secretory phospholipase A2 PLA2G2E Q9NZP8 Complement C1r subcomponent-like protein C1RL Q9NZV1 Cysteine-rich motor neuron 1 protein CRIM1 Q9NZW4 Dentin sialoprotein DSPP Q9P0G3 Kallikrein-14 KLK14 Q9P0W0 Interferon kappa IFNK Q9P218 Collagen alpha-1(XX) chain COL20A1 Q9P2C4 Transmembrane protein 181 TMEM181 Q9P2K2 Thioredoxin domain-containing protein 16 TXNDC16 Q9P2N4 A disintegrin and metalloproteinase with thrombospondin ADAMTS9 motifs 9 Q9UBC7 Galanin-like peptide GALP Q9UBD3 Cytokine SCM-1 beta XCL2 Q9UBD9 Cardiotrophin-like cytokine factor 1 CLCF1 Q9UBM4 Opticin OPTC Q9UBP4 Dickkopf-related protein 3 DKK3 Q9UBQ6 Exostosin-like 2 EXTL2 Q9UBR5 Chemokine-like factor CKLF Q9UBS5 Gamma-aminobutyric acid type B receptor subunit 1 GABBR1 Q9UBT3 Dickkopf-related protein 4 short form DKK4 Q9UBU2 Dickkopf-related protein 2 DKK2 Q9UBU3 Ghrelin-28 GHRL Q9UBV4 Protein Wnt-16 WNT16 Q9UBX5 Fibulin-5 FBLN5 Q9UBX7 Kallikrein-11 KLK11 Q9UEF7 Klotho KL Q9UFP1 Protein FAM198A FAM198A Q9UGM3 Deleted in malignant brain tumors 1 protein DMBT1 Q9UGM5 Fetuin-B FETUB Q9UGP8 Translocation protein SEC63 homolog SEC63 Q9UHF0 Neurokinin-B TAC3 Q9UHF1 Epidermal growth factor-like protein 7 EGFL7 Q9UHG2 ProSAAS PCSK1N

Q9UHI8 A disintegrin and metalloproteinase with thrombospondin ADAMTS1 motifs 1 Q9UHL4 Dipeptidyl peptidase 2 DPP7 Q9UI42 Carboxypeptidase A4 CPA4 Q9UIG4 Psoriasis susceptibility 1 candidate gene 2 protein PSORS1C2 Q9UIK5 Tomoregulin-2 TMEFF2 Q9UIQ6 Leucyl-cystinyl aminopeptidase, pregnancy serum form LNPEP Q9UJA9 Ectonucleotide pyrophosphatase/phosphodiesterase family ENPP5 member 5 Q9UJH8 Meteorin METRN Q9UJJ9 N-acetylglucosamine-1-phosphotransferase subunit gamma GNPTG Q9UJW2 Tubulointerstitial nephritis antigen TINAG Q9UK05 Growth/differentiation factor 2 GDF2 Q9UK55 Protein Z-dependent protease inhibitor SERPINA10 Q9UK85 Dickkopf-like protein 1 DKKL1 Q9UKJ1 Paired immunoglobulin-like type 2 receptor alpha PILRA Q9UKP4 A disintegrin and metalloproteinase with ADAMTS7 thrombospondin motifs 7 Q9UKP5 A disintegrin and metalloproteinase with ADAMTS6 thrombospondin motifs 6 Q9UKQ2 Disintegrin and metalloproteinase domain-containing protein 28 ADAM28 Q9UKQ9 Kallikrein-9 KLK9 Q9UKR0 Kallikrein-12 KLK12 Q9UKR3 Kallikrein-13 KLK13 Q9UKU9 Angiopoietin-related protein 2 ANGPTL2 Q9UKZ9 Procollagen C-endopeptidase enhancer 2 PCOLCE2 Q9UL52 Transmembrane protease serine 11E non-catalytic chain TMPRSS11E Q9ULC0 Endomucin EMCN Q9ULI3 Protein HEG homolog 1 HEG1 Q9ULZ1 Apelin-13 APLN Q9ULZ9 Matrix metalloproteinase-17 MMP17 Q9UM21 Alpha-1,3-mannosyl-glycoprotein 4-beta-N- MGAT4A acetylglucosaminyltransferase A soluble form Q9UM22 Mammalian ependymin-related protein 1 EPDR1 Q9UM73 ALK tyrosine kinase receptor ALK Q9UMD9 97 kDa linear IgA disease antigen COL17A1 Q9UMX5 Neudesin NENF Q9UN73 Protocadherin alpha-6 PCDHA6 Q9UNA0 A disintegrin and metalloproteinase with ADAMTS5 thrombospondin motifs 5 Q9UNI1 Chymotrypsin-like elastase family member 1 CELA1 Q9UNK4 Group IID secretory phospholipase A2 PLA2G2D Q9UP79 A disintegrin and metalloproteinase with thrombospondin ADAMTS8 motifs 8 Q9UPZ6 Thrombospondin type-1 domain-containing protein 7A THSD7A Q9UQ72 Pregnancy-specific beta-1-glycoprotein 11 PSG11 Q9UQ74 Pregnancy-specific beta-1-glycoprotein 8 PSG8 Q9UQC9 Calcium-activated chloride channel regulator 2 CLCA2 Q9UQE7 Structural maintenance of chromosomes protein 3 SMC3 Q9UQP3 Tenascin-N TNN Q9Y223 UDP-N-acetylglucosamine 2-epimerase GNE Q9Y240 C-type lectin domain family 11 member A CLEC11A Q9Y251 Heparanase 8 kDa subunit HPSE Q9Y258 C—C motif chemokine 26 CCL26 Q9Y264 Angiopoietin-4 ANGPT4 Q9Y275 Tumor necrosis factor ligand superfamily member 13b, TNFSF13B membrane form Q9Y287 BRI2 intracellular domain ITM2B Q9Y2E5 Epididymis-specific alpha-mannosidase MAN2B2 Q9Y334 von Willebrand factor A domain-containing protein 7 VWA7 Q9Y337 Kallikrein-5 KLK5 Q9Y3B3 Transmembrane emp24 domain-containing protein 7 TMED7 Q9Y3E2 BolA-like protein 1 BOLA1 Q9Y426 C2 domain-containing protein 2 C2CD2 Q9Y4K0 Lysyl oxidase homolog 2 LOXL2 Q9Y4X3 C—C motif chemokine 27 CCL27 Q9Y5C1 Angiopoietin-related protein 3 ANGPTL3 Q9Y5I2 Protocadherin alpha-10 PCDHA10 Q9Y5I3 Protocadherin alpha-1 PCDHA1 Q9Y5K2 Kallikrein-4 KLK4 Q9Y5L2 Hypoxia-inducible lipid droplet-associated protein HILPDA Q9Y5Q5 Atrial natriuretic peptide-converting enzyme CORIN Q9Y5R2 Matrix metalloproteinase-24 MMP24 Q9Y5U5 Tumor necrosis factor receptor superfamily member 18 TNFRSF18 Q9Y5W5 Wnt inhibitory factor 1 WIF1 Q9Y5X9 Endothelial lipase LIPG Q9Y625 Secreted glypican-6 GPC6 Q9Y646 Carboxypeptidase Q CPQ Q9Y6C2 EMILIN-1 EMILIN1 Q9Y6F9 Protein Wnt-6 WNT6 Q9Y6I9 Testis-expressed sequence 264 protein TEX264 Q9Y6L7 Tolloid-like protein 2 TLL2 Q9Y6N3 Calcium-activated chloride channel regulator family member 3 CLCA3P Q9Y6N6 Laminin subunit gamma-3 LAMC3 Q9Y6R7 IgGFc-binding protein FCGBP Q9Y6Y9 Lymphocyte antigen 96 LY96 Q9Y6Z7 Collectin-10 COLEC10

[0197] In some embodiments, the compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more additional exemplary proteins listed in Table 2; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 2 (or a homolog thereof) along with other components set out herein, and methods of the

invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from the proteins listed in Table 2 (or a homolog thereof) along with other components set out herein.

TABLE-US-00002 TABLE 2 Additional Exemplary Proteins Uniprot ID Protein Name Gene Name A6NGW2 Putative stereocilin-like protein STRCP1 A6NIE9 Putative serine protease 29 PRSS29P A6NJ16 Putative V-set and immunoglobulin IGHV4OR15-8 domain-containing-like protein IGHV4OR15-8 A6NJS3 Putative V-set and immunoglobulin IGHV1OR21-1 domain-containing-like protein IGHV1OR21-1 A6NMY6 Putative annexin A2-like protein ANXA2P2 A8MT79 Putative zinc-alpha-2-glycoprotein-like 1 A8MWS1 Putative killer cell immunoglobulin-like KIR3DP1 receptor like protein KIR3DP1 A8MXU0 Putative beta-defensin 108A DEFB108P1 C9JUS6 Putative adrenomedullin-5-like protein ADM5 P0C7V7 Putative signal peptidase complex SEC11B catalytic subunit SEC11B P0C854 Putative cat eye syndrome critical region CECR9 protein 9 Q13046 Putative pregnancy-specific beta-1- PSG7 glycoprotein 7 Q16609 Putative apolipoprotein(a)-like protein 2 LPAL2 Q2TV78 Putative macrophage-stimulating protein MST1P9 MSTP9 Q5JQD4 Putative peptide YY-3 PYY3 Q5R387 Putative inactive group IIC secretory PLA2G2C phospholipase A2 Q5VSP4 Putative lipocalin 1-like protein 1 LCN1P1 Q5W188 Putative cystatin-9-like protein CST9LP1 CST9LP1 Q6UXR4 Putative serpin A13 SERPINA13P Q86SH4 Putative testis-specific prion protein PRNT Q86YQ2 Putative latherin LATH Q8IVG9 Putative humanin peptide MT-RNR2 Q8NHM4 Putative trypsin-6 TRY6 Q8NHW4 C—C motif chemokine 4-like CCL4L2 Q9H7L2 Putative killer cell immunoglobulin-like KIR3DX1 receptor-like protein KIR3DX1 Q9NRI6 Putative peptide YY-2 PYY2 Q9UF72 Putative TP73 antisense gene protein 1 TP73-AS1 Q9UKY3 Putative inactive carboxylesterase 4 CES1P1

[0198] The Uniprot IDs set forth in Table 1 and Table 2 refer to the human versions the listed proteins and the sequences of each are available from the Uniprot database. Sequences of the listed proteins are also generally available for various animals, including various mammals and animals of veterinary or industrial interest. Accordingly, in some embodiments, compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more proteins chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of the secreted proteins listed in Table 1 or Table 2; thus, compositions of the invention may comprise an mRNA encoding a protein chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of a protein listed in Table 1 or Table 2 along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of a protein listed in Table 1 or Table 2 along with other components set out herein. In some embodiments, mammalian homologs are chosen from mouse, rat, hamster, gerbil, horse, pig, cow, llama, alpaca, mink, dog, cat, ferret, sheep, goat, or camel homologs. In some embodiments, the animal of veterinary or industrial interest is chosen from the mammals listed above and/or chicken, duck, turkey, salmon, catfish, or tilapia.

[0199] In embodiments, the compositions and methods of the invention provide for the delivery of mRNA encoding a lysosomal protein chosen from Table 3. In some embodiments, the compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more lysosomal and/or related proteins listed in Table 3; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 3 (or a homolog thereof) along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from the proteins listed in Table 3 (or a homolog thereof) along with other components set out herein.

TABLE-US-00003 TABLE 3 Lysosomal and Related Proteins α-fucosidase α-galactosidase α-glucosidase α-Iduronidase α-mannosidase α-N-acetylgalactosaminidase (α-galactosidase B) β-

galactosidase β-glucuronidase β-hexosaminidase β-mannosidase 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase 3-methylcrotonyl-CoA carboxylase 3-O-sulfogalactosyl cerebroside sulfatase (arylsulfatase A) acetyl-CoA transferase acid alpha-glucosidase acid ceramidase acid lipase acid phosphatase acid sphingomyelinase alpha-galactosidase A arylsulfatase A beta-galactosidase beta-glucocerebrosidase beta-hexosaminidase biotinidase cathepsin A cathepsin K CLN3 CLN5 CLN6 CLN8 CLN9 cystine transporter (cystinosin) cytosolic protein beta3A subunit of the adaptor protein-3 complex, AP3 formyl-Glycine generating enzyme (FGE) galactocerebrosidase galactose-1-phosphate uridyltransferase (GALT) galactose 6-sulfate sulfatase (also known as N-acetylgalactosamine-6-sulfatase) glucocerebrosidase glucuronate sulfatase glucuronidase glycoprotein cleaving enzymes glycosaminoglycan cleaving enzymes glycosylasparaginase (aspartylglucosaminidase) GM2-AP Heparan-alpha-glucosaminide N-acetyltransferase (HGSNAT, TMEM76) Heparan sulfatase hexosaminidase A lysosomal proteases methylmalonyl-CoA mutase hyaluronidase Iduronate sulfatase LAMP-2 lysosomal α-mannosidase Lysosomal p40 (C2orf18) Major facilitator superfamily domain containing 8 protein (MFSD8 or CLN7) N-acetylgalactosamine 4-sulfatase N-acetyl glucosamine 6-sulfatase N-acetyl glucosaminidase N-acetylglucosamine-1-phosphate transferase NPC1 NPC2 palmitoyl-protein thioesterase palmitoyl-protein thioesterase (CLN1) Saposin A (Sphingolipid activator protein A) Saposin B (Sphingolipid activator protein B) Saposin C (Sphingolipid activator protein C) Saposin D (Sphingolipid activator protein D) sialic acid transporter (sialin) sialidase Sialin sulfatase Transmembrane protein 74 (TMEM74) tripeptidyl-peptidase tripeptidyl-peptidase I (CLN2) UDP-N-acetylglucosamine-phosphotransferase

[0200] Information regarding lysosomal proteins is available from Lubke et al., “Proteomics of the Lysosome,” *Biochim Biophys Acta*. (2009) 1793: 625-635. In some embodiments, the protein listed in Table 3 and encoded by mRNA in the compositions and methods of the invention is a human protein. Sequences of the listed proteins are also available for various animals, including various mammals and animals of veterinary or industrial interest as described above.

[0201] In some embodiments, the compositions and methods of the invention provide for the delivery of mRNA encoding a therapeutic protein (e.g., cytosolic, transmembrane or secreted) such as those listed in Table 4. In some embodiments, the compositions and methods of the invention provide for the delivery of an mRNA encoding a therapeutic protein useful in treating a disease or disorder (i.e., indication) listed in Table 4; thus, compositions of the invention may comprise an mRNA encoding a therapeutic protein listed or not listed in Table 4 (or a homolog thereof, as discussed below) along with other components set out herein for treating a disease or disorder (i.e., indication) listed in Table 4, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a such a protein (or a homolog thereof, as discussed below) along with other components set out herein for treatment of a disease or disorder listed in Table 4.

TABLE-US-00004 TABLE 4 Exemplary Indications and Related Proteins Indication Therapeutic Protein 3-Methylcrotonyl-CoA carboxylase deficiency Methylcrotonoyl-CoA carboxylase 3-Methylglutaconic aciduria Methylglutaconyl-CoA hydratase Actinic keratosis Acute intermittent porphyria Porphobilinogen deaminase Acute lymphocytic leukemia Acute myeloid leukemia Addison's disease Adenosine deaminase deficiency Adenosine deaminase Adrenoleukodystrophy ABCD1 Adrenomyeloneuropathy AIDS/HIV Alcohol use disorders Alkaptonuria Homogentisate 1,2-dioxygenase Allergic asthma Anti-IgE mAb Allergies (dermatitis, rhinitis) Alopecia areata Alpers' disease POLG Alpers-Huttenlocher syndrome Alpha 1-antitrypsin deficiency Alpha 1 protease inhibitor Alpha-mannosidosis Alpha-D-mannosidase Alport syndrome Alzheimer's disease Amyloid light-chain amyloidosis Amyotrophic lateral sclerosis (ALS) Anemia Erythropoietin Aortic valve stenosis Argininemia Arginase Argininosuccinic acidemia Argininosuccinate lyase Arrhythmogenic right ventricular dysplasia Autism Autosomal dominant and recessive progressive external ophthalmoplegia with mitochondrial DNA deletions Autosomal recessive polycystic

kidney disease ARPKD Bacterial infections Basal cell carcinoma Batten disease Battenin + others B-cell chronic lymphocytic leukemia Becker muscular dystrophy Dystrophin Beta-thalassemia Beta globin Binge eating disorder Bipolar disorder Bladder cancer Blepharospasm, Cervical dystonia, Chronic migraine, Botulinum toxin more Bronchiolitis obliterans Brugada syndrome Buerger's disease CACNA1A CACNB4-related Episodic Ataxia Type 2 Cancer and depression Cancer and sexual dysfunction Cancer in pregnancy Carbamylphosphate synthetase deficiency Carbamylphosphate synthetase Carcinoma of the gallbladder Cardiomyopathy (diabetic) Cardiomyopathy (hypertrophic) Carnitine uptake defect SLC22A5 Catecholaminergic polymorphic ventricular tachycardia CDKL5-related Atypical Rett Syndrome Celiac disease Cellulitis Cerebrovascular disease Cervix uteri cancer Chronic fatigue syndrome Chronic graft versus host disease Chronic idiopathic urticaria Chronic immune thrombocytopenia Thrombopoietin Chronic kidney kisease Chronic liver disease Chronic lymphocytic leukemia Chronic myeloid leukemia Chronic pancreatitis Cirrhosis of the liver Citrullinemia, type I Argininosuccinate synthase Classic Rett Syndrome Classical galactosemia Galactose-1-phosphate uridylyltransferase *Clostridium difficile* associated diarrhea Clotting disorders COAD/COPD Cocaine addiction COL4A5-related disorders Cold contact urticaria Contraception, female Coronary artery diseases Corpus uteri cancer Corticobasal degeneration Crigler-Najjar syndrome UDP-glucuronosyltransferase Critical limb ischemia CTNS-related cystinosis Cutaneous lupus erythematosus Cutaneous neuroendocrine carcinoma (Merkel Cell) Cystic fibrosis CFTR Cystic fibrosis Deoxyribonuclease I Cystinosis Cystinosin Cystinuria SLC7A9 Dementia (Lewy body) Depression Diabetic foot infections Diabetic foot ulcer Diabetic peripheral neuropathy Diabetic ulcers Diarrhoeal diseases Diffuse large B-cell lymphoma DiGeorge syndrome Diverticulitis Drug use disorders Duchenne muscular dystrophy Dystrophin Dysarthria Dyskinesia (levodopa-induced) Early-onset autosomal dominant Alzheimer's disease Eczema Ehlers-Danlos syndrome, type 1 EIF2B1 EIF2B2 EIF2B3 EIF2B4 EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter Eosinophilic esophagitis Epilepsy Erectile dysfunction Erythropoietic protoporphyria Ferrochelatase Esophageal carcinoma Essential tremor Fabry disease Alpha galactosidase Familial adenomatous polyposis APC Familial chylomicronemia Lipoprotein lipase Familial dysbetalipoproteinemia Apolipoprotein E Familial isolated dilated cardiomyopathy Familial mediterranean fever Pyrin (MEFV) Familial melanoma Female infertility Follicle stimulating hormone Female sexual dysfunction Fibromyalgia FMR1-related disorders Fracture healing Fragile X Premature Ovarian Failure Syndrome Fragile X syndrome FMRP Fragile X-Associated Tremor/Ataxia Syndrome Friedreich's ataxia Frontotemporal dementia Fryns syndrome Galactocerebrosidase deficiencies GALE deficiency Galactose epimerase GALK deficiency Galactokinase GALT-related galactosemia Gastric cancer Gastroesophageal reflux disease Gaucher disease Glucocerebrosidase Gilbert syndrome UDP-glucuronosyltransferase Glioblastoma multiforme Glomerulonephritis Glutaric acidemia, type I Glutaryl-CoA dehydrogenase GM2 gangliosidosis HEXA, HEXB Gout Urate oxidase Graft versus host disease Growth hormone deficiency Growth hormone 1/Growth hormone 2 Head and neck cancer, Metastatic colorectal cancer Anti-EGFr mAb Hearing loss, adult onset Heart failure Hemachromatosis HFE protein Hemifacial spasm Hemolytic uremic syndrome Anti-complement factor C5 mAb Hemophilia A Factor VIII Hemophilia A, Hemophilia B Factor VII Hemophilia B Factor IX Hepatitis B, Hepatitis C Interferon alpha HER2+ breast cancer, gastric cancer Anti-HER2 mAb Hereditary angioedema C1 esterase inhibitor Hereditary hemorrhagic telangiectasia Hereditary hemorrhagic telangiectasia (AT) Hereditary spherocytosis Hidradenitis suppurativa Homocystinuria Cystathionine beta-synthase Homozygous familial hypercholesterolemia LDL receptor Hunter syndrome (MPS II) Iduronate-2-sulfatase Huntington disease Huntingtin Hurler syndrome (MPS I) Alpha-L iduronidase Hydrolethalus Hyperalgesia Hyperbilirubinemia Hyperhidrosis Hyperlipidemia Hypermethioninemia Methionine adenosyltransferase Hyperoxaluria, type I Serine-pyruvate aminotransferase Hypertension Hyperuricemia Hyponatremia Hypoparathyroidism Parathyroid

hormone Hypophosphatasia TNSALP Idiopathic pulmonary fibrosis Iminoglycinuria Immunoglobulin deficiency Immunoglobulin Infection (adenovirus) Infection (anthrax prophylaxis) Infection (BK virus) Infection (*Clostridium difficile* prophylaxis) Infection (Dengue fever prophylaxis) Infection (Epstein-Barr virus) Infection (Hepatitis-D) Infection (Lyme disease prophylaxis) Infection (Smallpox virus) Infectious diseases vaccines Infectious antigen Inflammatory heart diseases Insomnia Interstitial cystitis Iron-deficiency anaemia Irritable bowel disease Ischaemic heart disease Isovaleric aciduria Isovaleric acid CoA dehydrogenase deficiency Jansky-Bielschowsky disease Juvenile Batten disease Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) Juvenile rheumatoid arthritis TNF-alpha inhibitors Kennedy's disease (SBMA) Keratoconus Krabbe disease Galactocerebrosidase Leber's hereditary optic neuropathy NADH dehydrogenase Leiomyosarcoma Lennox-Gastaut syndrome Lesch-Nyhan syndrome Hypoxanthine phosphoribosyltransferase 1 Leukaemia Li-Fraumeni syndrome TP53 Lipoma Liposarcoma Liver cancer Long-chain 3-OH acyl-CoA dehydrogenase deficiency Long-chain-3-hydroxyacyl-CoA dehydrogenase Lower respiratory infections Lysosomal acid lipase deficiency Lysosomal acid lipase Macular degeneration Major depressive disorder Malignant fibrous histiocytoma Mantle cell lymphoma Maple syrup urine disease 3-methyl-2-oxobutanoate dehydrogenase Marfan syndrome FBN1 Maroteaux-Lamy syndrome (MPS VI) N-acetylgalactosamine 4-sulfatase Mastocytosis McArdle disease Muscle glycogen phosphorylase MECP2-related disorders MECP2-related Severe Neonatal Encephalopathy Medium-chain acyl-CoA dehydrogenase deficiency Acyl-CoA dehydrogenase Melanoma Anti-CTLA4 mAb Metachromatic leukodystrophy Arylsulfatase A Metastatic colorectal cancer, NSCLC, others Anti-VEGF mAb Methylmalonyl-CoA mutase deficiency Methylmalonyl-CoA mutase Migraine Mitochondrial oxidative phosphorylation disorders Morquio syndrome, type A (MPS IVA) Galactose 6-sulfate sulfatase Morquio syndrome, type B (MPS IVB) Beta-galactosidase Mouth and oropharynx cancers Multiple carboxylase deficiency Biotin-methylcrotonoyl-CoA-carboxylase ligase Multiple myeloma Multiple sclerosis Anti-VLA-4 mAb Multiple sclerosis Interferon beta Multiple system atrophy Myasthenia gravis Myelofibrosis Narcolepsy Neonatal bronchopulmonary dysplasia Neonatal infections Nephritis and nephrosis Neurofibromatosis, type 1 NF-1 Neuronal ceroid lipofuscinoses-related diseases Neutropenia G-CSF Niemann Pick disease, type A/B SMPD1 Niemann Pick disease, type C NPC1 Niemann-Pick disease Type C1 Nocturia Non-alcoholic fatty liver disease Non-Hodgkin lymphoma Anti-CD20 mAb Non-small cell lung cancer Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) Obesity Ophthalmoparesis Opioid induced constipation Ornithine transcarbamylase deficiency Ornithine transcarbamylase Osteoarthritis Osteopetrosis Osteoporosis Anti-RANKL mAb Ovarian cancer Paget disease of bone Sequestosome 1 Pain Pancreatic carcinoma Panic disorder Parkinson disease Paroxysmal nocturnal hemoglobinuria Anti-complement factor C5 Mab *Pediculosis capitis* (head lice) Pelizaeus-Merzbacher disease Pemphigus vulgaris Peptic ulcer disease Peripheral neuropathy Peyronie's disease Phenylketonuria Phenylalanine hydroxylase Pneumococcal infection prophylaxis POLG-related sensory ataxic neuropathy Polycystic kidney disease Polycystic ovary syndrome Polycythaemia vera Polymerase G-related disorders Polymorphous light eruption Pompe disease Alpha glucosidase Porphyria cutanea tarda Uroporphyrinogen decarboxylase Post herpetic neuralgia Post-organ transplant Pouchitis PPM-X Syndrome Prader-Willi syndrome Preeclampsia Premature ejaculation Prematurity and low birth weight Primary ciliary dyskinesia Primary glomerular diseases Primary humoral immune deficiencies (e.g., CVID) Immunoglobulin Proctitis Progressive multifocal leukoencephalopathy Progressive supranuclear palsy Propionic acidemia Propionyl-CoA carboxylase Prostate cancer Psoriasis Anti-IL-12 & IL-23 mAb Psoriatic arthritis TNF-alpha inhibitors PTT-1 Pulmonary arterial hypertension Pulmonary arterial hypertension Raynaud's phenomenon Refractive errors Renal cell carcinoma Restless leg syndrome Retinitis pigmentosa Rheumatic heart disease Rheumatoid arthritis Anti-interleukin-6 (IL-6) mAb Rheumatoid arthritis T-cell costimulation blocker Rheumatoid arthritis TNF-alpha inhibitor

Romano-Ward syndrome Rosacea Sanfilippo syndrome, type A (MPS IIIA) Heparan N-sulfatase Sanfilippo syndrome, type B (MPS IIIB) N-acetyl-alpha-D-glucosaminidase Santavuori-Haltia disease Schizophrenia Schnitzler syndrome Scleroderma SCN1A SCN1B-related seizure disorders Short-chain acyl-CoA dehydrogenase deficiency Butyryl-CoA dehydrogenase Sickle cell disease Hemoglobin SLC3A1-related disorders Small cell lung cancer SMN-1-related spinal muscular atrophy (SMA) Spinal muscular atrophy Survival motor neuron protein Squamous cell carcinoma of head and neck Stickler syndrome Stomach cancer Stroke prophylaxis Synovial sarcoma Systemic lupus erythematosus Anti-BAFF Systemic sclerosis Tetrahydrobiopterin-deficient hyperphenylalaninemia Tetrahydrobiopterin Thromboangiitis obliterans Thrombotic disorders Thyroid cancer TPP1 deficiencies Trachea, bronchus, lung cancers Tricuspid atresia TSC1 TSC2-related tuberous sclerosis Type 2 diabetes mellitus Glucagon-like peptide 1 (GLP-1) agonist Type 2 diabetes mellitus Insulin Tyrosinemia, type I Fumarylacetoacetase Ulcerative colitis Uterine fibroids Varicose veins Venous thromboembolism Very long-chain acyl-CoA dehydrogenase deficiency Long-chain-acyl-CoA dehydrogenase von Gierke's disease Glucose-6-phosphatase Von Hippel-Lindau disease pVHL Wegener granulomatosis Wilson disease Wilson disease protein X-Linked adrenal hypoplasia X-linked adrenoleukodystrophy X-linked agammaglobulinemia Bruton's tyrosine kinase

[0202] In some embodiments, the present invention is used to prevent, treat and/or cure a subject affected with a disease or disorder listed or associated with the proteins listed in Tables 1, 2, 3 or 4. In some embodiments, an mRNA encodes one or more of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), argininosuccinate synthetase (ASS1), Factor IX, survival motor neuron 1 (SMN1), or phenylalanine hydroxylase (PAH).

[0203] In some embodiments, an mRNA encoding any one of the proteins listed in Tables 1, 2, 3 or 4 is codon-optimized. In one embodiment, an mRNA encoding CFTR is codon-optimized.

TABLE-US-00005 TABLE 5 Human CFTR Codon-

AUGCAACGCUCUCCUCUUGAAAAGGCCUCGGUGGUGUCCAAGCUCUU Optimized
CUUCUCGUGGACUAGACCCAUCCUGAGAAAGGGGUACAGACAGCGCU Human
UGGAGCUGUCCGAUAUCUAUCAAAUCCCUUCCGUGGACUCCGCGGAC CFTR
AACCUGUCCGAGAAGCUCGAGAGAGAAUGGGACAGAGAACUCGCCUC mRNA
AAAGAAGAACCCGAAGCUGAUUAAUGCGCUUAGGCGGUGCUUUUUC coding
UGGCGGUUCAUGUUCUACGGCAUCUUCCUCUACCUGGGAGAGGUCAC sequence
CAAGGCCGUGCAGCCCCUGUUGCUGGGACGGAUUAUUGCCUCCUACG
ACCCCGACAACAAGGAAGAAAGAAGCAUCGCUAUCUACUUGGGCAUC
GGUCUGUGCCUGCUUUUCAUCGUCCGGACCCUCUUGUUGCAUCCUGC
UAUUUUCGGCCUGCAUCACAUUGGCAUGCAGAUGAGAAUUGCCAUG
UUUUCCCUGAUCUACAAGAAAACUCUGAAGCUCUCGAGCCGCGUGCU
UGACAAGAUUUCCAUCGGCCAGCUCGUGUCCCUGCUCUCCAACAAUC
UGAACAAGUUCGACGAGGGCCUCGCCCUGGCCCACUUCGUGUGGAUC
GCCCCUCUGCAAGUGGCGCUUCUGAUGGGCCUGAUCUGGGAGCUGCU
GCAAGCCUCGGCAUUCUGUGGGCUUGGAUUCCUGAUCGUGCUGGCAC
UGUUCCAGGCCGGACUGGGGCGGAUGAUGAUGAAGUACAGGGACCA
GAGAGCCGGAAAGAUUUCCGAACGGCUGGUGAUCACUUCGGAAAUG
AUCGAAAACAUCCAGUCAGUGAAGGCCUACUGCUGGGAAGAGGCCAU
GGAAAAGAUGAUUGAAAACCUCCGGCAAACCGAGCUGAAGCUGACCC
GCAAGGCCGCUUACGUGCGCUAUUUCAACUCGUCCGCUUUCUUCUUC
UCCGGGUUCUUCGUGGUGUUUCUCUCCGUGCUCCCCUACGCCCUGAU
UAAGGGAAUCAUCCUCAGGAAGAUCUUCACCACCAUUUCCUUCUGUA
UCGUGCUCCGCAUGGCCGUGACCCGGCAGUUCCCAUGGGCCGUGCAG
ACUUGGUACGACUCCCUGGGAGCCAUUAACAAGAUCCAGGACUUCCU
UCAAAAGCAGGAGUACAAGACCCUCGAGUACAACCUGACUACUACCG

AGGUCGUGAUGGAAAACGUCACCGCCUUUUGGGAGGAGGGAUUUGG
CGAACUGUUCGAGAAGGCCAAGCAGAACAACAACAACCGCAAGACCU
CGAACGGUGACGACUCCCUCUUCUUUUCAAACUUCAGCCUGCUCGGG
ACGCCCGUGCUGAAGGACAUUAACUUCAAGAUCGAAAGAGGACAGCU
CCUGGCGGUGGCCGGAUCGACCGGAGCCGGAAAGACUUCCCUGCUGA
UGGUGAUCAUGGGAGAGCUUGAACCUAGCGAGGGAAAGAUCAAGCA
CUCCGGCCGCAUCAGCUUCUGUAGCCAGUUUUCCUGGAUCAUGCCCG
GAACCAUUAAGGAAAACAUCAUCUUCGGCGUGUCCUACGAUGAAUAC
CGCUACCGGUCCGUGAUCAAAGCCUGCCAGCUGGAAGAGGAUAUUUC
AAAGUUCGCGGAGAAAGAUAACAUCGUGCUGGGCGAAGGGGGUAUU
ACCUUGUCGGGGGGCCAGCGGGCUAGAAUCUCGCUGGCCAGAGCCGU
GUAUAAGGACGCCGACCUGUAUCUCCUGGACUCCCCCUUCGGAUACC
UGGACGUCCUGACCGAAAAGGAGAUCUUCGAAUCGUGCGUGUGCAA
GCUGAUGGCUAACAAGACUCGCAUCCUCGUGACCUCCAAAAUGGAGC
ACCUGAAGAAGGCAGACAAGAUUCUGAUUCUGCAUGAGGGGUCCUCC
UACUUUUACGGCACCUUCUCGGAGUUGCAGAACUUGCAGCCCGACUU
CUCAUCGAAGCUGAUGGGUUGCGACAGCUUCGACCAGUUCUCCGCCG
AAAGAAGGAACUCGAUCCUGACGGAAACCUUGCACCGCUUCUCUUUG
GAAGGCGACGCCCCUGUGUCAUGGACCGAGACUAAGAAGCAGAGCUU
CAAGCAGACCGGGGAAUUCGGCGAAAAGAGGAAGAACAGCAUCUUG
AACCCCAUUAACUCCAUCCGCAAGUUCUCAAUCGUGCAAAAGACGCC
ACUGCAGAUGAACGGCAUUGAGGAGGACUCCGACGAACCCCUUGAGA
GGCGCCUGUCCCUGGUGCCGGACAGCGAGCAGGGAGAAGCCAUCCUG
CCUCGGAUUUCCGUGAUCUCCACUGGUCCGACGCUCCAAGCCCGGCG
GCGGCAGUCCGUGCUGAACCUGAUGACCCACAGCGUGAACCAGGGCC
AAAACAUUCACCGCAAGACUACCGCAUCCACCCGGAAAGUGUCCCUG
GCACCUCAAGCGAAUCUUACCGAGCUCGACAUCUACUCCCGGAGACU
GUCGCAGGAAACCGGGCUCGAAAUUUCCGAAGAAAUCAACGAGGAG
GAUCUGAAAGAGUGCUUCUUCGACGAUAUGGAGUCGAUACCCGCCGU
GACGACUUGGAACACUUAUCUGCGGUACAUCACUGUGCACAAGUCAU
UGAUCUUCGUGCUGAUUUGGUGCCUGGUGAUUUUCCUGGCCGAGGU
CGCGGCCUCACUGGUGGUGCUCUGGCUGUUGGGAAACACGCCUCUGC
AAGACAAGGGAAACUCCACGCACUCGAGAAACAACAGCUAUGCCGUG
AUUAUCACUUCCACCUCCUCUUAUUACGUGUUCUACAUCUACGUCGG
AGUGGCGGAUACCCUGCUCGCGAUGGGUUUCUUCAGAGGACUGCCGC
UGGUCCACACCUUGAUCACCGUCAGCAAGAUUCUUCACCACAAGAUG
UUGCAUAGCGUGCUGCAGGCCCCCAUGUCCACCCUCAACACUCUGAA
GGCCGGAGGCAUUCUGAACAGAUUCUCCAAGGACAUCGCUAUCCUGG
ACGAUCUCCUGCCGCUUACCAUCUUUGACUUCAUCCAGCUGCUGCUG
AUCGUGAUUGGAGCAAUCGCAGUGGUGGCGGUGCUGCAGCCUUACA
UUUUCGUGGCCACUGUGCCGGUCAUUGUGGCGUUCAUCAUGCUGCGG
GCCUACUUCCUCCAAACCAGCCAGCAGCUGAAGCAACUGGAAUCCGA
GGGACGAUCCCCCAUCUUCACUCACCUUGUGACGUCGUUGAAGGGAC
UGUGGACCCUCCGGGCUUUCGGACGGCAGCCCUACUUCGAAACCCUC
UUCCACAAGGCCCUGAACCUCCACACCGCCAAUUGGUUCCUGUACCU
GUCCACCCUGCGGUGGUUCCAGAUGCGCAUCGAGAUGAUUUUCGUCA
UCUUCUUCAUCGCGGUCACAUUCAUCAGCAUCCUGACUACCGGAGAG
GGAGAGGGACGGGUCGGAAUAAUCCUGACCCUCGCCAUGAACAUUAU
GAGCACCCUGCAGUGGGCAGUGAACAGCUCGAUCGACGUGGACAGCC
UGAUGCGAAGCGUCAGCCGCGUGUUCAAGUUCAUCGACAUGCCUACU

GAGGGAAAACCCACUAAGUCCACUAAGCCCUACAAAAAUGGCCAGCU
GAGCAAGGUCAUGAUCAUCGAAAACUCCCACGUGAAGAAGGACGAU
AUUUGGCCCUCCGGAGGUCAAAUGACCGUGAAGGACCUGACCGCAAA
GUACACCGAGGGAGGAAACGCCAUUCUCGAAAACAUCAGCUUCUCCA
UUUCGCCGGGACAGCGGGUCGGCCUUCUCGGGCGGACCGGUUCCGGG
AAGUCAACUCUGCUGUCGGCUUUCCUCCGGCUGCUGAAUACCGAGGG
GGAAAUCCAAAUUGACGGCGUGUCUUGGGAUUCCAUUACUCUGCAGC
AGUGGCGGAAGGCCUUCGGCGUGAUCCCCCAGAAGGUGUUCAUCUUC
UCGGGUACCUUCCGGAAGAACCUGGAUCCUUACGAGCAGUGGAGCGA
CCAAGAAAUCUGGAAGGUCGCCGACGAGGUCGGCCUGCGCUCCGUGA
UUGAACAAUUUCCUGGAAAGCUGGACUUCGUGCUCGUCGACGGGGG
AUGUGUCCUGUCGCACGGACAUAAGCAGCUCAUGUGCCUCGCACGGU
CCGUGCUCUCCAAGGCCAAGAUUCUGCUGCUGGACGAACCUUCGGCC
CACCUGGAUCCGGUCACCUACCAGAUCAUCAGGAGGACCCUGAAGCA
GGCCUUUGCCGAUUGCACCGUGAUUCUCUGCGAGCACCGCAUCGAGG
CCAUGCUGGAGUGCCAGCAGUUCCUGGUCAUCGAGGAGAACAAGGUC
CGCCAAUACGACUCCAUUCAAAAGCUCCUCAACGAGCGGUCGCUGUU
CAGACAAGCUAUUUCACCGUCCGAUAGAGUGAAGCUCUUCCCGCAUC
GGAACAGCUCAAAGUGCAAAUCGAAGCCGCAGAUCGCAGCCUUGAAG
GAAGAGACUGAGGAAGAGGUGCAGGACACCCGGCUU**UAA** (SEQ ID NO: 1)
Comparison **AUG**CAGCGGUCCCCGCUCGAAAAGGCCAGUGUCGUGUCCAAACUCUU
Codon- CUUCUCAUGGACUCGGCCUAUCCUUAGAAAGGGGUAUCGGCAGAGGC
Optimized UUGAGUUGUCUGACAUCUACCAGAUCCCCUCGGUAGAUUCGGCGGAU
Human AACCUCUCGGAGAAGCUCGAACGGGAAUGGGACCGCGAACUCGCGUC CFTR
UAAGAAAAACCCGAAGCUCAUCAACGCACUGAGAAGGUGCUUCUUCU mRNA
GGCGGUUCAUGUUCUACGGUAUCUUCUUGUAUCUCGGGGAGGUCAC coding
AAAAGCAGUCCAACCCCUGUUGUUGGGUCGCAUUAUCGCCUCGUACG sequence
ACCCCGAUAACAAAGAAGAACGGAGCAUCGCGAUCUACCUCGGGAUC
GGACUGUGUUUGCUUUUCAUCGUCAGAACACUUUUGUUGCAUCCAGC
AAUCUUCGGCCUCCAUCACAUCGGUAUGCAGAUGCGAAUCGCUAUGU
UUAGCUUGAUCUACAAAAAGACACUGAAACUCUCGUCGCGGGUGUU
GGAUAAGAUUUCCAUCGGUCAGUUGGUGUCCCUGCUUAGUAAUAAC
CUCAACAAAUUCGAUGAGGGACUGGCGCUGGCACAUUUCGUGUGGA
UUGCCCCGUUGCAAGUCGCCCUUUUGAUGGGCCUUAUUUGGGAGCUG
UUGCAGGCAUCUGCCUUUUGUGGCCUGGGAUUUCUGAUUGUGUUGG
CAUUGUUUCAGGCUGGGCUUGGGCGGAUGAUGAUGAAGUAUCGCGA
CCAGAGAGCGGGUAAAAUCUCGGAAAGACUCGUCAUCACUUCGGAAA
UGAUCGAAAACAUCCAGUCGGUCAAAGCCUAUUGCUGGGAAGAAGC
UAUGGAGAAGAUGAUUGAAAACCUCCGCCAAACUGAGCUGAAACUG
ACCCGCAAGGCGGCGUAUGUCCGGUAUUUCAAUUCGUCAGCGUUCUU
CUUUUCCGGGUUCUUCGUUGUCUUUCUCUCGGUUUUGCCUUAUGCCU
UGAUUAAGGGGAUUAUCCUCCGCAAGAUUUUCACCACGAUUUCGUUC
UGCAUUGUAUUGCGCAUGGCAGUGACACGGCAAUUUCCGUGGGCCGU
GCAGACAUGGUAUGACUCGCUUGGAGCGAUCAACAAAAUCCAAGACU
UCUUGCAAAAGCAAGAGUACAAGACCCUGGAGUACAAUCUUACUACU
ACGGAGGUAGUAAUGGAGAAUGUGACGGCUUUUUGGGAAGAGGGUU
UUGGAGAACUGUUUGAGAAAGCAAAGCAGAAUAACAACAACCGCAA
GACCUCAAAUGGGGACGAUUCCCUGUUUUUCUCGAACUUCUCCCUGC
UCGGAACACCCGUGUUGAAGGACAUCAAUUUCAAGAUUGAGAGGGG
ACAGCUUCUCGCGGUAGCGGGAAGCACUGGUGCGGGAAAAACUAGCC

UCUUGAUGGUGAUUAUGGGGGAGCUUGAGCCCAGCGAGGGGAAGAU
UAAACACUCCGGGCGUAUCUCAUUCUGUAGCCAGUUUUCAUGGAUCA
UGCCCGGAACCAUUAAAGAGAACAUCAUUUUCGGAGUAUCCUAUGA
UGAGUACCGAUACAGAUCGGUCAUUAAGGCGUGCCAGUUGGAAGAG
GACAUUUCUAAGUUCGCCGAGAAGGAUAACAUCGUCUUGGGAGAAG
GGGGUAUUACAUUGUCGGGAGGGCAGCGAGCGCGGAUCAGCCUCGCG
AGAGCGGUAUACAAAGAUGCAGAUUUGUAUCUGCUUGAUUCACCGU
UUGGAUACCUCGACGUAUUGACAGAAAAAGAAAUCUUCGAGUCGUG
CGUGUGUAAACUUAUGGCUAAUAAGACGAGAAUCCUGGUGACAUCA
AAAAUGGAACACCUUAAGAAGGCGGACAAGAUCCUGAUCCUCCACGA
AGGAUCGUCCUACUUUUACGGCACUUUCUCAGAGUUGCAAAACUUGC
AGCCGGACUUCUCAAGCAAACUCAUGGGGUGUGACUCAUUCGACCAG
UUCAGCGCGGAACGGCGGAACUCGAUCUUGACGGAAACGCUGCACCG
AUUCUCGCUUGAGGGUGAUGCCCCGGUAUCGUGGACCGAGACAAAGA
AGCAGUCGUUUAAGCAGACAGGAGAAUUUGGUGAGAAAAGAAAGAA
CAGUAUCUUGAAUCCUAUUAACUCAAUUCGCAAGUUCUCAAUCGUCC
AGAAAACUCCACUGCAGAUGAAUGGAAUUGAAGAGGAUUCGGACGA
ACCCCUGGAGCGCAGGCUUAGCCUCGUGCCGGAUUCAGAGCAAGGGG
AGGCCAUUCUUCCCCGGAUUUCGGUGAUUUCAACCGGACCUACACUU
CAGGCGAGGCGAAGGCAAUCCGUGCUCAACCUCAUGACGCAUUCGGU
AAACCAGGGGCAAAACAUUCACCGCAAAACGACGGCCUCAACGAGAA
AAGUGUCACUUGCACCCCAGGCGAAUUUGACUGAACUCGACAUCUAC
AGCCGUAGGCUUUCGCAAGAAACCGGACUUGAGAUCAGCGAAGAAA
UCAAUGAAGAAGAUUUGAAAGAGUGUUUCUUUGAUGACAUGGAAUC
AAUCCCAGCGGUGACAACGUGGAACACAUACUUGCGUUACAUCACGG
UGCACAAGUCCUUGAUUUUCGUCCUCAUCUGGUGUCUCGUGAUCUUU
CUCGCUGAGGUCGCAGCGUCACUUGUGGUCCUCUGGCUGCUUGGUAA
UACGCCCUUGCAAGACAAAGGCAAUUCUACACACUCAAGAAACAAUU
CCUAUGCCGUGAUUAUCACUUCUACAAGCUCGUAUUACGUGUUUUAC
AUCUACGUAGGAGUGGCCGACACUCUGCUCGCGAUGGGUUUCUUCCG
AGGACUCCCACUCGUUCACACGCUUAUCACUGUCUCCAAGAUUCUCC
ACCAUAAGAUGCUUCAUAGCGUACUGCAGGCUCCCAUGUCCACCUUG
AAUACGCUCAAGGCGGGAGGUAUUUUGAAUCGCUUCUCAAAAGAUA
UUGCAAUUUUUGGAUGACCUUCUGCCCCUGACGAUCUUCGACUUCAUC
CAGUUGUUGCUGAUCGUGAUUGGGGCUAUUGCAGUAGUCGCUGUCC
UCCAGCCUUACAUUUUUGUCGCGACCGUUCCGGUGAUCGUGGCGUUU
AUCAUGCUGCGGGCCUAUUUCUUGCAGACGUCACAGCAGCUUAAGCA
ACUGGAGUCUGAAGGGAGGUCGCCUAUCUUUACGCAUCUUGUGACCA
GUUUGAAGGGAUUGUGGACGUUGCGCGCCUUUGGCAGGCAGCCCUAC
UUUGAAACACUGUUCCACAAAGCGCUGAAUCUCCAUACGGCAAAUUG
GUUUUUGUAUUUGAGUACCCUCCGAUGGUUUCAGAUGCGCAUUGAG
AUGAUUUUUGUGAUCUUCUUUAUCGCGGUGACUUUUAUCUCCAUCU
UGACCACGGGAGAGGGCGAGGGACGGGUCGGUAUUAUCCUGACACUC
GCCAUGAACAUUAUGAGCACUUUGCAGUGGGCAGUGAACAGCUCGA
UUGAUGUGGAUAGCCUGAUGAGGUCCGUUUCGAGGGUCUUUAAGUU
CAUCGACAUGCCGACGGAGGGAAAGCCCACAAAAAGUACGAAACCCU
AUAAGAAUGGGCAAUUGAGUAAGGUAAUGAUCAUCGAGAACAGUCA
CGUGAAGAAGGAUGACAUCUGGCCUAGCGGGGGUCAGAUGACCGUG
AAGGACCUGACGGCAAAAUACACCGAGGGAGGGAACGCAAUCCUUGA
AAACAUCUCGUUCAGCAUUAGCCCCGGUCAGCGUGUGGGGUUGCUCG

GGAGGACCGGGUCAGGAAAAUCGACGUUGCUGUCGGCCUUCUUGAG
ACUUCUGAAUACAGAGGGUGAGAUCCAGAUCGACGGCGUUUCGUGG
GAUAGCAUCACCUUGCAGCAGUGGCGGAAAGCGUUUGGAGUAAUCCC
CCAAAAGGUCUUUAUCUUUAGCGGAACCUUCCGAAAGAAUCUCGAUC
CUUAUGAACAGUGGUCAGAUCAAGAGAUUUGGAAAGUCGCGGACGA
GGUUGGCCUUCGGAGUGUAAUCGAGCAGUUUCCGGGAAAACUCGAC
UUUGUCCUUGUAGAUGGGGGAUGCGUCCUGUCGCAUGGGCACAAGC
AGCUCAUGUGCCUGGCGCGAUCCGUCCUCUCUAAAGCGAAAAUUCUU
CUCUUGGAUGAACCUUCGGCCCAUCUGGACCCGGUAACGUAUCAGAU
CAUCAGAAGGACACUUAAGCAGGCGUUUGCCGACUGCACGGUGAUUC
UCUGUGAGCAUCGUAUCGAGGCCAUGCUCGAAUGCCAGCAAUUUCUU
GUCAUCGAAGAGAAUAAGGUCCGCCAGUACGACUCCAUCCAGAAGCU
GCUUAAUGAGAGAUCAUUGUUCCGGCAGGCGAUUUCACCAUCCGAUA
GGGUGAAACUUUUUCCACACAGAAAUUCGUCGAAGUGCAAGUCCAA
ACCGCAGAUCGCGGCCUUGAAAGAAGAGACUGAAGAAGAAGUUCAA
GACACGCGUCUU**UAA** (SEQ ID NO: 2) Codon-
AUGCAGCGGUCCUAGCUCGAAAAGGCCAGUGUCGUGUCCAAACUCUU Optimized
CUUCUCAUGGACUCGGCCUAUCCUUAGAAAGGGGUAUCGGCAGAGGC Human
UUGAGUUGUCUGACAUCUACCAGUGACCCUCGGUAGAUUCGGCGGAU CFTR
AACCUCUCGGAGAAGCUCGAACGGGAAUGGGACCGCGAACUCGCGUC ′STOP′
UAAGAAAAACCCGAAGCUCAUCAACGCACUGAGAAGGUGCUUCUUCU Coding
GGCGGUUCAUGUUCUACGGUAUCUUCUUGUAUCUCGGGGAGGUCAC Sequence
AAAAGCAGUCCAACCCCUGUUGUUGGGUCGCAUUAUCGCCUCGUACG
ACCCCGAUAACAAAGAAGAACGGAGCAUCGCGAUCUACCUCGGGAUC
GGACUGUGUUUGCUUUUCAUCGUCAGAACACUUUUGUUGCAUCCAGC
AAUCUUCGGCCUCCAUCACAUCGGUAUGCAGAUGCGAAUCGCUAUGU
UUAGCUUGAUCUACAAAAAGACACUGAAACUCUCGUCGCGGGUGUU
GGAUAAGAUUUCCAUCGGUCAGUUGGUGUCCCUGCUUAGUAAUAAC
CUCAACAAAUUCGAUGAGGGACUGGCGCUGGCACAUUUCGUGUGGA
UUGCCCCGUUGCAAGUCGCCCUUUUGAUGGGCCUUAUUUGGGAGCUG
UUGCAGGCAUCUGCCUUUUGUGGCCUGGGAUUUCUGAUUGUGUUGG
CAUUGUUUCAGGCUGGGCUUGGGCGGAUGAUGAUGAAGUAUCGCGA
CCAGAGAGCGGGUAAAAUCUCGGAAAGACUCGUCAUCACUUCGGAAA
UGAUCGAAAACAUCCAGUCGGUCAAAGCCUAUUGCUGGGAAGAAGC
UAUGGAGAAGAUGAUUGAAAACCUCCGCCAAACUGAGCUGAAACUG
ACCCGCAAGGCGGCGUAUGUCCGGUAUUUCAAUUCGUCAGCGUUCUU
CUUUUCCGGGUUCUUCGUUGUCUUUCUCUCGGUUUUGCCUUAUGCCU
UGAUUAAGGGGAUUAUCCUCCGCAAGAUUUUCACCACGAUUUCGUUC
UGCAUUGUAUUGCGCAUGGCAGUGACACGGCAAUUUCCGUGGGCCGU
GCAGACAUGGUAUGACUCGCUUGGAGCGAUCAACAAAAUCCAAGACU
UCUUGCAAAAGCAAGAGUACAAGACCCUGGAGUACAAUCUUACUACU
ACGGAGGUAGUAAUGGAGAAUGUGACGGCUUUUUGGGAAGAGGGUU
UUGGAGAACUGUUUGAGAAAGCAAAGCAGAAUAACAACAACCGCAA
GACCUCAAAUGGGGACGAUUCCCUGUUUUUCUCGAACUUCUCCCUGC
UCGGAACACCCGUGUUGAAGGACAUCAAUUUCAAGAUUGAGAGGGG
ACAGCUUCUCGCGGUAGCGGGAAGCACUGGUGCGGGAAAAACUAGCC
UCUUGAUGGUGAUUAUGGGGGAGCUUGAGCCCAGCGAGGGGAAGAU
UAAACACUCCGGGCGUAUCUCAUUCUGUAGCCAGUUUUCAUGGAUCA
UGCCCGGAACCAUUAAAGAGAACAUCAUUUUCGGAGUAUCCUAUGA
UGAGUACCGAUACAGAUCGGUCAUUAAGGCGUGCCAGUUGGAAGAG

GACAUUUCUAAGUUCGCCGAGAAGGAUAACAUCGUCUUGGGAGAAG
GGGGUAUUACAUUGUCGGGAGGGCAGCGAGCGCGGAUCAGCCUCGCG
AGAGCGGUAUACAAAGAUGCAGAUUUGUAUCUGCUUGAUUCACCGU
UUGGAUACCUCGACGUAUUGACAGAAAAAGAAAUCUUCGAGUCGUG
CGUGUGUAAACUUAUGGCUAAUAAGACGAGAAUCCUGGUGACAUCA
AAAAUGGAACACCUUAAGAAGGCGGACAAGAUCCUGAUCCUCCACGA
AGGAUCGUCCUACUUUUACGGCACUUUCUCAGAGUUGCAAAACUUGC
AGCCGGACUUCUCAAGCAAACUCAUGGGGUGUGACUCAUUCGACCAG
UUCAGCGCGGAACGGCGGAACUCGAUCUUGACGGAAACGCUGCACCG
AUUCUCGCUUGAGGGUGAUGCCCCGGUAUCGUGGACCGAGACAAAGA
AGCAGUCGUUUAAGCAGACAGGAGAAUUUGGUGAGAAAAGAAAGAA
CAGUAUCUUGAAUCCUAUUAACUCAAUUCGCAAGUUCUCAAUCGUCC
AGAAAACUCCACUGCAGAUGAAUGGAAUUGAAGAGGAUUCGGACGA
ACCCCUGGAGCGCAGGCUUAGCCUCGUGCCGGAUUCAGAGCAAGGGG
AGGCCAUUCUUCCCCGGAUUUCGGUGAUUUCAACCGGACCUACACUU
CAGGCGAGGCGAAGGCAAUCCGUGCUCAACCUCAUGACGCAUUCGGU
AAACCAGGGGCAAAACAUUCACCGCAAAACGACGGCCUCAACGAGAA
AAGUGUCACUUGCACCCCAGGCGAAUUUGACUGAACUCGACAUCUAC
AGCCGUAGGCUUUCGCAAGAAACCGGACUUGAGAUCAGCGAAGAAA
UCAAUGAAGAAGAUUUGAAAGAGUGUUUCUUUGAUGACAUGGAAUC
AAUCCCAGCGGUGACAACGUGGAACACAUACUUGCGUUACAUCACGG
UGCACAAGUCCUUGAUUUUCGUCCUCAUCUGGUGUCUCGUGAUCUUU
CUCGCUGAGGUCGCAGCGUCACUUGUGGUCCUCUGGCUGCUUGGUAA
UACGCCCUUGCAAGACAAAGGCAAUUCUACACACUCAAGAAACAAUU
CCUAUGCCGUGAUUAUCACUUCUACAAGCUCGUAUUACGUGUUUUAC
AUCUACGUAGGAGUGGCCGACACUCUGCUCGCGAUGGGUUUCUUCCG
AGGACUCCCACUCGUUCACACGCUUAUCACUGUCUCCAAGAUUCUCC
ACCAUAAGAUGCUUCAUAGCGUACUGCAGGCUCCCAUGUCCACCUUG
AAUACGCUCAAGGCGGGAGGUAUUUUGAAUCGCUUCUCAAAAGAUA
UUGCAAUUUUUGGAUGACCUUCUGCCCCUGACGAUCUUCGACUUCAUC
CAGUUGUUGCUGAUCGUGAUUGGGGCUAUUGCAGUAGUCGCUGUCC
UCCAGCCUUACAUUUUUGUCGCGACCGUUCCGGUGAUCGUGGCGUUU
AUCAUGCUGCGGGCCUAUUUCUUGCAGACGUCACAGCAGCUUAAGCA
ACUGGAGUCUGAAGGGAGGUCGCCUAUCUUUACGCAUCUUGUGACCA
GUUUGAAGGGAUUGUGGACGUUGCGCGCCUUUGGCAGGCAGCCCUAC
UUUGAAACACUGUUCCACAAAGCGCUGAAUCUCCAUACGGCAAAUUG
GUUUUUGUAUUUGAGUACCCUCCGAUGGUUUCAGAUGCGCAUUGAG
AUGAUUUUUGUGAUCUUCUUUAUCGCGGUGACUUUUAUCUCCAUCU
UGACCACGGGAGAGGGCGAGGGACGGGUCGGUAUUAUCCUGACACUC
GCCAUGAACAUUAUGAGCACUUUGCAGUGGGCAGUGAACAGCUCGA
UUGAUGUGGAUAGCCUGAUGAGGUCCGUUUCGAGGGUCUUUAAGUU
CAUCGACAUGCCGACGGAGGGAAAGCCCACAAAAAGUACGAAACCCU
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CGUGAAGAAGGAUGACAUCUGGCCUAGCGGGGGUCAGAUGACCGUG
AAGGACCUGACGGCAAAAUACACCGAGGGAGGGAACGCAAUCCUUGA
AAACAUCUCGUUCAGCAUUAGCCCCGGUCAGCGUGUGGGGUUGCUCG
GGAGGACCGGGUCAGGAAAAUCGACGUUGCUGUCGGCCUUCUUGAG
ACUUCUGAAUACAGAGGGUGAGAUCCAGAUCGACGGCGUUUCGUGG
GAUAGCAUCACCUUGCAGCAGUGGCGGAAAGCGUUUGGAGUAAUCCC
CCAAAAGGUCUUUAUCUUUAGCGGAACCUUCCGAAAGAAUCUCGAUC

CUUAUGAACAGUGGUCAGAUCAAGAGAUUUGGAAAGUCGCGGACGA
GGUUGGCCUUCGGAGUGUAAUCGAGCAGUUUCCGGGAAAACUCGAC
UUUGUCCUUGUAGAUGGGGGAUGCGUCCUGUCGCAUGGGCACAAGC
AGCUCAUGUGCCUGGCGCGAUCCGUCCUCUCUAAAGCGAAAAUUCUU
CUCUUGGAUGAACCUUCGGCCCAUCUGGACCCGGUAACGUAUCAGAU
CAUCAGAAGGACACUUAAGCAGGCGUUUGCCGACUGCACGGUGAUUC
UCUGUGAGCAUCGUAUCGAGGCCAUGCUCGAAUGCCAGCAAUUUCUU
GUCAUCGAAGAGAAUAAGGUCCGCCAGUACGACUCCAUCCAGAAGCU
GCUUAAUGAGAGAUCAUUGUUCCGGCAGGCGAUUUCACCAUCCGAUA
GGGUGAAACUUUUUCCACACAGAAAUUCGUCGAAGUGCAAGUCCAA
ACCGCAGAUCGCGGCCUUGAAAGAAGAGACUGAAGAAGAAGUUCAA
GACACGCGUCUUUAA (SEQ ID NO: 3) Human
MQRSPLEKASVVSKLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLSEK CFTR
LEREWDRELASKKNPKLINALRRCFFWRFMFYGIFLYLGEVTKAVQPLLL Protein
GRIIASYDPDNKEERSIAIYLGIGLCLLFIVRTLLLHPAIFGLHHIGMQMRIA Sequence
MFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAHFVWIAPLQ
VALLMGLIWELLQASAFCGLGFLIVLALFQAGLGRIVIMMKYRDQRAGKIS
ERLVITSEMIENIQSVKAYCWEEAMEKMIENLRQTELKLTRKAAYVRYFN
SSAFFFSGFFVVFLSVLPYALIKGIILRKIFTTISFCIVLRMAVTRQFPWAVQT
WYDSLGAINKIQDFLQKQEYKTLEYNLTTTEVVMENVTAFWEEGFGELFE
KAKQNNNNRKTSNGDDSLFFSNFSLLGTPVLKDINFKIERGQLLAVAGSTG
AGKTSLLMVIIVIGELEPSEGKIKHSGRISFCSQFSWIMPGTIKENIIFGVSYDE
YRYRSVIKACQLEEDISKFAEKDNIVLGEGGITLSGGQRARISLARAVYKD
ADLYLLDSPFGYLDVLTEKEIFESCVCKLMANKTRILVTSKMEHLKKADKI
LILHEGSSYFYGTFSELQNLQPDFSSKLMGCDSFDQFSAERRNSILTETLHR
FSLEGDAPVSWTETKKQSFKQTGEFGEKRKNSILNPINSIRKFSIVQKTPLQ
MNGIEEDSDEPLERRLSLVPDSEQGEAILPRISVISTGPTLQARRRQSVLNL
MTHSVNQGQNIHRKTTASTRKVSLAPQANLTELDIYSRRLSQETGLEISEEI
NEEDLKECFFDDMESIPAVTTWNTYLRYITVHKSLIFVLIWCLVIFLAEVAA
SLVVLWLLGNTPLQDKGNSTHSRNNSYAVIITSTSSYYVFYIYVGVADTLL
AMGFFRGLPLVHTLITVSKILHHKMLHSVLQAPMSTLNTLKAGGILNRFSK
DIAILDDLLPLTIFDFIQLLLIVIGAIAVVAVLQPYIFVATVPVIVAFIMLRAY
FLQTSQQLKQLESEGRSPIFTHLVTSLKGLWTLRAFGRQPYFETLFHKALN
LHTANWFLYLSTLRWFQMRIEMIFVIFFIAVTFISILTTGEGEGRVGIILTLA
MNEVISTLQWAVNSSIDVDSLIVIRSVSRVFKFIDMPTEGKPTKSTKPYKNGQ
LSKVMIIENSHVKKDDIWPSGGQMTVKDLTAKYTEGGNAILENISFSISPGQ
RVGLLGRTGSGKSTLLSAFLRLLNTEGEIQIDGVSWDSITLQQWRKAFGVIP
QKVFIFSGTFRKNLDPYEQWSDQEIWKVADEVGLRSVIEQFPGKLDFVLVD
GGCVLSHGHKQLMCLARSVLSKAKILLLDEPSAHLDPVTYQIIRRTLKQAF
ADCTVILCEHRIEAMLECQQFLVIEENKVRQYDSIQKLLNERSLFRQAISPS
DRVKLFPHRNSSKCKSKPQIAALKEETEEEVQDTRL (SEQ ID NO: 4)
Exemplary Codon-Optimized Human Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mRNAs
Construct design: [0204] X—Coding Sequence—Y
5′ and 3′ UTR Sequences:
TABLE-US-00006 X (5′ UTR Sequence) = (SEQ ID NO: 5)
GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAA
GACACCGGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAAC
GCGGAUUCCCCGUGCCAAGAGUGACUCACCGUCCUUGACACG Y (3′ UTR Sequence) = (SEQ ID NO: 6)

CGGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCUGG
AAGUUGCCACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUG
CAUCAAGCU OR (SEQ ID NO: 7)
GGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCUGGAAG
UUGCCACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAU CAAAGCU
[0205] In one embodiment, a codon-optimized human CFTR mRNA sequence includes SEQ ID NO: 1.
[0206] In one embodiment, a full-length codon-optimized human CFTR mRNA sequence is:
TABLE-US-00007 (SEQ ID NO: 8)
GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAA
GACACCGGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAAC
GCGGAUUCCCCGUGCCAAGAGUGACUCACCGUCCUUGACACG**AUG**CAAC
GCUCUCCUCUUGAAAAGGCCUCGGUGGUGUCCAAGCUCUUCUUCUCGUG
GACUAGACCCAUCCUGAGAAAGGGGUACAGACAGCGCUUGGAGCUGUCC
GAUAUCUAUCAAAUCCCUUCCGUGGACUCCGCGGACAACCUGUCCGAGA
AGCUCGAGAGAGAAUGGGACAGAGAACUCGCCUCAAAGAAGAACCCGAA
GCUGAUUAAUGCGCUUAGGCGGUGCUUUUUCUGGCGGUUCAUGUUCUAC
GGCAUCUUCCUCUACCUGGGAGAGGUCACCAAGGCCGUGCAGCCCCUGU
UGCUGGGACGGAUUAUUGCCUCCUACGACCCCGACAACAAGGAAGAAAG
AAGCAUCGCUAUCUACUUGGGCAUCGGUCUGUGCCUGCUUUUCAUCGUC
CGGACCCUCUUGUUGCAUCCUGCUAUUUUCGGCCUGCAUCACAUUGGCA
UGCAGAUGAGAAUUGCCAUGUUUUCCCUGAUCUACAAGAAAACUCUGAA
GCUCUCGAGCCGCGUGCUUGACAAGAUUUCCAUCGGCCAGCUCGUGUCC
CUGCUCUCCAACAAUCUGAACAAGUUCGACGAGGGCCUCGCCCUGGCCC
ACUUCGUGUGGAUCGCCCCUCUGCAAGUGGCGCUUCUGAUGGGCCUGAU
CUGGGAGCUGCUGCAAGCCUCGGCAUUCUGUGGGCUUGGAUUCCUGAUC
GUGCUGGCACUGUUCCAGGCCGGACUGGGGCGGAUGAUGAUGAAGUACA
GGGACCAGAGAGCCGGAAAGAUUUCCGAACGGCUGGUGAUCACUUCGGA
AAUGAUCGAAAACAUCCAGUCAGUGAAGGCCUACUGCUGGGAAGAGGCC
AUGGAAAAGAUGAUUGAAAACCUCCGGCAAACCGAGCUGAAGCUGACCC
GCAAGGCCGCUUACGUGCGCUAUUUCAACUCGUCCGCUUUCUUCUUCUC
CGGGUUCUUCGUGGUGUUUCUCUCCGUGCUCCCCUACGCCCUGAUUAAG
GGAAUCAUCCUCAGGAAGAUCUUCACCACCAUUUCCUUCUGUAUCGUGC
UCCGCAUGGCCGUGACCCGGCAGUUCCCAUGGGCCGUGCAGACUUGGUA
CGACUCCCUGGGAGCCAUUAACAAGAUCCAGGACUUCCUUCAAAAGCAG
GAGUACAAGACCCUCGAGUACAACCUGACUACUACCGAGGUCGUGAUGG
AAAACGUCACCGCCUUUUGGGAGGAGGGAUUUGGCGAACUGUUCGAGAA
GGCCAAGCAGAACAACAACAACCGCAAGACCUCGAACGGUGACGACUCC
CUCUUCUUUUCAAACUUCAGCCUGCUCGGGACGCCCGUGCUGAAGGACA
UUAACUUCAAGAUCGAAAGAGGACAGCUCCUGGCGGUGGCCGGAUCGAC
CGGAGCCGGAAAGACUUCCCUGCUGAUGGUGAUCAUGGGAGAGCUUGAA
CCUAGCGAGGGAAAGAUCAAGCACUCCGGCCGCAUCAGCUUCUGUAGCC
AGUUUUCCUGGAUCAUGCCCGGAACCAUUAAGGAAAACAUCAUCUUCGG
CGUGUCCUACGAUGAAUACCGCUACCGGUCCGUGAUCAAAGCCUGCCAG
CUGGAAGAGGAUAUUUCAAAGUUCGCGGAGAAAGAUAACAUCGUGCUGG
GCGAAGGGGGUAUUACCUUGUCGGGGGGCCAGCGGGCUAGAAUCUCGCU
GGCCAGAGCCGUGUAUAAGGACGCCGACCUGUAUCUCCUGGACUCCCCC
UUCGGAUACCUGGACGUCCUGACCGAAAAGGAGAUCUUCGAAUCGUGCG
UGUGCAAGCUGAUGGCUAACAAGACUCGCAUCCUCGUGACCUCCAAAAU
GGAGCACCUGAAGAAGGCAGACAAGAUUCUGAUUCUGCAUGAGGGGUCC

UCCUACUUUUACGGCACCUUCUCGGAGUUGCAGAACUUGCAGCCCGACU
UCUCAUCGAAGCUGAUGGGUUGCGACAGCUUCGACCAGUUCUCCGCCGA
AAGAAGGAACUCGAUCCUGACGGAAACCUUGCACCGCUUCUCUUUGGAA
GGCGACGCCCCUGUGUCAUGGACCGAGACUAAGAAGCAGAGCUUCAAGC
AGACCGGGGAAUUCGGCGAAAAGAGGAAGAACAGCAUCUUGAACCCCAU
UAACUCCAUCCGCAAGUUCUCAAUCGUGCAAAAGACGCCACUGCAGAUG
AACGGCAUUGAGGAGGACUCCGACGAACCCCUUGAGAGGCGCCUGUCCC
UGGUGCCGGACAGCGAGCAGGGAGAAGCCAUCCUGCCUCGGAUUUCCGU
GAUCUCCACUGGUCCGACGCUCCAAGCCCGGCGGCGGCAGUCCGUGCUG
AACCUGAUGACCCACAGCGUGAACCAGGGCCAAAACAUUCACCGCAAGA
CUACCGCAUCCACCCGGAAAGUGUCCCUGGCACCUCAAGCGAAUCUUAC
CGAGCUCGACAUCUACUCCCGGAGACUGUCGCAGGAAACCGGGCUCGAA
AUUUCCGAAGAAAUCAACGAGGAGGAUCUGAAAGAGUGCUUCUUCGACG
AUAUGGAGUCGAUACCCGCCGUGACGACUUGGAACACUUAUCUGCGGUA
CAUCACUGUGCACAAGUCAUUGAUCUUCGUGCUGAUUUGGUGCCUGGUG
AUUUUCCUGGCCGAGGUCGCGGCCUCACUGGUGGUGCUCUGGCUGUUGG
GAAACACGCCUCUGCAAGACAAGGGAAACUCCACGCACUCGAGAAACAA
CAGCUAUGCCGUGAUUAUCACUUCCACCUCCUCUUAUUACGUGUUCUAC
AUCUACGUCGGAGUGGCGGAUACCCUGCUCGCGAUGGGUUUCUUCAGAG
GACUGCCGCUGGUCCACACCUUGAUCACCGUCAGCAAGAUUCUUCACCA
CAAGAUGUUGCAUAGCGUGCUGCAGGCCCCCAUGUCCACCCUCAACACU
CUGAAGGCCGGAGGCAUUCUGAACAGAUUCUCCAAGGACAUCGCUAUCC
UGGACGAUCUCCUGCCGCUUACCAUCUUUGACUUCAUCCAGCUGCUGCU
GAUCGUGAUUGGAGCAAUCGCAGUGGUGGCGGUGCUGCAGCCUUACAUU
UUCGUGGCCACUGUGCCGGUCAUUGUGGCGUUCAUCAUGCUGCGGGCCU
ACUUCCUCCAAACCAGCCAGCAGCUGAAGCAACUGGAAUCCGAGGGACG
AUCCCCCAUCUUCACUCACCUUGUGACGUCGUUGAAGGGACUGUGGACC
CUCCGGGCUUUCGGACGGCAGCCCUACUUCGAAACCCUCUUCCACAAGG
CCCUGAACCUCCACACCGCCAAUUGGUUCCUGUACCUGUCCACCCUGCG
GUGGUUCCAGAUGCGCAUCGAGAUGAUUUUCGUCAUCUUCUUCAUCGCG
GUCACAUUCAUCAGCAUCCUGACUACCGGAGAGGGAGAGGGACGGGUCG
GAAUAAUCCUGACCCUCGCCAUGAACAUUAUGAGCACCCUGCAGUGGGC
AGUGAACAGCUCGAUCGACGUGGACAGCCUGAUGCGAAGCGUCAGCCGC
GUGUUCAAGUUCAUCGACAUGCCUACUGAGGGAAAACCCACUAAGUCCA
CUAAGCCCUACAAAAAUGGCCAGCUGAGCAAGGUCAUGAUCAUCGAAAA
CUCCCACGUGAAGAAGGACGAUAUUUGGCCCUCCGGAGGUCAAAUGACC
GUGAAGGACCUGACCGCAAAGUACACCGAGGGAGGAAACGCCAUUCUCG
AAAACAUCAGCUUCUCCAUUUCGCCGGGACAGCGGGUCGGCCUUCUCGG
GCGGACCGGUUCCGGGAAGUCAACUCUGCUGUCGGCUUUCCUCCGGCUG
CUGAAUACCGAGGGGGAAAUCCAAAUUGACGGCGUGUCUUGGGAUUCCA
UUACUCUGCAGCAGUGGCGGAAGGCCUUCGGCGUGAUCCCCCAGAAGGU
GUUCAUCUUCUCGGGUACCUUCCGGAAGAACCUGGAUCCUUACGAGCAG
UGGAGCGACCAAGAAAUCUGGAAGGUCGCCGACGAGGUCGGCCUGCGCU
CCGUGAUUGAACAAUUUCCUGGAAAGCUGGACUUCGUGCUCGUCGACGG
GGGAUGUGUCCUGUCGCACGGACAUAAGCAGCUCAUGUGCCUCGCACGG
UCCGUGCUCUCCAAGGCCAAGAUUCUGCUGCUGGACGAACCUUCGGCCC
ACCUGGAUCCGGUCACCUACCAGAUCAUCAGGAGGACCCUGAAGCAGGC
CUUUGCCGAUUGCACCGUGAUUCUCUGCGAGCACCGCAUCGAGGCCAUG
CUGGAGUGCCAGCAGUUCCUGGUCAUCGAGGAGAACAAGGUCCGCCAAU
ACGACUCCAUUCAAAAGCUCCUCAACGAGCGGUCGCUGUUCAGACAAGC

UAUUUCACCGUCCGAUAGAGUGAAGCUCUUCCCGCAUCGGAACAGCUCA
AAGUGCAAAUCGAAGCCGCAGAUCGCAGCCUUGAAGGAAGAGACUGAGG
AAGAGGUGCAGGACACCCGGCUU**UAA**CGGGUGGCAUCCCUGUGACCCCU
CCCCAGUGCCUCUCCUGGCCCUGGAAGUUGCCACUCCAGUGCCCACCAG
CCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU

[0207] In one embodiment, another full-length codon-optimized human CFTR mRNA sequence is:

TABLE-US-00008 (SEQ ID NO: 9)

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAA
GACACCGGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAAC
GCGGAUUCCCCGUGCCAAGAGUGACUCACCGUCCUUGACACG**AUG**CAAC
GCUCUCCUCUUGAAAAGGCCUCGGUGGUGUCCAAGCUCUUCUUCUCGUG
GACUAGACCCAUCCUGAGAAAGGGGUACAGACAGCGCUUGGAGCUGUCC
GAUAUCUAUCAAAUCCCUUCCGUGGACUCCGCGGACAACCUGUCCGAGA
AGCUCGAGAGAGAAUGGGACAGAGAACUCGCCUCAAAGAAGAACCCGAA
GCUGAUUAAUGCGCUUAGGCGGUGCUUUUUCUGGCGGUUCAUGUUCUAC
GGCAUCUUCCUCUACCUGGGAGAGGUCACCAAGGCCGUGCAGCCCCUGU
UGCUGGGACGGAUUAUUGCCUCCUACGACCCCGACAACAAGGAAGAAAG
AAGCAUCGCUAUCUACUUGGGCAUCGGUCUGUGCCUGCUUUUCAUCGUC
CGGACCCUCUUGUUGCAUCCUGCUAUUUUCGGCCUGCAUCACAUUGGCA
UGCAGAUGAGAAUUGCCAUGUUUUCCCUGAUCUACAAGAAAACUCUGAA
GCUCUCGAGCCGCGUGCUUGACAAGAUUUCCAUCGGCCAGCUCGUGUCC
CUGCUCUCCAACAAUCUGAACAAGUUCGACGAGGGCCUCGCCCUGGCCC
ACUUCGUGUGGAUCGCCCCUCUGCAAGUGGCGCUUCUGAUGGGCCUGAU
CUGGGAGCUGCUGCAAGCCUCGGCAUUCUGUGGGCUUGGAUUCCUGAUC
GUGCUGGCACUGUUCCAGGCCGGACUGGGGCGGAUGAUGAUGAAGUACA
GGGACCAGAGAGCCGGAAAGAUUUCCGAACGGCUGGUGAUCACUUCGGA
AAUGAUCGAAAACAUCCAGUCAGUGAAGGCCUACUGCUGGGAAGAGGCC
AUGGAAAAGAUGAUUGAAAACCUCCGGCAAACCGAGCUGAAGCUGACCC
GCAAGGCCGCUUACGUGCGCUAUUUCAACUCGUCCGCUUUCUUCUUCUC
CGGGUUCUUCGUGGUGUUUCUCUCCGUGCUCCCCUACGCCCUGAUUAAG
GGAAUCAUCCUCAGGAAGAUCUUCACCACCAUUUCCUUCUGUAUCGUGC
UCCGCAUGGCCGUGACCCGGCAGUUCCCAUGGGCCGUGCAGACUUGGUA
CGACUCCCUGGGAGCCAUUAACAAGAUCCAGGACUUCCUUCAAAAGCAG
GAGUACAAGACCCUCGAGUACAACCUGACUACUACCGAGGUCGUGAUGG
AAAACGUCACCGCCUUUUGGGAGGAGGGAUUUGGCGAACUGUUCGAGAA
GGCCAAGCAGAACAACAACAACCGCAAGACCUCGAACGGUGACGACUCC
CUCUUCUUUUCAAACUUCAGCCUGCUCGGGACGCCCGUGCUGAAGGACA
UUAACUUCAAGAUCGAAAGAGGACAGCUCCUGGCGGUGGCCGGAUCGAC
CGGAGCCGGAAAGACUUCCCUGCUGAUGGUGAUCAUGGGAGAGCUUGAA
CCUAGCGAGGGAAAGAUCAAGCACUCCGGCCGCAUCAGCUUCUGUAGCC
AGUUUUCCUGGAUCAUGCCCGGAACCAUUAAGGAAAACAUCAUCUUCGG
CGUGUCCUACGAUGAAUACCGCUACCGGUCCGUGAUCAAAGCCUGCCAG
CUGGAAGAGGAUAUUUCAAAGUUCGCGGAGAAAGAUAACAUCGUGCUGG
GCGAAGGGGGUAUUACCUUGUCGGGGGGCCAGCGGGCUAGAAUCUCGCU
GGCCAGAGCCGUGUAUAAGGACGCCGACCUGUAUCUCCUGGACUCCCCC
UUCGGAUACCUGGACGUCCUGACCGAAAAGGAGAUCUUCGAAUCGUGCG
UGUGCAAGCUGAUGGCUAACAAGACUCGCAUCCUCGUGACCUCCAAAAU
GGAGCACCUGAAGAAGGCAGACAAGAUUCUGAUUCUGCAUGAGGGGUCC
UCCUACUUUUACGGCACCUUCUCGGAGUUGCAGAACUUGCAGCCCGACU
UCUCAUCGAAGCUGAUGGGUUGCGACAGCUUCGACCAGUUCUCCGCCGA

AAGAAGGAACUCGAUCCUGACGGAAACCUUGCACCGCUUCUCUUUGGAA
GGCGACGCCCCUGUGUCAUGGACCGAGACUAAGAAGCAGAGCUUCAAGC
AGACCGGGGAAUUCGGCGAAAAGAGGAAGAACAGCAUCUUGAACCCCAU
UAACUCCAUCCGCAAGUUCUCAAUCGUGCAAAAGACGCCACUGCAGAUG
AACGGCAUUGAGGAGGACUCCGACGAACCCCUUGAGAGGCGCCUGUCCC
UGGUGCCGGACAGCGAGCAGGGAGAAGCCAUCCUGCCUCGGAUUUCCGU
GAUCUCCACUGGUCCGACGCUCCAAGCCCGGCGGCGGCAGUCCGUGCUG
AACCUGAUGACCCACAGCGUGAACCAGGGCCAAAACAUUCACCGCAAGA
CUACCGCAUCCACCCGGAAAGUGUCCCUGGCACCUCAAGCGAAUCUUAC
CGAGCUCGACAUCUACUCCCGGAGACUGUCGCAGGAAACCGGGCUCGAA
AUUUCCGAAGAAAUCAACGAGGAGGAUCUGAAAGAGUGCUUCUUCGACG
AUAUGGAGUCGAUACCCGCCGUGACGACUUGGAACACUUAUCUGCGGUA
CAUCACUGUGCACAAGUCAUUGAUCUUCGUGCUGAUUUGGUGCCUGGUG
AUUUUCCUGGCCGAGGUCGCGGCCUCACUGGUGGUGCUCUGGCUGUUGG
GAAACACGCCUCUGCAAGACAAGGGAAACUCCACGCACUCGAGAAACAA
CAGCUAUGCCGUGAUUAUCACUUCCACCUCCUCUUAUUACGUGUUCUAC
AUCUACGUCGGAGUGGCGGAUACCCUGCUCGCGAUGGGUUUCUUCAGAG
GACUGCCGCUGGUCCACACCUUGAUCACCGUCAGCAAGAUUCUUCACCA
CAAGAUGUUGCAUAGCGUGCUGCAGGCCCCCAUGUCCACCCUCAACACU
CUGAAGGCCGGAGGCAUUCUGAACAGAUUCUCCAAGGACAUCGCUAUCC
UGGACGAUCUCCUGCCGCUUACCAUCUUUGACUUCAUCCAGCUGCUGCU
GAUCGUGAUUGGAGCAAUCGCAGUGGUGGCGGUGCUGCAGCCUUACAUU
UUCGUGGCCACUGUGCCGGUCAUUGUGGCGUUCAUCAUGCUGCGGGCCU
ACUUCCUCCAAACCAGCCAGCAGCUGAAGCAACUGGAAUCCGAGGGACG
AUCCCCCAUCUUCACUCACCUUGUGACGUCGUUGAAGGGACUGUGGACC
CUCCGGGCUUUCGGACGGCAGCCCUACUUCGAAACCCUCUUCCACAAGG
CCCUGAACCUCCACACCGCCAAUUGGUUCCUGUACCUGUCCACCCUGCG
GUGGUUCCAGAUGCGCAUCGAGAUGAUUUUCGUCAUCUUCUUCAUCGCG
GUCACAUUCAUCAGCAUCCUGACUACCGGAGAGGGAGAGGGACGGGUCG
GAAUAAUCCUGACCCUCGCCAUGAACAUUAUGAGCACCCUGCAGUGGGC
AGUGAACAGCUCGAUCGACGUGGACAGCCUGAUGCGAAGCGUCAGCCGC
GUGUUCAAGUUCAUCGACAUGCCUACUGAGGGAAAACCCACUAAGUCCA
CUAAGCCCUACAAAAAUGGCCAGCUGAGCAAGGUCAUGAUCAUCGAAAA
CUCCCACGUGAAGAAGGACGAUAUUUGGCCCUCCGGAGGUCAAAUGACC
GUGAAGGACCUGACCGCAAAGUACACCGAGGGAGGAAACGCCAUUCUCG
AAAACAUCAGCUUCUCCAUUUCGCCGGGACAGCGGGUCGGCCUUCUCGG
GCGGACCGGUUCCGGGAAGUCAACUCUGCUGUCGGCUUUCCUCCGGCUG
CUGAAUACCGAGGGGGAAAUCCAAAUUGACGGCGUGUCUUGGGAUUCCA
UUACUCUGCAGCAGUGGCGGAAGGCCUUCGGCGUGAUCCCCCAGAAGGU
GUUCAUCUUCUCGGGUACCUUCCGGAAGAACCUGGAUCCUUACGAGCAG
UGGAGCGACCAAGAAAUCUGGAAGGUCGCCGACGAGGUCGGCCUGCGCU
CCGUGAUUGAACAAUUUCCUGGAAAGCUGGACUUCGUGCUCGUCGACGG
GGGAUGUGUCCUGUCGCACGGACAUAAGCAGCUCAUGUGCCUCGCACGG
UCCGUGCUCUCCAAGGCCAAGAUUCUGCUGCUGGACGAACCUUCGGCCC
ACCUGGAUCCGGUCACCUACCAGAUCAUCAGGAGGACCCUGAAGCAGGC
CUUUGCCGAUUGCACCGUGAUUCUCUGCGAGCACCGCAUCGAGGCCAUG
CUGGAGUGCCAGCAGUUCCUGGUCAUCGAGGAGAACAAGGUCCGCCAAU
ACGACUCCAUUCAAAAGCUCCUCAACGAGCGGUCGCUGUUCAGACAAGC
UAUUUCACCGUCCGAUAGAGUGAAGCUCUUCCCGCAUCGGAACAGCUCA
AAGUGCAAAUCGAAGCCGCAGAUCGCAGCCUUGAAGGAAGAGACUGAGG

AAGAGGUGCAGGACACCCGGCUU**UAA**GGGUGGCAUCCCUGUGACCCCUC
CCCAGUGCCUCUCCUGGCCCUGGAAGUUGCCACUCCAGUGCCCACCAGC
CUUGUCCUAAUAAAAUUAAGUUGCAUCAAAGCU

[0208] In one embodiment, a codon-optimized human CFTR mRNA sequence includes SEQ ID NO: 2.

[0209] In one embodiment, a full-length codon-optimized human CFTR mRNA sequence is:

TABLE-US-00009 (SEQ ID NO: 10)

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAA
GACACCGGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAAC
GCGGAUUCCCCGUGCCAAGAGUGACUCACCGUCCUUGACACG**AUG**CAGC
GGUCCCCGCUCGAAAAGGCCAGUGUCGUGUCCAAACUCUUCUUCUCAUG
GACUCGGCCUAUCCUUAGAAAGGGGUAUCGGCAGAGGCUUGAGUUGUCU
GACAUCUACCAGAUCCCCUCGGUAGAUUCGGCGGAUAACCUCUCGGAGA
AGCUCGAACGGGAAUGGGACCGCGAACUCGCGUCUAAGAAAAACCCGAA
GCUCAUCAACGCACUGAGAAGGUGCUUCUUCUGGCGGUUCAUGUUCUAC
GGUAUCUUCUUGUAUCUCGGGGAGGUCACAAAAGCAGUCCAACCCCUGU
UGUUGGGUCGCAUUAUCGCCUCGUACGACCCCGAUAACAAAGAAGAACG
GAGCAUCGCGAUCUACCUCGGGAUCGGACUGUGUUUGCUUUUCAUCGUC
AGAACACUUUUGUUGCAUCCAGCAAUCUUCGGCCUCCAUCACAUCGGUA
UGCAGAUGCGAAUCGCUAUGUUUAGCUUGAUCUACAAAAAGACACUGAA
ACUCUCGUCGCGGGUGUUGGAUAAGAUUUCCAUCGGUCAGUUGGUGUCC
CUGCUUAGUAAUAACCUCAACAAAUUCGAUGAGGGACUGGCGCUGGCAC
AUUUCGUGUGGAUUGCCCCGUUGCAAGUCGCCCUUUUGAUGGGCCUUAU
UUGGGAGCUGUUGCAGGCAUCUGCCUUUUGUGGCCUGGGAUUUCUGAUU
GUGUUGGCAUUGUUUCAGGCUGGGCUUGGGCGGAUGAUGAUGAAGUAUC
GCGACCAGAGAGCGGGUAAAAUCUCGGAAAGACUCGUCAUCACUUCGGA
AAUGAUCGAAAACAUCCAGUCGGUCAAAGCCUAUUGCUGGGAAGAAGCU
AUGGAGAAGAUGAUUGAAAACCUCCGCCAAACUGAGCUGAAACUGACCC
GCAAGGCGGCGUAUGUCCGGUAUUUCAAUUCGUCAGCGUUCUUCUUUUC
CGGGUUCUUCGUUGUCUUUCUCUCGGUUUUGCCUUAUGCCUUGAUUAAG
GGGAUUAUCCUCCGCAAGAUUUUCACCACGAUUUCGUUCUGCAUUGUAU
UGCGCAUGGCAGUGACACGGCAAUUUCCGUGGGCCGUGCAGACAUGGUA
UGACUCGCUUGGAGCGAUCAACAAAAUCCAAGACUUCUUGCAAAAGCAA
GAGUACAAGACCCUGGAGUACAAUCUUACUACUACGGAGGUAGUAAUGG
AGAAUGUGACGGCUUUUUGGGAAGAGGGUUUUGGAGAACUGUUUGAGAA
AGCAAAGCAGAAUAACAACAACCGCAAGACCUCAAAUGGGGACGAUUCC
CUGUUUUUCUCGAACUUCUCCCUGCUCGGAACACCCGUGUUGAAGGACA
UCAAUUUCAAGAUUGAGAGGGGACAGCUUCUCGCGGUAGCGGGAAGCAC
UGGUGCGGGAAAAACUAGCCUCUUGAUGGUGAUUAUGGGGGAGCUUGAG
CCCAGCGAGGGGAAGAUUAAACACUCCGGGCGUAUCUCAUUCUGUAGCC
AGUUUUCAUGGAUCAUGCCCGGAACCAUUAAAGAGAACAUCAUUUUCGG
AGUAUCCUAUGAUGAGUACCGAUACAGAUCGGUCAUUAAGGCGUGCCAG
UUGGAAGAGGACAUUUCUAAGUUCGCCGAGAAGGAUAACAUCGUCUUGG
GAGAAGGGGGUAUUACAUUGUCGGGAGGGCAGCGAGCGCGGAUCAGCCU
CGCGAGAGCGGUAUACAAAGAUGCAGAUUUGUAUCUGCUUGAUUCACCG
UUUGGAUACCUCGACGUAUUGACAGAAAAAGAAAUCUUCGAGUCGUGCG
UGUGUAAACUUAUGGCUAAUAAGACGAGAAUCCUGGUGACAUCAAAAAU
GGAACACCUUAAGAAGGCGGACAAGAUCCUGAUCCUCCACGAAGGAUCG
UCCUACUUUUACGGCACUUUCUCAGAGUUGCAAAACUUGCAGCCGGACU
UCUCAAGCAAACUCAUGGGGUGUGACUCAUUCGACCAGUUCAGCGCGGA

ACGGCGGAACUCGAUCUUGACGGAAACGCUGCACCGAUUCUCGCUUGAG
GGUGAUGCCCCGGUAUCGUGGACCGAGACAAAGAAGCAGUCGUUUAAGC
AGACAGGAGAAUUUGGUGAGAAAAGAAAGAACAGUAUCUUGAAUCCUAU
UAACUCAAUUCGCAAGUUCUCAAUCGUCCAGAAAACUCCACUGCAGAUG
AAUGGAAUUGAAGAGGAUUCGGACGAACCCCUGGAGCGCAGGCUUAGCC
UCGUGCCGGAUUCAGAGCAAGGGGAGGCCAUUCUUCCCCGGAUUUCGGU
GAUUUCAACCGGACCUACACUUCAGGCGAGGCGAAGGCAAUCCGUGCUC
AACCUCAUGACGCAUUCGGUAAACCAGGGGCAAAACAUUCACCGCAAAA
CGACGGCCUCAACGAGAAAAGUGUCACUUGCACCCCAGGCGAAUUUGAC
UGAACUCGACAUCUACAGCCGUAGGCUUUCGCAAGAAACCGGACUUGAG
AUCAGCGAAGAAAUCAAUGAAGAAGAUUUGAAAGAGUGUUUCUUUGAUG
ACAUGGAAUCAAUCCCAGCGGUGACAACGUGGAACACAUACUUGCGUUA
CAUCACGGUGCACAAGUCCUUGAUUUUCGUCCUCAUCUGGUGUCUCGUG
AUCUUUCUCGCUGAGGUCGCAGCGUCACUUGUGGUCCUCUGGCUGCUUG
GUAAUACGCCCUUGCAAGACAAAGGCAAUUCUACACACUCAAGAAACAA
UUCCUAUGCCGUGAUUAUCACUUCUACAAGCUCGUAUUACGUGUUUUAC
AUCUACGUAGGAGUGGCCGACACUCUGCUCGCGAUGGGUUUCUUCCGAG
GACUCCCACUCGUUCACACGCUUAUCACUGUCUCCAAGAUUCUCCACCA
UAAGAUGCUUCAUAGCGUACUGCAGGCUCCCAUGUCCACCUUGAAUACG
CUCAAGGCGGGAGGUAUUUUGAAUCGCUUCUCAAAAGAUAUUGCAAUUU
UGGAUGACCUUCUGCCCCUGACGAUCUUCGACUUCAUCCAGUUGUUGCU
GAUCGUGAUUGGGGCUAUUGCAGUAGUCGCUGUCCUCCAGCCUUACAUU
UUUGUCGCGACCGUUCCGGUGAUCGUGGCGUUUAUCAUGCUGCGGGCCU
AUUUCUUGCAGACGUCACAGCAGCUUAAGCAACUGGAGUCUGAAGGGAG
GUCGCCUAUCUUUACGCAUCUUGUGACCAGUUUGAAGGGAUUGUGGACG
UUGCGCGCCUUUGGCAGGCAGCCCUACUUUGAAACACUGUUCCACAAAG
CGCUGAAUCUCCAUACGGCAAAUUGGUUUUUGUAUUUGAGUACCCUCCG
AUGGUUUCAGAUGCGCAUUGAGAUGAUUUUUGUGAUCUUCUUUAUCGCG
GUGACUUUUAUCUCCAUCUUGACCACGGGAGAGGGCGAGGGACGGGUCG
GUAUUAUCCUGACACUCGCCAUGAACAUUAUGAGCACUUUGCAGUGGGC
AGUGAACAGCUCGAUUGAUGUGGAUAGCCUGAUGAGGUCCGUUUCGAGG
GUCUUUAAGUUCAUCGACAUGCCGACGGAGGGAAAGCCCACAAAAAGUA
CGAAACCCUAUAAGAAUGGGCAAUUGAGUAAGGUAAUGAUCAUCGAGAA
CAGUCACGUGAAGAAGGAUGACAUCUGGCCUAGCGGGGGUCAGAUGACC
GUGAAGGACCUGACGGCAAAAUACACCGAGGGAGGGAACGCAAUCCUUG
AAAACAUCUCGUUCAGCAUUAGCCCCGGUCAGCGUGUGGGGUUGCUCGG
GAGGACCGGGUCAGGAAAAUCGACGUUGCUGUCGGCCUUCUUGAGACUU
CUGAAUACAGAGGGUGAGAUCCAGAUCGACGGCGUUUCGUGGGAUAGCA
UCACCUUGCAGCAGUGGCGGAAAGCGUUUGGAGUAAUCCCCCAAAAGGU
CUUUAUCUUUAGCGGAACCUUCCGAAAGAAUCUCGAUCCUUAUGAACAG
UGGUCAGAUCAAGAGAUUUGGAAAGUCGCGGACGAGGUUGGCCUUCGGA
GUGUAAUCGAGCAGUUUCCGGGAAAACUCGACUUUGUCCUUGUAGAUGG
GGGAUGCGUCCUGUCGCAUGGGCACAAGCAGCUCAUGUGCCUGGCGCGA
UCCGUCCUCUCUAAAGCGAAAAUUCUUCUCUUGGAUGAACCUUCGGCCC
AUCUGGACCCGGUAACGUAUCAGAUCAUCAGAAGGACACUUAAGCAGGC
GUUUGCCGACUGCACGGUGAUUCUCUGUGAGCAUCGUAUCGAGGCCAUG
CUCGAAUGCCAGCAAUUUCUUGUCAUCGAAGAGAAUAAGGUCCGCCAGU
ACGACUCCAUCCAGAAGCUGCUUAAUGAGAGAUCAUUGUUCCGGCAGGC
GAUUUCACCAUCCGAUAGGGUGAAACUUUUUCCACACAGAAAUUCGUCG
AAGUGCAAGUCCAAACCGCAGAUCGCGGCCUUGAAAGAAGAGACUGAAG

AAGAAGUUCAAGACACGCGUCUU**UAA**CGGGUGGCAUCCCUGUGACCCCU
CCCCAGUGCCUCUCCUGGCCCUGGAAGUUGCCACUCCAGUGCCCACCAG
CCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU

[0210] In one embodiment, another full-length codon-optimized human CFTR mRNA sequence is:

TABLE-US-00010 (SEQ ID NO: 11)

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAA
GACACCGGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAAC
GCGGAUUCCCCGUGCCAAGAGUGACUCACCGUCCUUGACACG**AUG**CAGC
GGUCCCCGCUCGAAAAGGCCAGUGUCGUGUCCAAACUCUUCUUCUCAUG
GACUCGGCCUAUCCUUAGAAAGGGGUAUCGGCAGAGGCUUGAGUUGUCU
GACAUCUACCAGAUCCCCUCGGUAGAUUCGGCGGAUAACCUCUCGGAGA
AGCUCGAACGGGAAUGGGACCGCGAACUCGCGUCUAAGAAAAACCCGAA
GCUCAUCAACGCACUGAGAAGGUGCUUCUUCUGGCGGUUCAUGUUCUAC
GGUAUCUUCUUGUAUCUCGGGGAGGUCACAAAAGCAGUCCAACCCCUGU
UGUUGGGUCGCAUUAUCGCCUCGUACGACCCCGAUAACAAAGAAGAACG
GAGCAUCGCGAUCUACCUCGGGAUCGGACUGUGUUUGCUUUUCAUCGUC
AGAACACUUUUGUUGCAUCCAGCAAUCUUCGGCCUCCAUCACAUCGGUA
UGCAGAUGCGAAUCGCUAUGUUUAGCUUGAUCUACAAAAAGACACUGAA
ACUCUCGUCGCGGGUGUUGGAUAAGAUUUCCAUCGGUCAGUUGGUGUCC
CUGCUUAGUAAUAACCUCAACAAAUUCGAUGAGGGACUGGCGCUGGCAC
AUUUCGUGUGGAUUGCCCCGUUGCAAGUCGCCCUUUUGAUGGGCCUUAU
UUGGGAGCUGUUGCAGGCAUCUGCCUUUUGUGGCCUGGGAUUUCUGAUU
GUGUUGGCAUUGUUUCAGGCUGGGCUUGGGCGGAUGAUGAUGAAGUAUC
GCGACCAGAGAGCGGGUAAAAUCUCGGAAAGACUCGUCAUCACUUCGGA
AAUGAUCGAAAACAUCCAGUCGGUCAAAGCCUAUUGCUGGGAAGAAGCU
AUGGAGAAGAUGAUUGAAAACCUCCGCCAAACUGAGCUGAAACUGACCC
GCAAGGCGGCGUAUGUCCGGUAUUUCAAUUCGUCAGCGUUCUUCUUUUC
CGGGUUCUUCGUUGUCUUUCUCUCGGUUUUGCCUUAUGCCUUGAUUAAG
GGGAUUAUCCUCCGCAAGAUUUUCACCACGAUUUCGUUCUGCAUUGUAU
UGCGCAUGGCAGUGACACGGCAAUUUCCGUGGGCCGUGCAGACAUGGUA
UGACUCGCUUGGAGCGAUCAACAAAAUCCAAGACUUCUUGCAAAAGCAA
GAGUACAAGACCCUGGAGUACAAUCUUACUACUACGGAGGUAGUAAUGG
AGAAUGUGACGGCUUUUUGGGAAGAGGGUUUUGGAGAACUGUUUGAGAA
AGCAAAGCAGAAUAACAACAACCGCAAGACCUCAAAUGGGGACGAUUCC
CUGUUUUUCUCGAACUUCUCCCUGCUCGGAACACCCGUGUUGAAGGACA
UCAAUUUCAAGAUUGAGAGGGGACAGCUUCUCGCGGUAGCGGGAAGCAC
UGGUGCGGGAAAAACUAGCCUCUUGAUGGUGAUUAUGGGGGAGCUUGAG
CCCAGCGAGGGGAAGAUUAAACACUCCGGGCGUAUCUCAUUCUGUAGCC
AGUUUUCAUGGAUCAUGCCCGGAACCAUUAAAGAGAACAUCAUUUUCGG
AGUAUCCUAUGAUGAGUACCGAUACAGAUCGGUCAUUAAGGCGUGCCAG
UUGGAAGAGGACAUUUCUAAGUUCGCCGAGAAGGAUAACAUCGUCUUGG
GAGAAGGGGGUAUUACAUUGUCGGGAGGGCAGCGAGCGCGGAUCAGCCU
CGCGAGAGCGGUAUACAAAGAUGCAGAUUUGUAUCUGCUUGAUUCACCG
UUUGGAUACCUCGACGUAUUGACAGAAAAAGAAAUCUUCGAGUCGUGCG
UGUGUAAACUUAUGGCUAAUAAGACGAGAAUCCUGGUGACAUCAAAAAU
GGAACACCUUAAGAAGGCGGACAAGAUCCUGAUCCUCCACGAAGGAUCG
UCCUACUUUUACGGCACUUUCUCAGAGUUGCAAAACUUGCAGCCGGACU
UCUCAAGCAAACUCAUGGGGUGUGACUCAUUCGACCAGUUCAGCGCGGA
ACGGCGGAACUCGAUCUUGACGGAAACGCUGCACCGAUUCUCGCUUGAG
GGUGAUGCCCCGGUAUCGUGGACCGAGACAAAGAAGCAGUCGUUUAAGC

AGACAGGAGAAUUUGGUGAGAAAAGAAAGAACAGUAUCUUGAAUCCUAU
UAACUCAAUUCGCAAGUUCUCAAUCGUCCAGAAAACUCCACUGCAGAUG
AAUGGAAUUGAAGAGGAUUCGGACGAACCCCUGGAGCGCAGGCUUAGCC
UCGUGCCGGAUUCAGAGCAAGGGGAGGCCAUUCUUCCCCGGAUUUCGGU
GAUUUCAACCGGACCUACACUUCAGGCGAGGCGAAGGCAAUCCGUGCUC
AACCUCAUGACGCAUUCGGUAAACCAGGGGCAAAACAUUCACCGCAAAA
CGACGGCCUCAACGAGAAAAGUGUCACUUGCACCCCAGGCGAAUUUGAC
UGAACUCGACAUCUACAGCCGUAGGCUUUCGCAAGAAACCGGACUUGAG
AUCAGCGAAGAAAUCAAUGAAGAAGAUUUGAAAGAGUGUUUCUUUGAUG
ACAUGGAAUCAAUCCCAGCGGUGACAACGUGGAACACAUACUUGCGUUA
CAUCACGGUGCACAAGUCCUUGAUUUUCGUCCUCAUCUGGUGUCUCGUG
AUCUUUCUCGCUGAGGUCGCAGCGUCACUUGUGGUCCUCUGGCUGCUUG
GUAAUACGCCCUUGCAAGACAAAGGCAAUUCUACACACUCAAGAAACAA
UUCCUAUGCCGUGAUUAUCACUUCUACAAGCUCGUAUUACGUGUUUUAC
AUCUACGUAGGAGUGGCCGACACUCUGCUCGCGAUGGGUUUCUUCCGAG
GACUCCCACUCGUUCACACGCUUAUCACUGUCUCCAAGAUUCUCCACCA
UAAGAUGCUUCAUAGCGUACUGCAGGCUCCCAUGUCCACCUUGAAUACG
CUCAAGGCGGGAGGUAUUUUGAAUCGCUUCUCAAAAGAUAUUGCAAUUU
UGGAUGACCUUCUGCCCCUGACGAUCUUCGACUUCAUCCAGUUGUUGCU
GAUCGUGAUUGGGGCUAUUGCAGUAGUCGCUGUCCUCCAGCCUUACAUU
UUUGUCGCGACCGUUCCGGUGAUCGUGGCGUUUAUCAUGCUGCGGGCCU
AUUUCUUGCAGACGUCACAGCAGCUUAAGCAACUGGAGUCUGAAGGGAG
GUCGCCUAUCUUUACGCAUCUUGUGACCAGUUUGAAGGGAUUGUGGACG
UUGCGCGCCUUUGGCAGGCAGCCCUACUUUGAAACACUGUUCCACAAAG
CGCUGAAUCUCCAUACGGCAAAUUGGUUUUUGUAUUUGAGUACCCUCCG
AUGGUUUCAGAUGCGCAUUGAGAUGAUUUUUGUGAUCUUCUUUAUCGCG
GUGACUUUUAUCUCCAUCUUGACCACGGGAGAGGGCGAGGGACGGGUCG
GUAUUAUCCUGACACUCGCCAUGAACAUUAUGAGCACUUUGCAGUGGGC
AGUGAACAGCUCGAUUGAUGUGGAUAGCCUGAUGAGGUCCGUUUCGAGG
GUCUUUAAGUUCAUCGACAUGCCGACGGAGGGAAAGCCCACAAAAAGUA
CGAAACCCUAUAAGAAUGGGCAAUUGAGUAAGGUAAUGAUCAUCGAGAA
CAGUCACGUGAAGAAGGAUGACAUCUGGCCUAGCGGGGGUCAGAUGACC
GUGAAGGACCUGACGGCAAAAUACACCGAGGGAGGGAACGCAAUCCUUG
AAAACAUCUCGUUCAGCAUUAGCCCCGGUCAGCGUGUGGGGUUGCUCGG
GAGGACCGGGUCAGGAAAAUCGACGUUGCUGUCGGCCUUCUUGAGACUU
CUGAAUACAGAGGGUGAGAUCCAGAUCGACGGCGUUUCGUGGGAUAGCA
UCACCUUGCAGCAGUGGCGGAAAGCGUUUGGAGUAAUCCCCCAAAAGGU
CUUUAUCUUUAGCGGAACCUUCCGAAAGAAUCUCGAUCCUUAUGAACAG
UGGUCAGAUCAAGAGAUUUGGAAAGUCGCGGACGAGGUUGGCCUUCGGA
GUGUAAUCGAGCAGUUUCCGGGAAAACUCGACUUUGUCCUUGUAGAUGG
GGGAUGCGUCCUGUCGCAUGGGCACAAGCAGCUCAUGUGCCUGGCGCGA
UCCGUCCUCUCUAAAGCGAAAAUUCUUCUCUUGGAUGAACCUUCGGCCC
AUCUGGACCCGGUAACGUAUCAGAUCAUCAGAAGGACACUUAAGCAGGC
GUUUGCCGACUGCACGGUGAUUCUCUGUGAGCAUCGUAUCGAGGCCAUG
CUCGAAUGCCAGCAAUUUCUUGUCAUCGAAGAGAAUAAGGUCCGCCAGU
ACGACUCCAUCCAGAAGCUGCUUAAUGAGAGAUCAUUGUUCCGGCAGGC
GAUUUCACCAUCCGAUAGGGUGAAACUUUUUCCACACAGAAAUUCGUCG
AAGUGCAAGUCCAAACCGCAGAUCGCGGCCUUGAAAGAAGAGACUGAAG
AAGAAGUUCAAGACACGCGUCUU**UAA**GGGUGGCAUCCCUGUGACCCCUC
CCCAGUGCCUCUCCUGGCCCUGGAAGUUGCCACUCCAGUGCCCACCAGC

CUUGUCCUAAUAAAAUUAAGUUGCAUCAAAGCU
[0211] SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11 include 5′ and 3′ untranslated regions framing a codon-optimized hCFTR-encoding mRNA.
[0212] In some embodiments, a suitable mRNA sequence may be an mRNA sequence a homolog or an analog of human CFTR (hCFTR) protein. For example, a homolog or an analog of hCFTR protein may be a modified hCFTR protein containing one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring hCFTR protein while retaining substantial hCFTR protein activity. In some embodiments, an mRNA suitable for the present invention encodes an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO: 4. In some embodiments, an mRNA suitable for the present invention encodes a protein substantially identical to hCFTR protein. In some embodiments, an mRNA suitable for the present invention encodes an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO: 4. In some embodiments, an mRNA suitable for the present invention encodes a fragment or a portion of hCFTR protein. In some embodiments, an mRNA suitable for the present invention encodes a fragment or a portion of hCFTR protein, wherein the fragment or portion of the protein still maintains CFTR activity similar to that of the wild-type protein. In some embodiments, an mRNA suitable for the present invention has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical SEQ ID NO: 1, SEQ ID NO: 8 or SEQ ID NO: 9. In some embodiments, an mRNA suitable for the present invention has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical SEQ ID NO: 2, SEQ ID NO: 10 or SEQ ID NO: 11. In some embodiments, an mRNA suitable for the present invention comprises a nucleotide sequence identical to SEQ ID NO: 1. In some embodiments, an mRNA suitable for the present invention comprises a nucleotide sequence identical to SEQ ID NO: 8. In some embodiments, an mRNA suitable for the present invention comprises a nucleotide sequence identical to SEQ ID NO: 9. In some embodiments, a suitable mRNA encodes a fusion protein comprising a full length, fragment or portion of an hCFTR protein fused to another protein (e.g., an N or C terminal fusion). In some embodiments, the protein fused to the mRNA encoding a full length, fragment or portion of an hCFTR protein encodes a signal or a cellular targeting sequence.
[0213] mRNAs according to the present invention may be synthesized according to any of a variety of known methods. For example, mRNAs according to the present invention may be synthesized via in vitro transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7 or SP6 RNA polymerase), DNAse I, pyrophosphatase, and/or RNAse inhibitor. The exact conditions will vary according to the specific application.
Non-Coding Regions
[0214] In some embodiments, mRNAs include a 5′ and/or 3′ untranslated region. In some embodiments, a 5′ untranslated region includes one or more elements that affect an mRNA's stability or translation, for example, an iron responsive element. In some embodiments, a 5′ untranslated region may be between about 50 and 500 nucleotides in length.
[0215] In some embodiments, a 3′ untranslated region includes one or more of a polyadenylation signal, a binding site for proteins that affect an mRNA's stability of location in a cell, or one or more binding sites for miRNAs. In some embodiments, a 3′ untranslated region may be between 50 and 500 nucleotides in length or longer.
[0216] Exemplary 3′ and/or 5′ UTR sequences can be derived from mRNA molecules which are stable (e.g., globin, actin, GAPDH, tubulin, histone, or citric acid cycle enzymes) to increase the stability of the sense mRNA molecule. For example, a 5′ UTR sequence may include a partial

sequence of a CMV immediate-early 1 (IE1) gene, or a fragment thereof to improve the nuclease resistance and/or improve the half-life of the polynucleotide. Also contemplated is the inclusion of a sequence encoding human growth hormone (hGH), or a fragment thereof to the 3′ end or untranslated region of the polynucleotide (e.g., mRNA) to further stabilize the polynucleotide. Generally, these modifications improve the stability and/or pharmacokinetic properties (e.g., half-life) of the polynucleotide relative to their unmodified counterparts, and include, for example modifications made to improve such polynucleotides' resistance to in vivo nuclease digestion.
[0217] According to various embodiments, any size mRNA may be encapsulated by provided liposomes. In some embodiments, the provided liposomes may encapsulate mRNA of greater than about 0.5 kb, 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, or 5 kb in length.
[0218] Typically, mRNA synthesis includes the addition of a “cap” on the N-terminal (5′) end, and a “tail” on the C-terminal (3′) end. The presence of the cap is important in providing resistance to nucleases found in most eukaryotic cells. The presence of a “tail” serves to protect the mRNA from exonuclease degradation.
[0219] Thus, in some embodiments, mRNAs (e.g., mRNAs encoding CFTR) include a 5′ cap structure. A 5′ cap is typically added as follows: first, an RNA terminal phosphatase removes one of the terminal phosphate groups from the 5′ nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP) is then added to the terminal phosphates via a guanylyl transferase, producing a 5′5′5 triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. Examples of cap structures include, but are not limited to, m7G(5′)ppp(5′(A,G(5′)ppp(5′)A and G(5′)ppp(5′)G. Additional cap structures are described in published US Application No. US 2016/0032356 and U.S. Provisional Application 62/464,327, filed Feb. 27, 2017, which are incorporated herein by reference.
[0220] In some embodiments, the cap is a Cap0 structure. Cap0 structures lack a 2′-O-methyl residue of the ribose attached to bases 1 and 2. In some embodiments, the cap is a Cap1 structure. Cap1 structures have a 2′-O-methyl residue at base 2. In some embodiments, the cap is a Cap2 structure. Cap2 structures have a 2′-O-methyl residue attached to both bases 2 and 3.
[0221] In some embodiments, mRNAs (e.g., mRNAs encoding CFTR) include a 3′ tail structure. Typically, a tail structure includes a poly(A) and/or poly(C) tail. A poly-A or poly-C tail on the 3′ terminus of mRNA typically includes at least 50 adenosine or cytosine nucleotides, at least 150 adenosine or cytosine nucleotides, at least 200 adenosine or cytosine nucleotides, at least 250 adenosine or cytosine nucleotides, at least 300 adenosine or cytosine nucleotides, at least 350 adenosine or cytosine nucleotides, at least 400 adenosine or cytosine nucleotides, at least 450 adenosine or cytosine nucleotides, at least 500 adenosine or cytosine nucleotides, at least 550 adenosine or cytosine nucleotides, at least 600 adenosine or cytosine nucleotides, at least 650 adenosine or cytosine nucleotides, at least 700 adenosine or cytosine nucleotides, at least 750 adenosine or cytosine nucleotides, at least 800 adenosine or cytosine nucleotides, at least 850 adenosine or cytosine nucleotides, at least 900 adenosine or cytosine nucleotides, at least 950 adenosine or cytosine nucleotides, or at least 1 kb adenosine or cytosine nucleotides, respectively. In some embodiments, a poly-A or poly-C tail may be about 10 to 800 adenosine or cytosine nucleotides (e.g., about 10 to 200 adenosine or cytosine nucleotides, about 10 to 300 adenosine or cytosine nucleotides, about 10 to 400 adenosine or cytosine nucleotides, about 10 to 500 adenosine or cytosine nucleotides, about 10 to 550 adenosine or cytosine nucleotides, about 10 to 600 adenosine or cytosine nucleotides, about 50 to 600 adenosine or cytosine nucleotides, about 100 to 600 adenosine or cytosine nucleotides, about 150 to 600 adenosine or cytosine nucleotides, about 200 to 600 adenosine or cytosine nucleotides, about 250 to 600 adenosine or cytosine nucleotides, about 300 to 600 adenosine or cytosine nucleotides, about 350 to 600 adenosine or cytosine nucleotides, about 400 to 600 adenosine or cytosine nucleotides, about 450 to 600 adenosine or cytosine nucleotides, about 500 to 600 adenosine or cytosine nucleotides, about 10 to 150 adenosine or cytosine nucleotides, about 10 to 100 adenosine or cytosine nucleotides, about 20 to

70 adenosine or cytosine nucleotides, or about 20 to 60 adenosine or cytosine nucleotides) respectively. In some embodiments, a tail structure includes is a combination of poly(A) and poly(C) tails with various lengths described herein. In some embodiments, a tail structure includes at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% adenosine nucleotides. In some embodiments, a tail structure includes at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% cytosine nucleotides.

Modified mRNA

[0222] In some embodiments, mRNA according to the present invention may be synthesized as unmodified or modified mRNA. Typically, mRNAs are modified to enhance stability. Modifications of mRNA can include, for example, modifications of the nucleotides of the RNA. A modified mRNA according to the invention can thus include, for example, backbone modifications, sugar modifications or base modifications. In some embodiments, mRNAs may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as e.g. 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5′-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queosine, .beta.-D-mannosyl-queosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to a person skilled in the art e.g., from the U.S. Pat. Nos. 4,373,071, 4,401,796, 4,415,732, 4,458,066, 4,500,707, 4,668,777, 4,973,679, 5,047,524, 5,132,418, 5,153,319, 5,262,530 and 5,700,642, the disclosures of which are incorporated by reference in their entirety.

[0223] In some embodiments, mRNAs (e.g., mRNAs encoding CFTR) may contain RNA backbone modifications. Typically, a backbone modification is a modification in which the phosphates of the backbone of the nucleotides contained in the RNA are modified chemically. Exemplary backbone modifications typically include, but are not limited to, modifications from the group consisting of methylphosphonates, methylphosphoramidates, phosphoramidates, phosphorothioates (e.g., cytidine 5′-O-(1-thiophosphate)), boranophosphates, positively charged guanidinium groups etc., which means by replacing the phosphodiester linkage by other anionic, cationic or neutral groups.

[0224] In some embodiments, mRNAs (e.g., mRNAs encoding CFTR) may contain sugar modifications. A typical sugar modification is a chemical modification of the sugar of the nucleotides it contains including, but not limited to, sugar modifications chosen from the group consisting of 2′-deoxy-2′-fluoro-oligoribonucleotide (2′-fluoro-2′-deoxycytidine 5′-triphosphate, 2′-fluoro-2′-deoxyuridine 5′-triphosphate), 2′-deoxy-2′-deamine-oligoribonucleotide (2′-amino-2′-deoxycytidine 5′-triphosphate, 2′-amino-2′-deoxyuridine 5′-triphosphate), 2′-O-alkyloligoribonucleotide, 2′-deoxy-2′-C-alkyloligoribonucleotide (2′-O-methylcytidine 5′-triphosphate, 2′-methyluridine 5′-triphosphate), 2′-C-alkyloligoribonucleotide, and isomers thereof (2′-aracytidine 5′-triphosphate, 2′-arauridine 5′-triphosphate), azidotriphosphates (2′-azido-2′-deoxycytidine 5′-triphosphate, 2′-azido-2′-deoxyuridine 5′-triphosphate) or 4′-thio-substituted ribonucleotides.

[0225] In some embodiments, mRNAs (e.g., mRNAs encoding CFTR) may contain modifications of the bases of the nucleotides (base modifications). A modified nucleotide which contains a base modification is also called a base-modified nucleotide. Exemples of such base-modified

nucleotides include, but are not limited to, 2-amino-6-chloropurine riboside 5′-triphosphate, 2-aminoadenosine 5′-triphosphate, 2-thiocytidine 5′-triphosphate, 2-thiouridine 5′-triphosphate, 4-thiouridine 5′-triphosphate, 5-aminoallylcytidine 5′-triphosphate, 5-aminoallyluridine 5′-triphosphate, 5-bromocytidine 5′-triphosphate, 5-bromouridine 5′-triphosphate, 5-iodocytidine 5′-triphosphate, 5-iodouridine 5′-triphosphate, 5-methylcytidine 5′-triphosphate, 5-methyluridine 5′-triphosphate, 6-azacytidine 5′-triphosphate, 6-azauridine 5′-triphosphate, 6-chloropurine riboside 5′-triphosphate, 7-deazaadenosine 5′-triphosphate, 7-deazaguanosine 5′-triphosphate, 8-azaadenosine 5′-triphosphate, 8-azidoadenosine 5′-triphosphate, benzimidazole riboside 5′-triphosphate, N1-methyladenosine 5′-triphosphate, N1-methylguanosine 5′-triphosphate, N6-methyladenosine 5′-triphosphate, 06-methylguanosine 5′-triphosphate, pseudouridine 5′-triphosphate, puromycin 5′-triphosphate or xanthosine 5′-triphosphate.

Pharmaceutical Compositions

[0226] To facilitate expression of mRNA in vivo, delivery vehicles such as liposomes can be formulated in combination with one or more additional nucleic acids, carriers, targeting ligands or stabilizing reagents, or in pharmacological compositions where it is mixed with suitable excipients. Techniques for formulation and administration of drugs may be found in “Remington's Pharmaceutical Sciences,” Mack Publishing Co., Easton, Pa., latest edition.

[0227] Provided liposomally-encapsulated or associated mRNAs, and compositions containing the same, may be administered and dosed in accordance with current medical practice, taking into account the clinical condition of the subject, the site and method of administration, the scheduling of administration, the subject's age, sex, body weight and other factors relevant to clinicians of ordinary skill in the art. The “effective amount” for the purposes herein may be determined by such relevant considerations as are known to those of ordinary skill in experimental clinical research, pharmacological, clinical and medical arts. In some embodiments, the amount administered is effective to achieve at least some stabilization, improvement or elimination of symptoms and other indicators as are selected as appropriate measures of disease progress, regression or improvement by those of skill in the art. For example, a suitable amount and dosing regimen is one that causes at least transient protein (e.g., enzyme) production.

[0228] Suitable routes of administration include, for example, oral, rectal, vaginal, transmucosal, pulmonary including intratracheal or inhaled, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, and/or intranasal administration.

[0229] Alternately or additionally, liposomally encapsulated mRNAs and compositions of the invention may be administered in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a targeted tissue, preferably in a sustained release formulation. Local delivery can be affected in various ways, depending on the tissue to be targeted. For example, aerosols containing compositions of the present invention can be inhaled (for nasal, tracheal, or bronchial delivery); compositions of the present invention can be injected into the site of injury, disease manifestation, or pain, for example; compositions can be provided in lozenges for oral, tracheal, or esophageal application; can be supplied in liquid, tablet or capsule form for administration to the stomach or intestines, can be supplied in suppository form for rectal or vaginal application; or can even be delivered to the eye by use of creams, drops, or even injection. Formulations containing provided compositions complexed with therapeutic molecules or ligands can even be surgically administered, for example in association with a polymer or other structure or substance that can allow the compositions to diffuse from the site of implantation to surrounding cells. Alternatively, they can be applied surgically without the use of polymers or supports.

[0230] In some embodiments, provided liposomes and/or compositions are formulated such that they are suitable for extended-release of the mRNA contained therein. Such extended-release compositions may be conveniently administered to a subject at extended dosing intervals. For

example, in one embodiment, the compositions of the present invention are administered to a subject twice day, daily or every other day. In a preferred embodiment, the compositions of the present invention are administered to a subject twice a week, once a week, every ten days, every two weeks, every three weeks, or more preferably every four weeks, once a month, every six weeks, every eight weeks, every other month, every three months, every four months, every six months, every eight months, every nine months or annually. Also contemplated are compositions and liposomes which are formulated for depot administration (e.g., intramuscularly, subcutaneously, intravitreally) to either deliver or release mRNA over extended periods of time. Preferably, the extended-release means employed are combined with modifications made to the mRNA to enhance stability.

[0231] Also contemplated herein are lyophilized pharmaceutical compositions comprising one or more of the liposomes disclosed herein and related methods for the use of such compositions as disclosed for example, in U.S. Provisional Application No. 61/494,882, filed Jun. 8, 2011, the teachings of which are incorporated herein by reference in their entirety. For example, lyophilized pharmaceutical compositions according to the invention may be reconstituted prior to administration or can be reconstituted in vivo. For example, a lyophilized pharmaceutical composition can be formulated in an appropriate dosage form (e.g., an intradermal dosage form such as a disk, rod or membrane) and administered such that the dosage form is rehydrated over time in vivo by the individual's bodily fluids.

[0232] Provided liposomes and compositions may be administered to any desired tissue. In some embodiments, the mRNA delivered by provided liposomes or compositions is expressed in the tissue in which the liposomes and/or compositions were administered. In some embodiments, the mRNA delivered is expressed in a tissue different from the tissue in which the liposomes and/or compositions were administered. Exemplary tissues in which delivered mRNA may be delivered and/or expressed include, but are not limited to the lungs, liver, kidney, heart, spleen, serum, brain, skeletal muscle, lymph nodes, skin, and/or cerebrospinal fluid.

[0233] In some embodiments, a target tissue is lung. In some embodiments, a target tissue is the upper (i.e., superior) lobe of the right or left lung. In some embodiments, a target tissue is the lower (i.e., inferior) lobe of the right or left lung. In some embodiments, a target tissue is the middle lobe of the right lung.

[0234] In some embodiments, a target tissue is the apical segment of the right lung or the apicoposterior segment of the left lung. In some embodiments, a target tissue is the posterior segment of the right lung. In some embodiments, a target tissue is the anterior segment of the right or left lung. In some embodiments, a target tissue is the superior segment of the right or left lung. In some embodiments, a target tissue is the lateral basal segment of the right or left lung. In some embodiments, a target tissue is the anterior basal segment of the right lung. In some embodiments, a target tissue is the anteromedial basal segment of the left lung. In some embodiments, a target tissue is the lateral segment of the right lung. In some embodiments, a target tissue is the medial segment of the right lung. In some embodiments, a target tissue is the superior lingular segment of the left lung. In some embodiments, a target tissue is the inferior lingular segment of the left lung. In some embodiments, a target tissue is the posterior basal segment of the right or left lung. In some embodiments, a target tissue is the medial basal segment of the right lung.

[0235] In particular embodiments, a target tissue is epithelial cells in the lung. In some embodiments, a target tissue is smooth muscle cells in the lung. In some embodiment, a target tissue is pancreatic duct epithelial cells. In some embodiment, a target tissue is bile-duct epithelial cells. In some embodiment, a target tissue is epithelial cells of the salivary glands. In some embodiment, a target tissue is renal epithelial cells. In some embodiment, a target tissue is beta-S cells in sweat gland secretory coils of sweat glands. In some embodiment, a target tissue is epithelial cells of the reproductive tract.

[0236] According to various embodiments, the timing of expression of delivered mRNAs can be

tuned to suit a particular medical need. In some embodiments, the expression of the protein encoded by delivered mRNA is detectable 1, 2, 3, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and/or 72 hours in serum or target tissues after a single administration of provided liposomes or compositions. In some embodiments, the expression of the protein encoded by the mRNA is detectable 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, and/or 7 days in serum or target tissues after a single administration of provided liposomes or compositions. In some embodiments, the expression of the protein encoded by the mRNA is detectable 1 week, 2 weeks, 3 weeks, and/or 4 weeks in serum or target tissues after a single administration of provided liposomes or compositions. In some embodiments, the expression of the protein encoded by the mRNA is detectable after a month or longer after a single administration of provided liposomes or compositions.

[0237] In some embodiments, mRNA (e.g., encoding CFTR protein) in a formulation as provided herein (e.g. encapsulated in a lipid nanoparticle consisting of 3 distinct lipid components, one of which is a sterol-based cationic lipid) delivered to the lung (e.g., by nebulization) is expressed in lung tissue for at least 7 days, at least 14 days, at least 21 days, or at least 28 days. In some embodiments, mRNA (e.g., encoding CFTR protein) in a formulation as provided herein (e.g. encapsulated in a lipid nanoparticle consisting of 3 distinct lipid components, one of which is a sterol-based cationic lipid) delivered to the lung (e.g., by nebulization) is expressed in lung tissue for up to 7 days, up to 14 days, up to 21 days, or up to 28 days. In some embodiments, a protein (e.g., CFTR) encoded by an mRNA (e.g., encoding CFTR protein) in a formulation as provided herein (e.g. encapsulated in a lipid nanoparticle consisting of 3 distinct lipid components, one of which is a sterol-based cationic lipid) delivered to the lung (e.g., by nebulization) is expressed in lung tissue for at least 7 days, at least 14 days, at least 21 days, or at least 28 days. In some embodiments, a protein (e.g., CFTR) encoded by an mRNA (e.g., encoding CFTR protein) in a formulation as provided herein (e.g. encapsulated in a lipid nanoparticle consisting of 3 distinct lipid components, one of which is a sterol-based cationic lipid) delivered to the lung (e.g., by nebulization) is expressed in lung tissue for up to 7 days, up to 14 days, up to 21 days, or up to 28 days.

[0238] The present invention can be used to deliver mRNA at various doses. In some embodiments, an mRNA is administered at a dose ranging from about 0.1-5.0 mg/kg body weight, for example about 0.1-4.5, 0.1-4.0, 0.1-3.5, 0.1-3.0, 0.1-2.5, 0.1-2.0, 0.1-1.5, 0.1-1.0, 0.1-0.5, 0.1-0.3, 0.3-5.0, 0.3-4.5, 0.3-4.0, 0.3-3.5, 0.3-3.0, 0.3-2.5, 0.3-2.0, 0.3-1.5, 0.3-1.0, 0.3-0.5, 0.5-5.0, 0.5-4.5, 0.5-4.0, 0.5-3.5, 0.5-3.0, 0.5-2.5, 0.5-2.0, 0.5-1.5, or 0.5-1.0 mg/kg body weight. In some embodiments, an mRNA is administered at a dose of or less than about 5.0, 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.8, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 mg/kg body weight.

EXAMPLES

[0239] While certain compounds, compositions and methods of the present invention have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same. Lipid Materials

[0240] The formulations described herein included a multi-component lipid mixture of varying ratios employing cationic lipids, helper lipids and PEGy-modified lipids designed to encapsulate various nucleic acid-based materials. Cationic lipids can include (but not exclusively) sterol-based cationic lipids, ICE (imidazole cholesterol ester), 1,2-dioleoyl-3-dimethylammonium-propane (DODAP), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-Dioleyloxy-3-dimethylaminopropane (DODMA), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), C12-200, DLinSSDMA, Target 24, etc. Helper lipids can include (but not exclusively) DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleyl-sn-glycero-3-phosphoethanolamine), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (2-dioleoyl-sn-glycero-3-phospho-(1′-rac-glycerol)), cholesterol, etc. The PEG-modified

lipids can include (but not exclusively) a poly(ethylene) glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C.sub.6-C.sub.20 length.
[0241] N/P ratio is the molar ratios of the nitrogen (amine) groups of cationic carriers to those of the phosphate ones of mRNA.
Messenger RNA Material
[0242] The formulations described herein included messenger RNA (mRNA). mRNA can (but not exclusively) encode human Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) and Firefly Luciferase (FFL). Exemplary mRNA coding sequences as disclosed herein include SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. An exemplary 5′ UTR mRNA sequence as disclosed herein is SEQ ID NO: 5. Exemplary 3′ UTR mRNA sequences as disclosed herein are SEQ ID NO: 6 and SEQ ID NO: 7. Exemplary full length mRNA sequences (i.e., a coding sequence plus a 5′ UTR and a 3′ UTR) as disclosed herein include SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11.
Exemplary LNP Formulations Comprising Different Cationic Lipids
[0243] In some embodiments, one particular application of the novel lipid composition comprising a sterol-based cationic lipid, a helper lipid, and a PEG or PEG-modified lipid is pulmonary delivery of mRNA, such as CFTR mRNA. Lipid nanoparticle formulations composed of ICE lipid, DOPE lipid and DMG-PEG lipid (molar ratio of 60:35:5) exhibit high percent encapsulation values for mRNA, such as CFTR mRNA (>80%), as determined by fluorescence-based detection of mRNA. As shown in Table 5, this particular formulation composition is superior because conventional pH titrable cationic lipids with DOPE and DMG-PEG 2K do not show such high percent encapsulation.
TABLE-US-00011 TABLE 5 The encapsulation percentage of mRNA for a sterol-based cationic lipid nanoparticle formulation and other cationic lipid-based nanoparticle formulations. All lipid formulations were prepared with the composition of cationic lipid:DOPE:DMG-PEG 2K (molar ratio 60:35:5). pH titratable % mRNA Cationic Lipid encapsulation ICE 90 DODMA 51 DODAP 49 C12-200 39 DLinSSDMA 57 Target 24 59
[0244] All of these exemplary formulations were prepared as 5 mg mRNA LNP preparations.
Formulation Example #1 (ICE Lipid)
[0245] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.
[0246] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 90%. Size: 60 nm. PDI: 0.14.
Formulation Example #2 (DODMA Lipid)
[0247] Aliquots of 10 mg/mL ethanolic solutions of DODMA, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.
In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The

formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 51%. Size: 62 nm. PDI: 0.18.

Formulation Example #3 (DODAP Lipid)

[0248] Aliquots of 10 mg/mL ethanolic solutions of DODAP, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL. In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 49%. Size: 78 nm. PDI: 0.19.

Formulation Example #4 (C12-200 Lipid)

[0249] Aliquots of 10 mg/mL ethanolic solutions of C12-200, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.

[0250] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 39%. Size: 98 nm. PDI: 0.22.

Formulation Example #5 (DLin-SS-DMA Lipid)

[0251] Aliquots of 10 mg/mL ethanolic solutions of DLin-SS-DMA, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.

[0252] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 57%.

Formulation Example #6 (Target 24 Lipid)

[0253] Aliquots of 10 mg/mL ethanolic solutions of Target 24 (3-(5-(bis(2-hydroxydodecyl)amino)pentan-2-yl)-6-(5-((2-hydroxydodecyl)(2-hydroxyundecyl)amino)pentan-2-yl)-1,4-dioxane-2,5-dione), DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.

[0254] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic

attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 59%. Size: 92 nm. PDI: 0.24.

Exemplary ICE Formulation Protocols

A. Formulation Example—1 mg Scale, N/P=4

[0255] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE, cholesterol and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 3 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution. The lipid solution was injected rapidly into the aqueous mRNA solution and shaken to yield a final suspension in 20% ethanol. The resulting nanoparticle suspension was filtered, diafiltrated, concentrated and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 89% mRNA encapsulation (89% of the lipid nanoparticles contained mRNA) and the lipid nanoparticles had an average size of around 67 nm with polydispersibility index (PDI) of 0.19.

B. Formulation Example—15 mg Scale, N/P=2

[0256] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 45 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 180 mL.

[0257] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der Waals forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The lipid nanoparticles had an average size of around 70 nm with PDI of 0.17.

C. Formulation Example—15 mg Scale, N/P=4

[0258] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 45 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 180 mL.

[0259] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 85% mRNA encapsulation and the lipid nanoparticles had an average size of around 65 nm with PDI of 0.13.

D. Formulation Example—30 mg Scale, N/P=4

[0260] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 90 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 360 mL.

[0261] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in

formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 86% of mRNA encapsulation and the lipid nanoparticles had an average size of around 89 nm with PDI of 0.12.

E. Formulation Example—30 mg Scale, 3% PEG, N/P=4

[0262] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:37:3) were mixed and diluted with ethanol to 90 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 360 mL.

[0263] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The lipid nanoparticles had an average size of around 94 nm with PDI of 0.15.

F. Formulation Example—60 mg Scale, N/P=4

[0264] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 180 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 720 mL.

[0265] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR STOP mRNA (encapsulated). The formulation had about 97% mRNA encapsulation and the lipid nanoparticles had an average size of around 67 nm with PDI of 0.12.

G. Formulation Example—3.5 Gram Scale, N/P=4

[0266] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 42 L final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 42 L.

[0267] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation is adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 91% mRNA encapsulation and the lipid nanoparticles had an average size of around 50 nm with PDI of 0.17.

H. Formulation Example—200 mg Scale, N/P=4

[0268] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 600 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of FFL mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 2.4 L.

[0269] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer

were mixed using a pump system. The instantaneous mixing of the two streams results in formation of lipid nanoparticles. The resultant formulation mixture is in citrate buffer containing 20% ethanol. The formulation is subjected to a buffer exchange using TFF system and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 95% mRNA encapsulation and the lipid nanoparticles had an average size of around 59 nm with PDI of 0.15.

I. Formulation Example—200 mg Scale, N/P=4

[0270] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 600 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of hCFTR-STOP mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 2.4 L.

[0271] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation is adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR STOP mRNA (encapsulated). The formulation had about 88% mRNA encapsulation and the lipid nanoparticles had an average size of around 57 nm with PDI of 0.18.

Pulmonary Delivery of ICE LNPs

[0272] Several exemplary studies were performed to demonstrate successful mRNA delivery and subsequent human CFTR protein production from ICE-based LNPs encapsulating codon-optimized hCFTR mRNA. Immunohistochemical analysis was performed on all lung sections using an anti-human CFTR antibody to specifically detect human CFTR protein within the lungs of treated animals.

Example 1. In Vivo Expression of hCFTR in Rat Lungs after Intratracheal Administration

[0273] FIG. **1** shows exemplary immunohistochemical detection of hCFTR protein in mice lungs 24 hours after pulmonary delivery of hCFTR mRNA lipid nanoparticles prepared by the process described above (Formulation Example #1).

[0274] Mice were administered LNP formulations containing hCFTR mRNA via microsprayer (intratracheal aerosol). The LNP formulations were made using ICE lipid as the cationic lipid. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0275] Protein was detected throughout the entire lung including both the bronchial epithelial cells and the alveolar regions. Positive (brown) staining was observed in all mRNA lipid nanoparticle test article groups, as compared to the lack of brown staining in the lungs of untreated control mice. Panel A depicts untreated mouse lung at 10× magnification. Panel B depicts untreated mouse lung at 20× magnification. Panel C depicts codon-optimized hCFTR (CO-hCFTR) mRNA ICE LNP-treated mouse lung at 10× magnification. Panel D depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 20× magnification.

Example 2. In Vivo Expression of hCFTR in Rat Lungs after Nebulization Administration

[0276] FIG. **2** shows exemplary immunohistochemical detection of hCFTR protein in mice lungs 24 hours after pulmonary delivery of hCFTR mRNA lipid nanoparticles prepared by the process described above (Formulation Example #1).

[0277] Mice were administered, via nebulization, LNP formulations containing hCFTR mRNA. The LNP formulations were made using ICE lipid as the cationic lipid. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0278] Protein was detected throughout the entire lung including both the bronchial epithelial cells and the alveolar regions. Positive (brown) staining was observed in all mRNA lipid nanoparticle

test article groups, as compared to the lack of brown staining in the lungs of untreated control mice. Panel A depicts untreated mouse lung at 10× magnification. Panel B depicts untreated mouse lung at 20× magnification. Panel C depicts codon-optimized hCFTR (CO-hCFTR) mRNA ICE LNP-treated mouse lung at 10× magnification. Panel D depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 20× magnification.

Example 3. In Vivo Expression of hCFTR in Rat Lungs (10 mg, 5% PEG)

[0279] FIG. **3** shows exemplary immunohistochemical analysis of hCFTR protein in rat lungs 24 hours after pulmonary delivery of codon-optimized hCFTR (CO-hCFTR) mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0280] In some representative studies, rats were placed in an aerosol chamber which allowed for full motion of the rats. An aerosol was produced via nebulization of 10 mg (as measured by encapsulated mRNA) CO-hCFTR mRNA encapsulated in ICE LNPs (containing 5% [mol] of PEG-modified lipid) which filled the chamber containing the rats. The rats were exposed for a given period of time with during which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the rats were removed and placed back into their cages. The rats were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0281] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. **3**. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated rat lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 10× magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 20× magnification.

Example 4. In Vivo Expression of hCFTR in Rat Lungs (50 μg mRNA, 5% PEG)

[0282] FIG. **4** shows exemplary immunohistochemical analysis of hCFTR protein in rat lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0283] In some representative studies, rats were exposed to CO-hCFTR mRNA ICE LNPs using direct instillation via a MicroSprayer apparatus. An aerosol was produced via a MicroSprayer apparatus during intratracheal administration of 50 μg CO-hCFTR mRNA encapsulated ICE LNPs (containing 5% [mol] of PEG-modified lipid) in rats. The rats were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0284] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. **4**. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated rat lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 10× magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 20× magnification.

Example 5. In Vivo Expression of hCFTR in Rat Lungs (10 mg, 3% PEG)

[0285] FIG. **5** shows exemplary immunohistochemical analysis of hCFTR protein in rat lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0286] In some representative studies, rats were placed in an aerosol chamber which allowed for full motion of the rats. An aerosol was produced via nebulization of 10 mg CO-hCFTR mRNA encapsulated in ICE LNPs (containing 3% [mol] of PEG-modified lipid) which filled the chamber containing the rats. The rats were exposed for a given period of time during which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the rats were removed and placed back into their cages. The rats were then sacrificed 24 hours after exposure and the lungs

were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0287] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. **5**. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated rat lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 10× magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 20× magnification.

Example 6. In Vivo Expression of hCFTR in Rat Lungs (50 μg mRNA, 3% PEG)

[0288] FIG. **6** shows exemplary immunohistochemical analysis of hCFTR protein in rat lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0289] In some representative studies, rats were exposed to CO-hCFTR mRNA ICE LNPs using direct instillation via a MicroSprayer apparatus. An aerosol was produced via a MicroSprayer apparatus during intratracheal administration of 50 μg CO-hCFTR mRNA encapsulated ICE LNPs (containing 3% [mol] of PEG-modified lipid) in rats. The rats were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0290] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. **6**. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated rat lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 10× magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 20× magnification.

Example 7. In Vivo Expression of hCFTR in Mouse Lungs (10 mg, N/P=2)

[0291] FIG. **7** shows exemplary immunohistochemical analysis of hCFTR protein in CFTR KO mouse lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE based lipid nanoparticles.

[0292] In some representative studies, mice were placed in an aerosol chamber which allowed for full motion of the mice. An aerosol was produced via nebulization of 10 mg CO-hCFTR mRNA encapsulated in ICE LNPs (N/P=2) which filled the chamber containing the mice. The mice were exposed for a given period of time with which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the mice were removed and placed back into their cages. The mice were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0293] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. **7**. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated mouse lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 10× magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 20× magnification.

Example 8. In Vivo Expression of hCFTR in Mouse Lungs (10 mg, N/P=4)

[0294] FIG. **8** shows exemplary immunohistochemical analysis of hCFTR protein in CFTR KO mouse lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0295] In some representative studies, mice were placed in an aerosol chamber which allowed for full motion of the mice. An aerosol was produced via nebulization of 10 mg CO-hCFTR mRNA encapsulated in ICE LNPs (N/P=4) which filled the chamber containing the mice. The mice were

exposed for a given period of time with which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the mice were removed and placed back into their cages. The mice were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0296] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. **8**. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated mouse lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 10× magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 20× magnification. Widespread distribution of hCFTR protein is observed in both example 5 (N/P=2) and example 6 (N/P=4).

Example 9. In Vivo Expression of hCFTR in Mouse Lungs with Different Formulations

[0297] FIG. **9** shows exemplary immunohistochemical analysis of hCFTR protein in wild-type mouse lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0298] In some representative studies, several groups of wild-type mice were separated and placed in individual aerosol chambers. Each group was treated with a different formulation. The formulations consisted of ICE-based LNPs (N/P=4) encapsulating the following mRNA constructs: 1) CO-hCFTR mRNA ICE LNP; 2) CO-hCFTR “STOP” mRNA (nonsense mutated CO-hCFTR mRNA unable to translate protein) ICE LNP; 3) FFL (Firefly Luciferase) mRNA ICE LNP; and 4) Buffer (Vehicle).

[0299] In some embodiments, mice were placed in an aerosol chamber which allowed for full motion of the mice. An aerosol was produced via nebulizer of CO-hCFTR mRNA encapsulated in ICE LNPs (N/P=4) which filled the chamber containing the mice. The mice were exposed for a total of 50 mg (shown in FIG. **7**) aerosolized CO-hCFTR mRNA (as measured by encapsulated mRNA) for a given period of time with which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the mice were removed and placed back into their cages. The mice were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0300] In FIG. **9**, positive (brown) staining was observed only in the CO-hCFTR mRNA LNP test article group. The first panel depicts the CO-hCFTR mRNA ICE LNP-treated mouse lung showing widespread distribution of human CFTR protein observed throughout the entire lung, including both bronchial epithelial and alveolar regions. The second panel depicts CO-hCFTR “STOP” mRNA ICE LNP-treated mouse lung with no positive (brown) staining observed. The third panel depicts FFL mRNA ICE LNP-treated mouse lung with no positive (brown) staining observed. The fourth panel depicts buffer treated mouse lung with no positive (brown) staining observed.

Example 10. In Vivo Expression of hCFTR in Mouse Lungs at Different Time Points

[0301] FIG. **10** shows exemplary immunohistochemical analysis of hCFTR protein in wild-type mouse lungs at predetermined time points after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0302] In some representative studies, the pharmacokinetic behavior of hCFTR protein after treatment of CO-hCFTR mRNA encapsulated ICE LNPs was performed in CFTR KO mice. In some embodiments, mice were placed in an aerosol chamber which allowed for full motion of the mice. An aerosol was produced via nebulization of CO-hCFTR mRNA encapsulated in ICE LNPs (N/P=4) which filled the chamber containing the mice. The mice were exposed to 10 mg aerosolized CO-hCFTR mRNA for a given period of time durinig which the aerosol was freely

taken into the lungs via normal breathing. After the exposure, the mice were removed and placed back into their cages. Selected cohorts of treated mice were sacrificed at pre-determined time points after aerosol exposure. The time range for sacrificing the mice post-administration ranged from 30 minutes to one week, specifically, 30 minutes, 2 hours, 4 hours, 6 hours, 24 hours, 48 hours, 72 hours and 1 week as shown in FIG. **10**. The mouse lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0303] As shown in FIG. **10**, protein or positive (brown) staining was detected throughout the entire lung, including both the bronchial epithelial cells and the alveolar regions, in the hCFTR mRNA treated mouse groups sacrificed more than 30 minutes after administration. The most positive or brown staining was observed at 24 hours, 48 hours and 72 hours after hCFTR mRNA delivery. No positive (brown) staining was observed in the saline treated control group.

Example 11. In Vivo Expression of hCFTR in Rat Lungs at Different Dose Levels

[0304] In some representative studies, rats were placed in an aerosol chamber which allowed for full motion of the rats. An aerosol was produced via nebulization of CO-hCFTR mRNA encapsulated in ICE LNPs which filled the chamber containing the rats. The rats were exposed for a given period of time with which the aerosol was freely taken into the lungs via normal breathing. Several groups were dosed at different dose levels as following: 1) Buffer; 2) Empty ICE LNP (˜8.0 mg/kg equivalent); 3) ICE LNP (0.5 mg/kg); 4) ICE LNP (2.1 mg/kg); 5) ICE LNP (4.1 mg/kg); and 6) ICE LNP (6.2 mg/kg).

[0305] After administration, the rats were removed and placed back into their cages. Furthermore, each group of rats was split into selected cohorts that were sacrificed at different predetermined time points post-administration. The time range for sacrificing the rats post-administration was 24 hours to 28 days, specifically 24 hours, 7 days, 14 days and 28 days.

[0306] Quantitative PCR, which measures copies of CO-hCFTR mRNA per total RNA, was performed on snap frozen lungs of all treated rats in each group. The results are represented in FIG. **11**, showing the ratio of copies of CO-hCFTR mRNA per micrograms of total RNA. Results were analyzed across the different doses administered and at the selected sacrifice time points for each dose level. Background levels of CO-hCFTR mRNA are indicated by the control groups' (Buffer and Empty LNP) values. Copies of CO-hCFTR mRNA per total RNA in frozen lung sections of rats increased as the dose level increased. At the same dose level, copies of CO-hCFTR mRNA per total RNA in frozen lung sections of rats decreased as the sacrifice time post-administration increased.

[0307] FIG. **12** shows a comparison of the levels of copies of exogenous CO-hCFTR mRNA per copy of endogenous CFTR mRNA. The fold-increase or ratio of copies of CO-hCFTR mRNA per copy of endogenous CFTR mRNA in frozen lung sections of rats was assessed and plotted as a function of dose and sacrifice time. The data was also obtained using Quantitative PCR. Fold-increase of copies of CO-hCFTR mRNA over endogenous levels of CFTR mRNA increased as the dose level increased. At the same mRNA dosage, fold-increase of copies of CO-hCFTR mRNA over endogenous levels of CFTR mRNA decreased as the sacrifice time post-administration increased.

[0308] Immunohistochemical analysis of the rat lungs was also performed and is shown in FIG. **13**. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining. A dose-dependent increase in hCFTR protein was observed as determined by positive (brown) staining throughout the entire lung, including both bronchial epithelial and alveolar regions. A decrease in hCFTR protein was observed as determined by positive (brown) staining as the time after the single dose of administration increased beyond 24 hours. However, detectable levels of hCFTR protein were observed 28 days after a single exposure of hCFTR mRNA ICE LNPs. No positive (brown) staining was observed in the saline treated

control group or in the empty LNP group.

Example 12. In Vivo Effect of hCFTR Formulations on Respiration

[0309] In some representative studies, rats were exposed to a single dose of hCFTR mRNA via inhalation. The hCFTR mRNA was formulated with either ICE as described above or with branched PEI (bPEI). As shown in FIG. **14**A, the various dosages of hCFTR mRNA formulated with bPEI caused an adverse respiratory rate increase relative to a saline control in the rats studied. When the rats were sacrificed, it was also found that the weight of the lungs was also increased. By comparison, as shown in FIG. **14**B, the various dosages of hCFTR mRNA formulated with ICE did not cause an increase in respiratory rate relative to a buffer control in the rats studied. There was also no increase in the weight of the rat lungs observed when the rats were sacrificed.

Example 13. In Vitro Expression and Activity of hCFTR at Different Dose Levels

[0310] In some representative experiments, hCFTR mRNA was transfected into cultured Fischer rat thyroid cells using Lipofectamine according to standard procedures. As is shown in FIG. **15**, there was a dose response of chloride-ion channel activity induced by hCFTR mRNA. This indicates that the hCFTR mRNA produced active CFTR protein in the transfected cells.

EQUIVALENTS

[0311] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

Claims

1-**88**. (canceled)

89. A method of delivering messenger RNAs (mRNAs) encoding a protein or a peptide in vivo comprising administering by pulmonary delivery to a subject in need of delivery a composition comprising: the mRNAs; and lipid nanoparticles encapsulating the mRNAs, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, wherein the three lipid components are a non-cationic lipid, a PEG-modified lipid, and a sterol-based cationic lipid, wherein the sterol-based cationic lipid has a structure according to Formula (A),

B-L.sup.1-S (Formula A), or a protonated form thereof, wherein: B is a basic functional group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl wherein the protonated form has a pKa that is no more than about 8.0; L.sup.1 is an optionally substituted linker group; and S is a sterol.

90. The method of claim 89, wherein the mRNAs are codon-optimized and/or comprise one or more modified nucleotides.

91. The method of claim 89, wherein the lipid nanoparticles have a size less than about 100 nm.

92. The method of claim 89, wherein the lipid nanoparticles have a lipid/mRNA (N/P) ratio of 2 or 4.

93. The method of claim 89, wherein the molar percentage of the PEG-modified lipid in the lipid nanoparticles is no more than 5%.

94. The method of claim 89, wherein the molar percentage of the sterol-based cationic lipid in the lipid nanoparticles is no more than 70%.

95. The method of claim 89, wherein the molar percentage of the sterol-based cationic lipid in the lipid nanoparticles is at least 40%.

96. The method of claim 89, wherein the composition is administered intravenously, or by pulmonary delivery, wherein the pulmonary delivery comprises nebulization.

97. A composition comprising: messenger RNAs (mRNAs) encoding a protein or a peptide; and lipid nanoparticles encapsulating the mRNAs, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, wherein the three lipid components are a non-cationic

lipid, a PEG-modified lipid, and a sterol-based cationic lipid, wherein the sterol-based cationic lipid has a structure according to Formula (A),

B-L.sup.1-S (Formula A), or a protonated form thereof, wherein: B is a basic functional group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl wherein the protonated form has a pKa that is no more than about 8.0; L.sup.1 is an optionally substituted linker group; and S is a sterol, and further wherein the lipid nanoparticles have an encapsulation percentage for mRNAs of at least 70%.

98. The composition of claim 97, wherein the sterol-based cationic lipid constitutes no more than 70% of the total lipids.

99. The composition of claim 97, wherein L.sup.1 is an optionally substituted linker group that is a C.sub.1-C.sub.20 alkylene or a 2- to 20-membered heteroalkylene.

100. The composition of claim 97, wherein L.sup.1 does not comprise substituents having the structure —N(R′).sub.2, or a positively charged form thereof, wherein each R′ is independently hydrogen or optionally substituted C.sub.1-C.sub.20 alkyl.

101. The composition of claim 97, wherein S is a sterol selected from a zoosterol, or an oxidized or reduced form thereof, a phytosterol, or an oxidized or reduced form thereof, a synthetic sterol, or an oxidized or reduced form thereof, cholesterol, an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol.

102. The composition of claim 97, wherein the mRNAs are codon-optimized and/or comprise one or more modified nucleotides.

103. The composition of claim 97, wherein the lipid nanoparticles have a lipid/mRNA (N/P) ratio of 2 or 4.

104. The composition of claim 97, wherein the molar percentage of the PEG-modified lipid in the lipid nanoparticles is no more than 5%.

105. The composition of claim 97, wherein the molar percentage of the sterol-based cationic lipid in the lipid nanoparticles is no more than 70%.

106. The composition of claim 97, wherein the molar percentage of the sterol-based cationic lipid in the lipid nanoparticles is at least 40%.

107. A composition formulated for nebulization comprising: messenger RNAs (mRNAs) encoding a protein or a peptide; and lipid nanoparticles encapsulating the mRNAs, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, wherein the three lipid components are a non-cationic lipid, a PEG-modified lipid, and a sterol-based cationic lipid, wherein the sterol-based cationic lipid has a structure according to Formula (A),

B-L.sup.1-S (Formula A), or a protonated form thereof, wherein: B is a basic functional group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl wherein the protonated form has a pKa that is no more than about 8.0; L.sup.1 is an optionally substituted linker group; and S is a sterol, and further wherein the lipid nanoparticles have an encapsulation percentage for mRNAs of at least 70%.

108. The composition of claim 107, wherein the mRNAs are codon-optimized and/or comprise one or more modified nucleotides.