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(54) **MODIFIED MESSENGER RNA COMPRISING FUNCTIONAL RNA ELEMENTS**

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(60) Provisional application No. 62/667,824, filed on May 7, 2018, provisional application No. 62/519,800, filed on Jun. 14, 2017, provisional application No. 62/508,318, filed on May 18, 2017.

(51) **Int. Cl.**

C07H 21/02	(2006.01)
C07H 21/04	(2006.01)
C12N 15/11	(2006.01)
A61K 47/69	(2017.01)
C12N 15/85	(2006.01)
C12N 15/88	(2006.01)

(52) **U.S. Cl.**
CPC ***C12N 15/11*** (2013.01); ***A61K 47/6929*** (2017.08); ***C12N 15/85*** (2013.01); ***C12N 15/88*** (2013.01); *C12N 2310/321* (2013.01); *C12N 2310/322* (2013.01); *C12N 2310/335* (2013.01)

(58) **Field of Classification Search**
CPC C12N 15/11; A61K 31/7105
See application file for complete search history.

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(57) **ABSTRACT**

The present disclosure provides messenger RNAs (mRNAs) having chemical and/or structural modifications, including RNA elements and/or modified nucleotides, which provide a desired translational regulatory activity to the mRNA.

30 Claims, 12 Drawing Sheets

Specification includes a Sequence Listing.

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Figure 1A

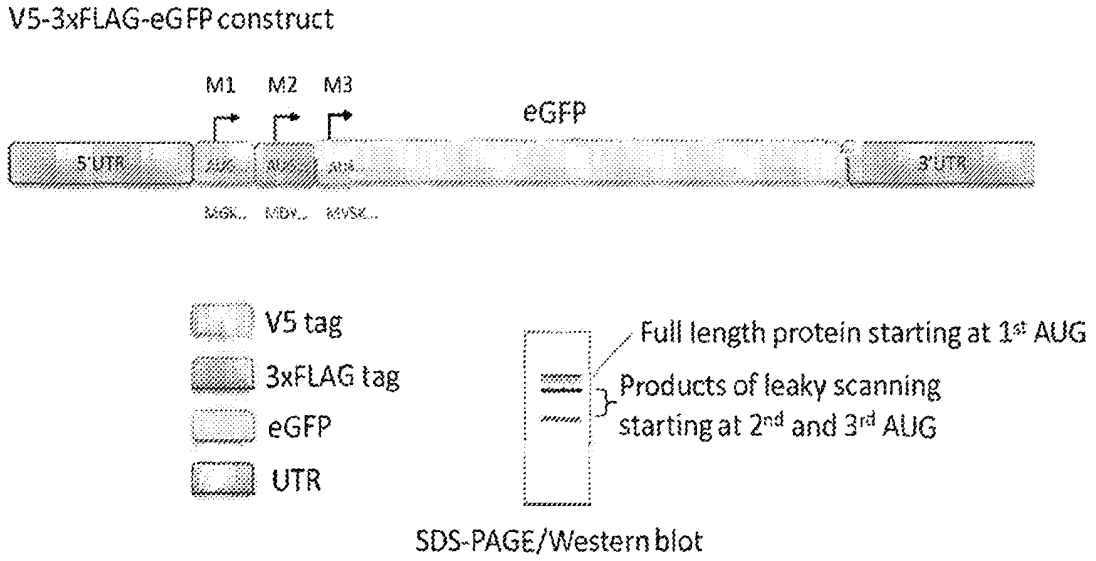


Figure 1B

5' UTRs

1. Standard	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC **ATGG**
2. 6nt	GGGAAA **ATGG**
3. 6nt (TISU)	GGCAAG **ATGG**
4. Tubulin-like	GTACACCGGCATCGACTAATCAGGGCCAGGCTCGAGGCTTTGTCTCCCTACCGCGCGCCGATTCTCCCGCCTCCCAG CCCCGGCGCACGCGCGCCCCGCCCAGCCTGCTTTCCCTCCGCGCCCTCCCCTCTCCTTTCTCCCTCTCAGAACCTTCCT GCCGTCGCGTTTGCACCTCGCTGCTCCAGCCTCTCGCATTCCAACCTTCCAGCCTGCGACCTGCGGAGACTTAGCCCC ATACATACCTTGAGGCGAGCTTTTAACC **ATGG**

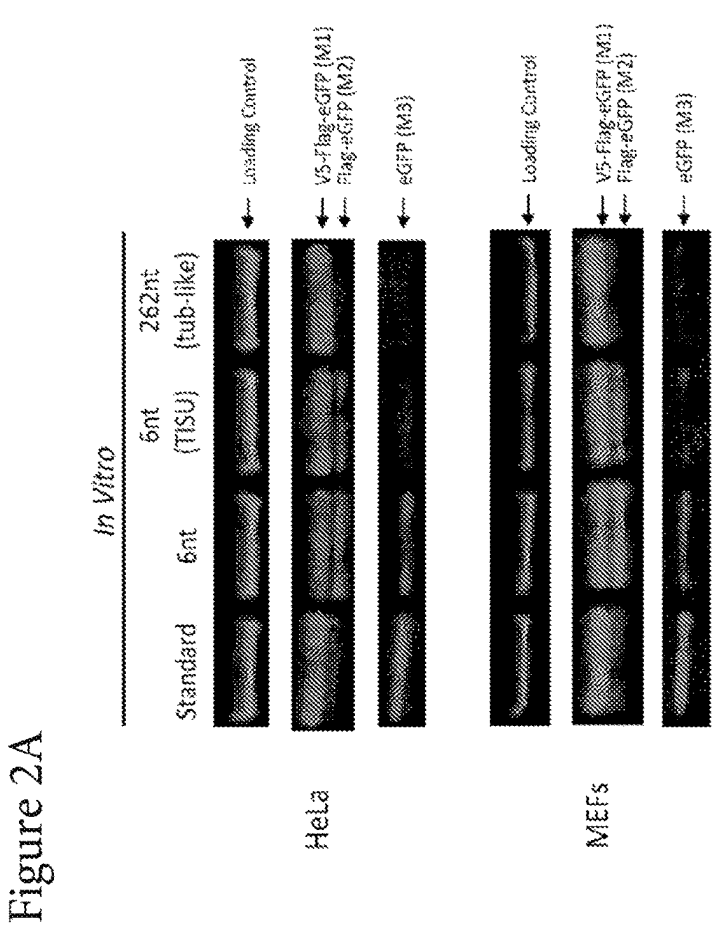


Figure 2A
In Vitro
Standard
6nt
6nt (TISU)
262nt (tub-like)
Loading Control
V5-Flag-eGFP (M1)
Flag-eGFP (M2)
eGFP (M3)
HeLa
MEFs

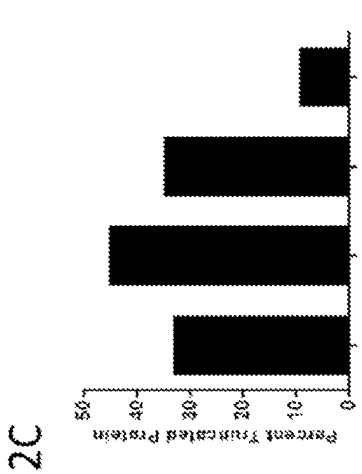


Figure 2C
Percent Truncated Protein
0
10
20
30
40
50
Standard
6nt
6nt (TISU)
262nt (tub-like)

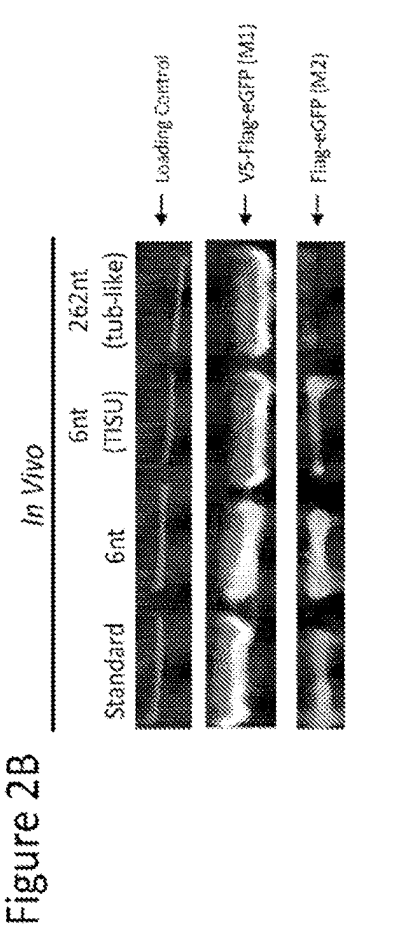


Figure 2B
In Vivo
Standard
6nt
6nt (TISU)
262nt (tub-like)
Loading Control
V5-Flag-eGFP (M1)
Flag-eGFP (M2)

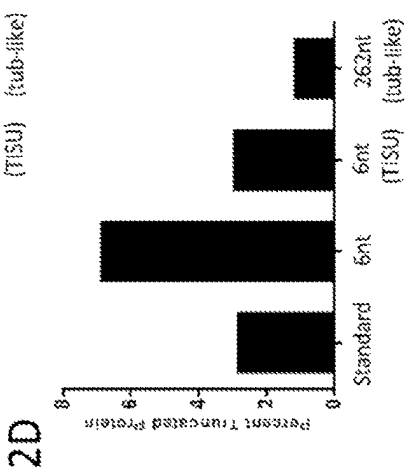


Figure 2D
Percent Truncated Protein
0
2
4
6
8
Standard
6nt
6nt (TISU)
262nt (tub-like)

Figure 3A

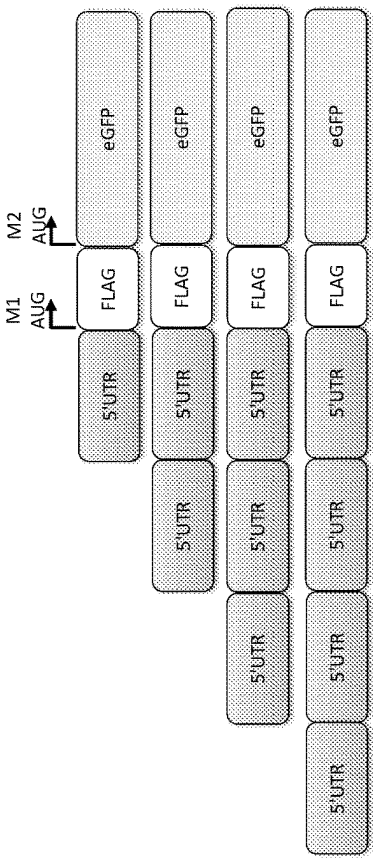


Figure 3B

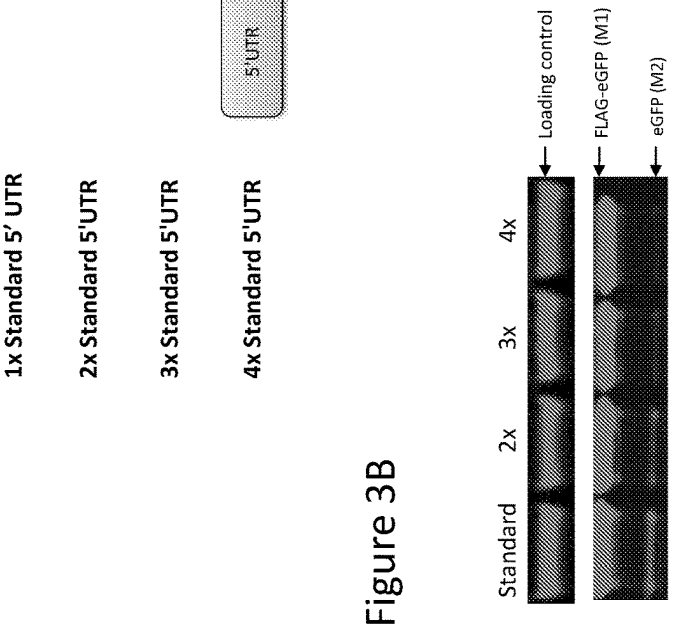


Figure 3C

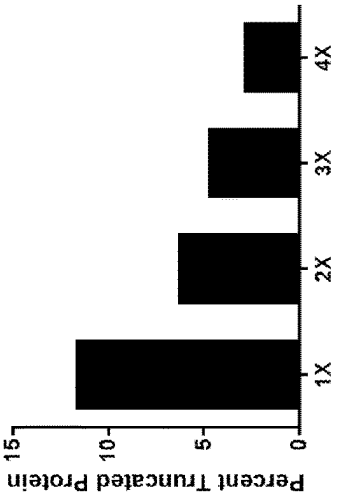


Figure 3D

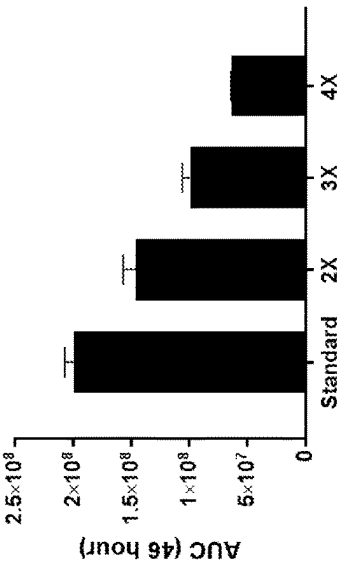


Figure 4B Hepatocytes

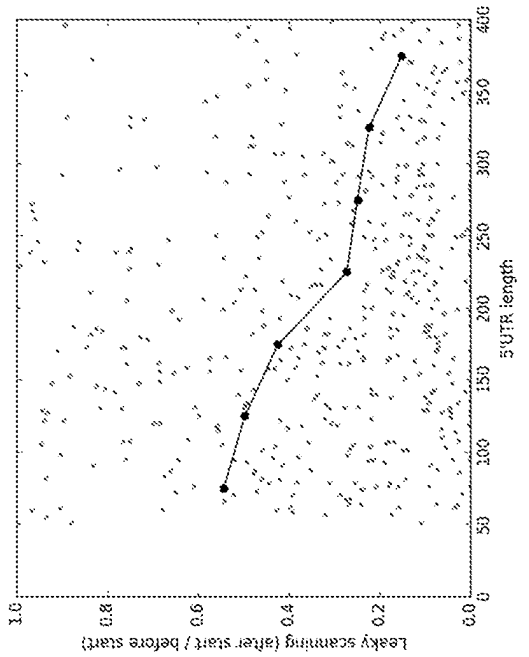
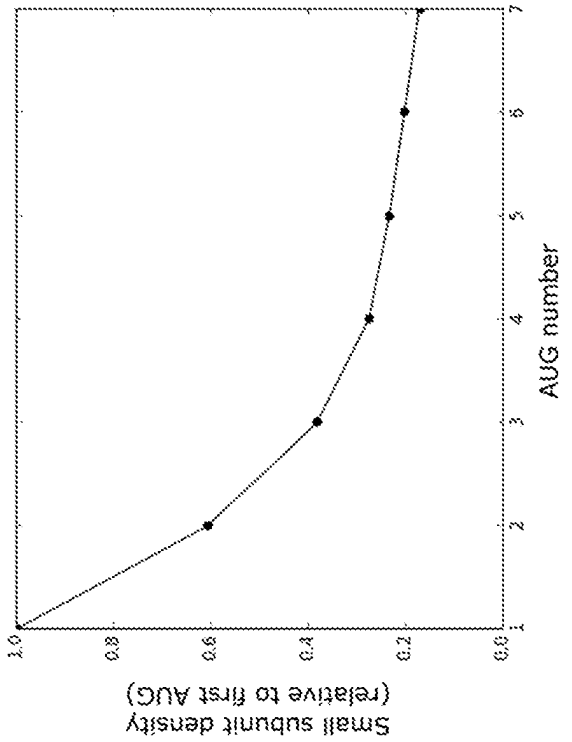


Figure 4A HeLa



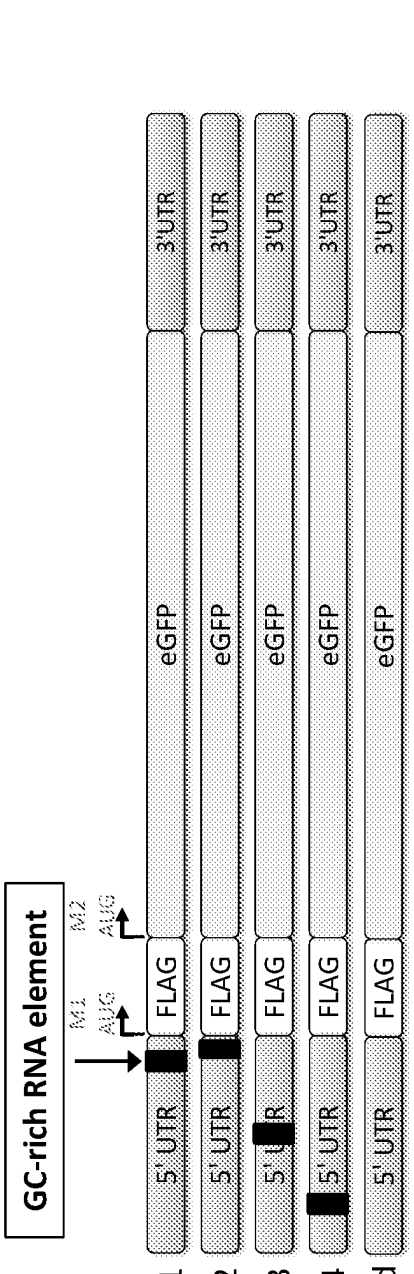


Figure 5A

Figure 5B

Figure 6A

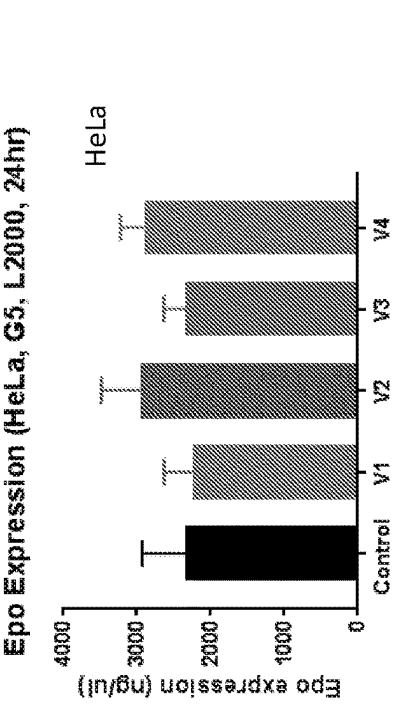


Figure 6B

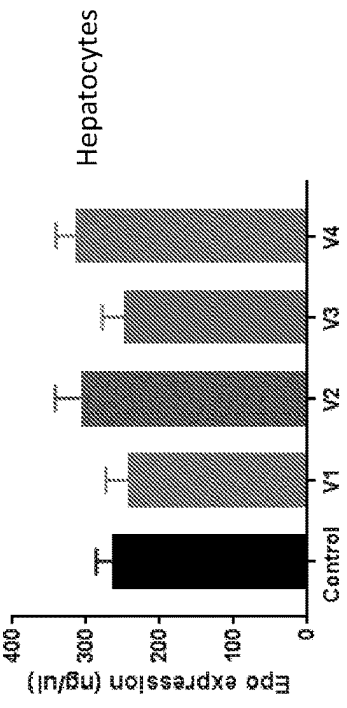


Figure 6C

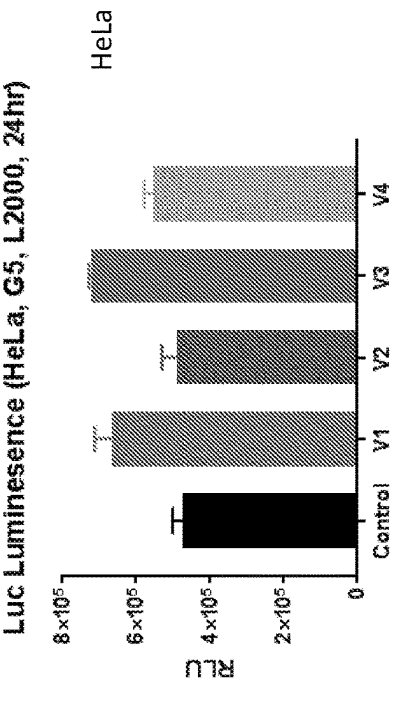


Figure 6D

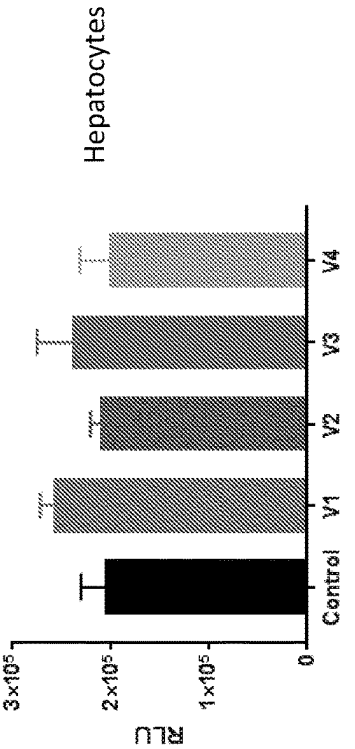


Figure 7B

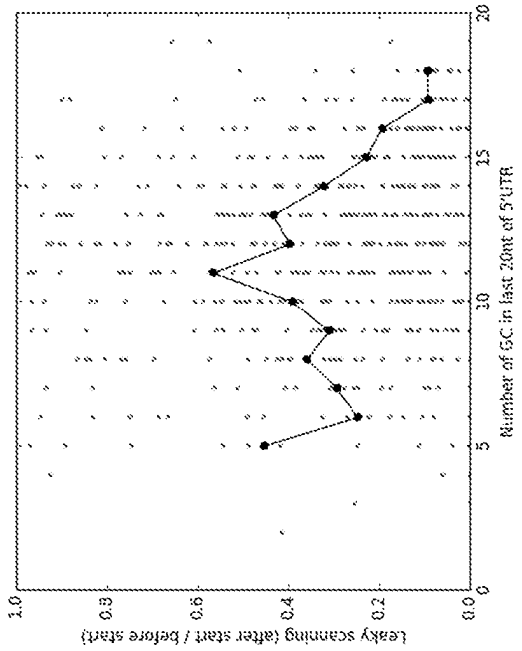


Figure 7A

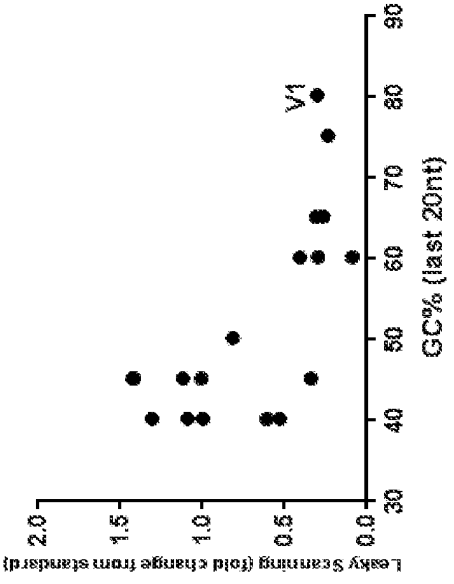
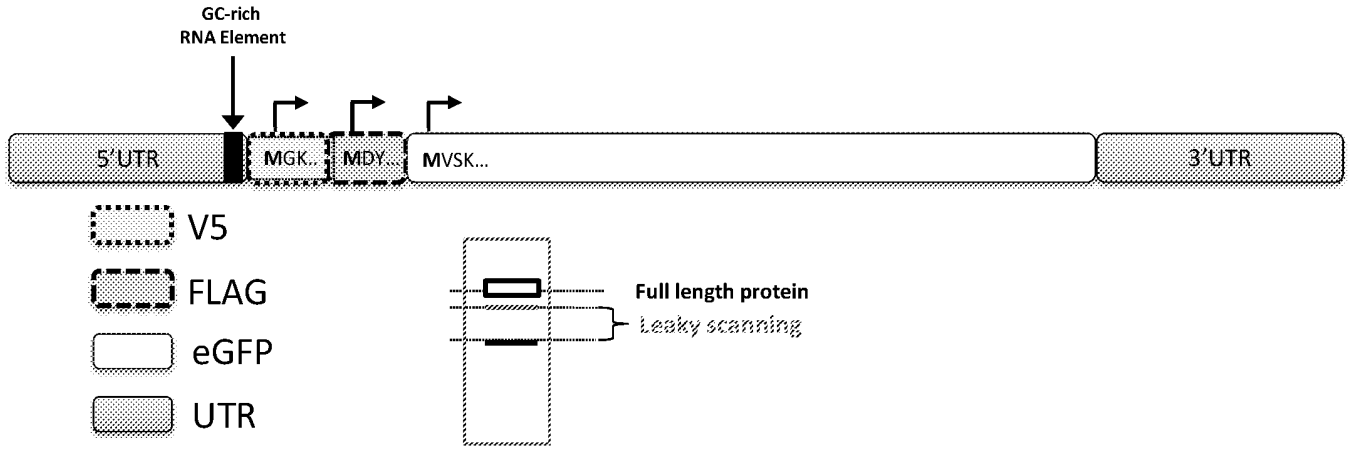


Figure 8A

#	5'UTR	5'UTR Sequence	% Cytosine of GC-Rich RNA Element
1	Standard	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC	0%
2	V1-UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA**CCCCGGCGCC**GCCACC	70%
3	GC Scramble #1-UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA**GGGGCGCCCG**GCCACC	40%
4	GC Scramble #2-UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA**GCCCGCCCGC**GCCACC	70%
5	GC Scramble #3-UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA**GCGCCCCGCG**GCCACC	60%
6	GC1-UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA**GCGCCCCGCGGCGCCCCGCG**GCCACC	60%

Figure 8B



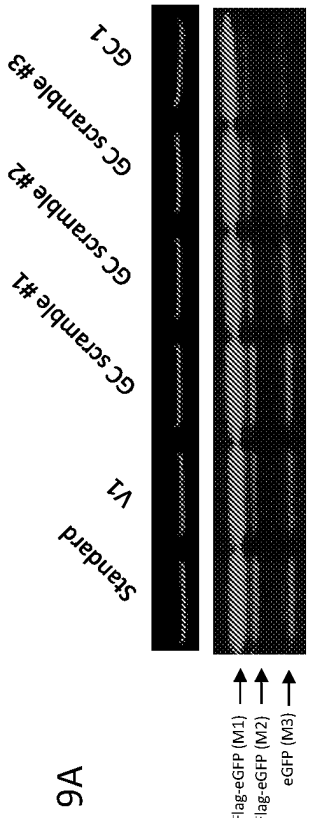


Figure 9A

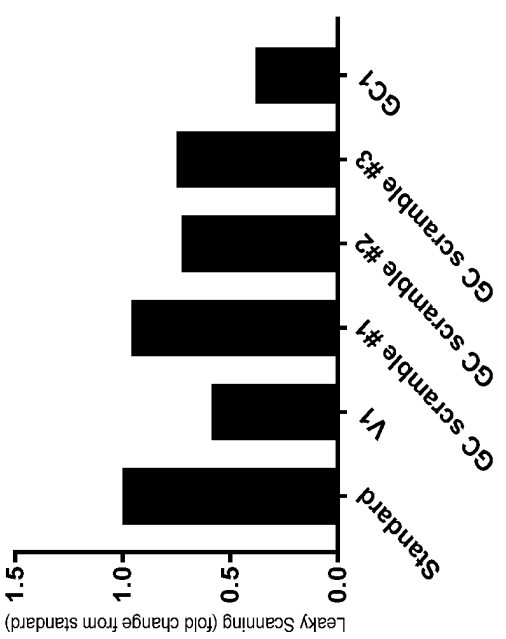


Figure 9B

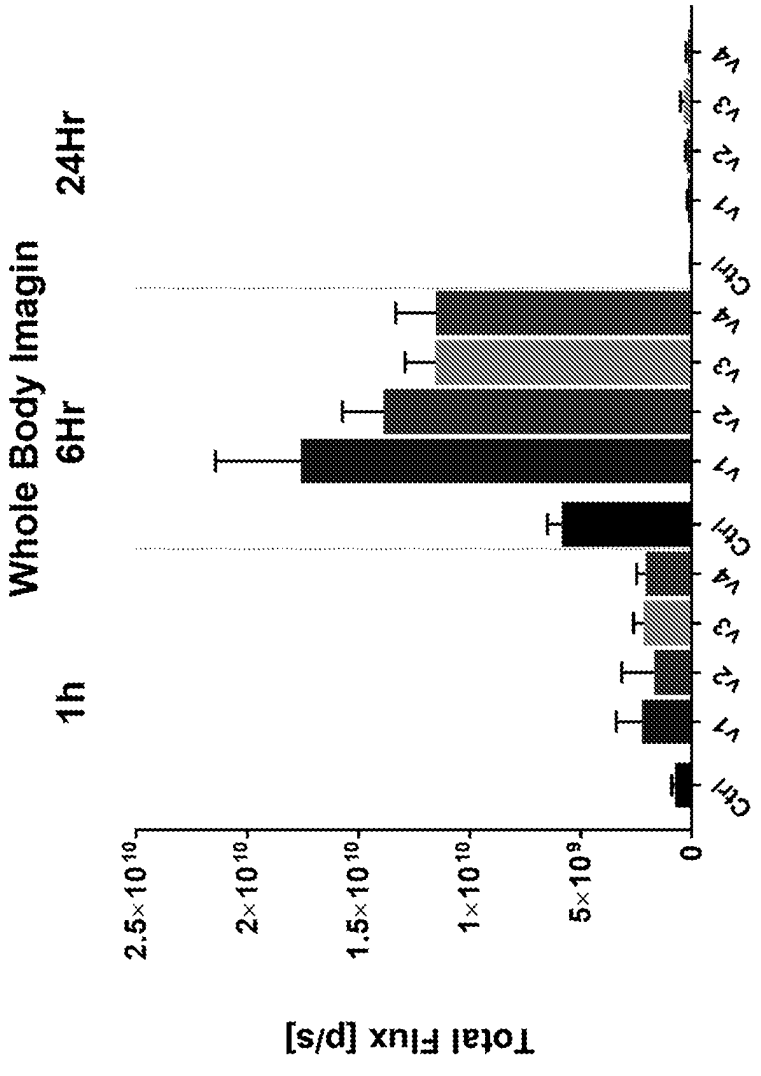

Whole Body Imagin
1h
6Hr
24Hr
Total Flux [p/s]
2.5×10^10
2×10^10
1.5×10^10
1×10^10
5×10^9
0
Ctrl
V1
V2
V3
V4
Ctrl
V1
V2
V3
V4
Ctrl
V1
V2
V3
V4

Figure 10

Figure 11A

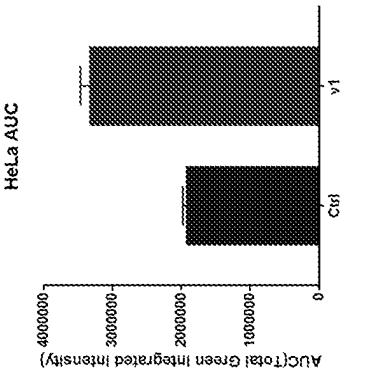


Figure 11B

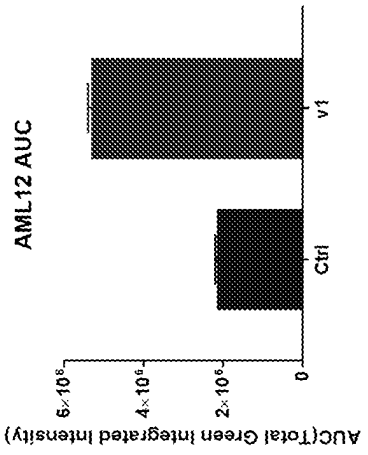


Figure 11C

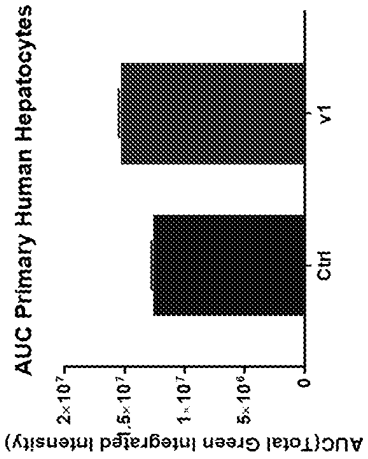


Figure 12B

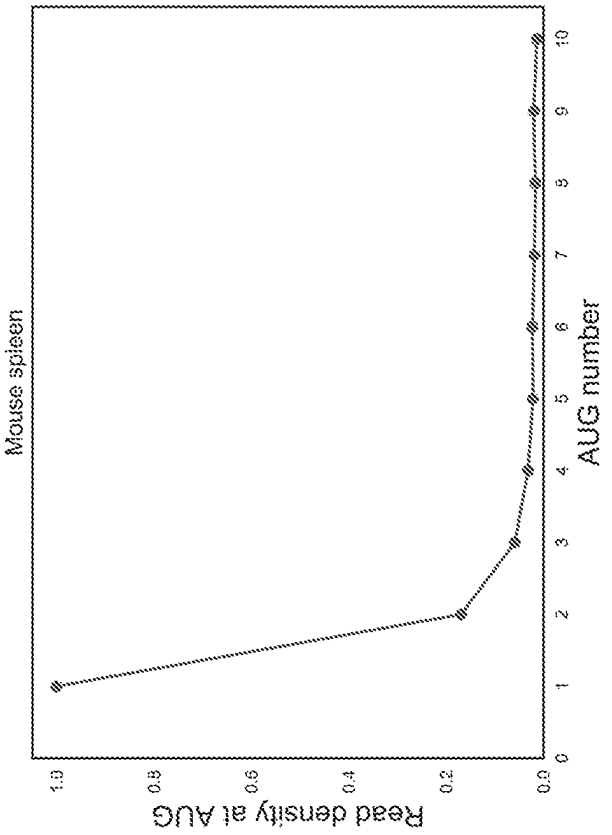
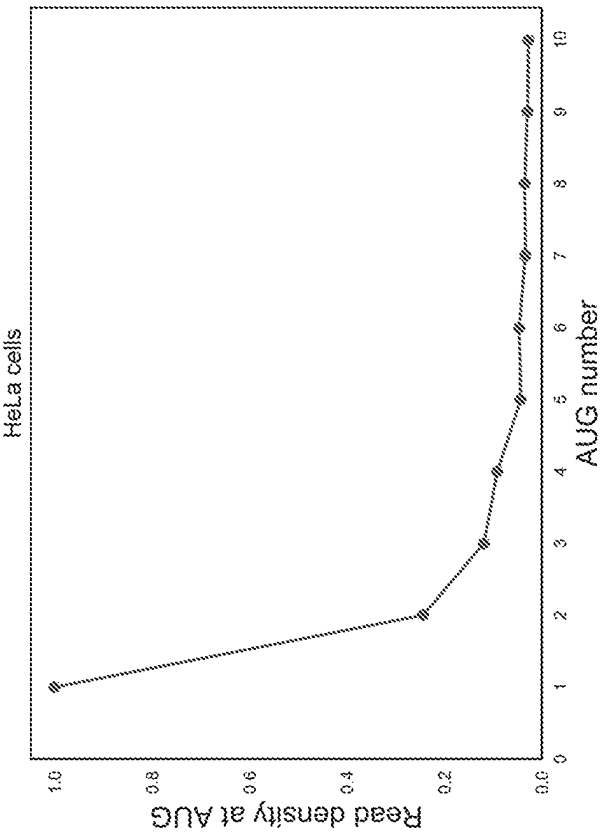


Figure 12A



MODIFIED MESSENGER RNA COMPRISING FUNCTIONAL RNA ELEMENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 35 U.S.C. § 371 national stage filing of International Application No. PCT/US2018/033519, filed May 18, 2018, which claims the benefit of U.S. Provisional Application Ser. No. 62/508,318 filed on May 18, 2017; U.S. Provisional Application Ser. No. 62/519,800 filed on Jun. 14, 2017; and U.S. Provisional Application 62/667,824 filed on May 7, 2018. The entire contents of the above-referenced applications are incorporated herein by this reference.

REFERENCE TO SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format via EFS-Web, and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Mar. 21, 2022, is named MRNA_108_N01US.ST25.txt and is 148529 bytes in size.

BACKGROUND

Messenger RNA (mRNA) designed to encode and transiently express a pharmacologically active protein or peptide product is the quintessence of a novel class of mRNA-based therapeutics. Administration of a synthetic and/or in vitro-generated mRNA that structurally resembles natural mRNA can result in the controlled production of therapeutic proteins or peptides via the endogenous and constitutively-active translation machinery (e.g. ribosomes) that exists within the patient's own cells. In recent years, the development and use of mRNA as a therapeutic agent has demonstrated potential for treatment of numerous diseases and for the development of novel approaches in regenerative medicine and vaccination (Sahin et al., (2014) Nat Rev Drug Discov 13(10):759-780).

It is recognized that the control and regulation of mRNA translation is an important development component in order for this class of drugs to establish the desired therapeutic effect. Within the field of mRNA therapeutics, there exists a need to develop mRNA with improved therapeutic effect.

SUMMARY OF THE INVENTION

The present disclosure provides messenger RNAs (mRNAs), including modified mRNAs (mmRNAs) having chemical and/or structural modifications, including RNA elements and/or modified nucleotides, which provide a desired translational regulatory activity to the mRNA. In one aspect, the mRNAs of the disclosure comprise modifications that reduce leaky scanning of 5' UTRs by the cellular translation machinery. Leaky scanning can result in the bypass of the desired initiation codon that begins the open reading frame encoding a polypeptide of interest or a translation product. This bypass can further result in the initiation of polypeptide synthesis from an alternate or alternative initiation codon, and thereby promote the translation of partial, aberrant, or otherwise undesirable open reading frames within the mRNA. The negative impact caused by the failure to initiate translation of the therapeutic protein or peptide at the desired initiator codon, as a consequence of leaky scanning or other mechanisms, poses a challenge in the development of mRNA therapeutics.

Accordingly, the present disclosure provides mRNAs, including mmRNAs having novel chemical and/or structural modifications, which provide a desired translational regulatory activity, including promoting translation of only one open reading frame encoding a desired polypeptide or translation product. In some aspects, the desired translational regulatory activity reduces, inhibits or eliminates the failure to initiate translation of the therapeutic protein or peptide at the desired initiator codon, as a consequence of leaky scanning or other mechanisms, Thus, the present disclosure provides mRNA having chemical and/or structural modifications (e.g., mmRNAs) which are useful to modulate (e.g., control) translation of an mmRNA to produce a desired translation product.

Accordingly, in one aspect the disclosure provides, mRNAs comprising a 5' untranslated region (UTR), an initiation codon, a full open reading frame encoding a polypeptide, a 3' UTR, and at least one modification, wherein the at least one modification provides a translational regulatory activity. In one embodiment, the translational regulatory activity comprises increasing residence time of a 43S pre-initiation complex (PIC) or ribosome at, or proximal to, the initiation codon. In another embodiment, the translational regulatory activity comprises increasing initiation of polypeptide synthesis at or from the initiation codon. In another embodiment, the translational regulatory activity comprises increasing an amount of polypeptide translated from the full open reading frame. In another embodiment, the translational regulatory activity comprises increasing fidelity of initiation codon decoding by the PIC or ribosome. In another embodiment, the translational regulatory activity comprises inhibiting or reducing leaky scanning by the PIC or ribosome. In another embodiment, the translational regulatory activity comprises decreasing a rate of decoding the initiation codon by the PIC or ribosome. In another embodiment, the translational regulatory activity comprises inhibiting or reducing initiation of polypeptide synthesis at any codon within the mmRNA other than the initiation codon. In another embodiment, the translational regulatory activity comprises inhibiting or reducing the amount of polypeptide translated from any open reading frame within the mmRNA other than the full open reading frame. In another embodiment, the translational regulatory activity comprises inhibiting or reducing the production of aberrant translation products. In another embodiment, the translational regulatory activity comprises any combination of the foregoing activities.

In another aspect, the disclosure provides an mRNA comprising at least one modification (e.g., mmRNA), wherein the at least one modification is a structural modification. In one embodiment, the structural modification is a RNA element. In another embodiment, the structural modification is a GC-rich RNA element. In another embodiment, the structural modification is a viral RNA element. In another embodiment, the structural modification is a protein-binding RNA element. In another embodiment, the structural modification is a translation initiation element. In another embodiment, the structural modification is a translation enhancer element. In another embodiment, the structural modification is a translation fidelity enhancing element. In another embodiment, the structural modification is an mRNA nuclear export element. In another embodiment, the structural modification is a codon optimized open reading frame. In another embodiment, the structural modification is a modification of base composition.

In another aspect, the disclosure provides an mRNA comprising at least one modification (e.g., mmRNA),

wherein the at least one modification is a chemical modification. In one embodiment, the chemical modification is one or more chemically modified nucleotides. In another embodiment, the chemical modification is one or more deoxyribonucleotides. In another embodiment, the chemical modification is one or more chemical modifications to the mRNA backbone.

In some aspects, the modification in the mRNA is in a 5' UTR, an initiation codon, a full open reading frame, a 3' UTR, or any combination thereof. Thus, in one embodiment, the 5' UTR of an mRNA comprises at least one modification as described herein. In another embodiment, the initiation codon of an mRNA comprises at least one modification as described herein. In another embodiment, the full open reading frame encoding a polypeptide of an mRNA comprises at least one modification as described herein. In another embodiment, the 3' UTR of an mRNA comprises at least one modification as described herein. In another embodiment, a modification comprises any one of the sequences set forth in Table 1. In another embodiment, a 5' UTR comprises any one of the sequences set forth in Table 1. In yet another embodiment, a 5' UTR comprises the sequence V1-UTR as set forth in Table 1.

In another aspect, the disclosure provides an mRNA comprising at least one modification, wherein the at least one modification is a GC-rich element comprising a sequence of linked nucleotides, or derivatives or analogs thereof, located upstream of a Kozak consensus sequence in the 5' UTR. In one embodiment, the GC-rich element is located about 30, about 25, about 20, about 15, about 10, about 5, about 4, about 3, about 2, or about 1 nucleotide(s) upstream of a Kozak consensus sequence in the 5' UTR. In another embodiment, the GC-rich element is located about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides upstream of a Kozak consensus sequence in the 5' UTR. In another embodiment, the GC-rich element is located upstream of and immediately adjacent to a Kozak consensus sequence in the 5' UTR. In another embodiment, the GC-rich element comprises a sequence of about 30, about 20-30, about 20, about 10-20, about 15, about 10-15, about 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, or 3 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence composition is about 70% cytosine, about 60%-70% cytosine, about 60% cytosine, about 50%-60% cytosine, about 50% cytosine, about 40%-50% cytosine, about 40% cytosine, about 30%-40% cytosine, about 30% cytosine. In one embodiment, the GC-rich element comprises a sequence of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence composition is >50% cytosine. In another embodiment, the GC-rich element comprises a sequence of about 3-30 nucleotides, or derivatives or analogues thereof, wherein the sequence comprises a repeating GC-motif, wherein the repeating GC-motif is [CCG]n, wherein n=1 to 10, 1 to 5, 3, 2, or 1. In another embodiment, the GC-motif is [GCC]n. In another embodiment, the GC-rich element comprises any one of the sequences set forth in Table 1. In a preferred embodiment, the GC-rich element comprises the sequence V1 as set forth in Table 1. In another aspect, the disclosure provides an mRNA comprising at least one modification, wherein the at least one modification is a GC-rich element comprising a stable RNA secondary structure located upstream of a Kozak consensus sequence in the 5' UTR. In one embodiment, the GC-rich RNA element comprising a stable RNA secondary structure is located about 30, about 25, about 20, about 15, about 10, about 5, about 4, about 3, about 3, or about 1 nucleotide(s) upstream of a Kozak consensus sequence in the 5' UTR. In another embodiment, the GC-rich RNA element comprising a stable RNA secondary structure is located about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides upstream of a Kozak consensus sequence in the 5' UTR. In another embodiment, the GC-rich RNA element comprising a stable RNA secondary structure is located upstream of and immediately adjacent to a Kozak consensus sequence in the 5' UTR.

In another aspect, the disclosure provides an mRNA comprising at least one modification, wherein the at least one modification is a GC-rich RNA element comprising a stable RNA secondary structure located downstream of the initiation codon. In one embodiment, the GC-rich RNA element comprising a stable RNA secondary structure is located about 30, about 25, about 20, about 15, about 10, about 5, about 4, about 3, about 2, or about 1 nucleotide(s) downstream of the initiation codon. In another embodiment, the GC-rich RNA element comprising a stable RNA secondary structure is located about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides downstream of the initiation codon. In another embodiment, the GC-rich RNA element comprising a stable RNA secondary structure is located 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 nucleotides downstream of the initiation codon.

In another aspect, the disclosure provides an mRNA comprising at least one modification, wherein the at least one modification is a GC-rich RNA element comprising a stable RNA secondary structure located upstream of the initiation codon. In one embodiment, the GC-rich RNA element comprising a stable RNA secondary structure is located about 40, about 35, about 30, about 25, about 20, about 15, about 10, about 5, about 4, about 3, about 2, about 1 nucleotide upstream of the initiation codon. In another embodiment, the GC-rich RNA element comprising a stable RNA secondary structure is located about 15-40, about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides upstream of the initiation codon.

In another aspect, the disclosure provides an mRNA comprising at least one modification, wherein the at least one modification is a GC-rich RNA element comprising a stable RNA secondary structure, wherein the stable RNA secondary structure comprises the initiation codon and one or more additional nucleotides upstream, downstream, or upstream and downstream of the initiation codon. In another embodiment, the GC-rich RNA element comprising a stable RNA secondary structure comprises any one of the sequences set forth in Table 1. In another embodiment, the stable RNA secondary structure comprises a hairpin or a stem-loop. In another embodiment, the stable RNA secondary structure has a deltaG of about −30 kcal/mol, about −20 to −30 kcal/mol, about −20 kcal/mol, about −10 to −20 kcal/mol, about −10 kcal/mol, about −5 to ~10 kcal/mol.

In another aspect, the disclosure provides an mRNA comprising at least one modification, wherein the at least one modification is one or more modified nucleotides, wherein the sequence comprising the initiation codon comprises one or more modified nucleotides that increases binding affinity with the initiator Met-tRNA$_i^{Mct}$. In one embodiment, the one or more modified nucleotides to comprises 2-thiouridine, 2'-O-methyl-2-thiouridine, 2-selenouridine, 2'-O-methyl ribose, a modified nucleotide in which the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon, inosine, 2-methylguanosine, 6-methyl-adenosine, a deoxyribonucleotide.

In another aspect, the disclosure provides an mRNA, including mmRNAs, wherein the mRNA comprises a first polynucleotide, wherein the first polynucleotide is chemically synthesized, and wherein the first polynucleotide comprises a 5' UTR, an initiation codon, and at least one modification, and a second polynucleotide, wherein the second polynucleotide is synthesized by in vitro transcription, and, wherein the second polynucleotide comprises a full open reading frame encoding a polypeptide, and a 3' UTR. In one embodiment, the first polynucleotide and the second polynucleotide are chemically cross-linked. In another embodiment, the first polynucleotide and the second polynucleotide are enzymatically ligated. In another embodiment, the first polynucleotide and the second polynucleotide are operably linked.

In another aspect, the disclosure provides mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the sequence of the 5' UTR comprises any of the sequences set forth in Table 1.

Another aspect, the disclosure provides a method of isolating a modification having translational regulatory activity, the method comprising synthesizing a 1^{st} control mRNA comprising a polynucleotide sequence comprising an open reading frame encoding eGFP and a 1^{st} AUG codon upstream of, in-frame, and operably linked to, the open reading frame encoding eGFP, and, a coding sequence for a 3×FLAG epitope tag upstream of, in-frame, and operably linked to the 1^{st} AUG codon, a 2nd AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the 3×FLAG epitope tag, a coding sequence for a V5 epitope tag upstream of, in-frame, and operably linked to the 2^{nd} AUG codon, a 3^{rd} AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the V5 epitope tag, and a 5' UTR and a 3' UTR. The method further comprising synthesizing a 2^{nd} test mmRNA comprising a polynucleotide sequence comprising an open reading frame encoding eGFP, a 1^{st} AUG codon upstream of, in-frame, and operably linked to, the open reading frame encoding eGFP, a coding sequence for a 3×FLAG epitope tag upstream of, in-frame, and operably linked to the 1^{st} AUG codon, a 2^{nd} AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the 3×FLAG epitope tag, a coding sequence for a V5 epitope tag upstream of, in-frame, and operably linked to the 2^{nd} AUG codon, a 3^{nd} AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the V5 epitope tag, a 5' UTR, a 3' UTR, and a candidate modification. The method further comprising introducing the 1^{st} control mmRNA and 2^{nd} test mmRNA to conditions suitable for translation of the polynucleotide sequence encoding the reporter polypeptide. The method further comprising measuring the effect of the candidate modification on the initiation of translation of the polynucleotide sequence encoding the reporter polypeptide from each of the three AUG codons.

In some aspects, the disclosure provides messenger RNA (mRNA) comprising

(i) a 5' untranslated region (UTR) comprising at least one RNA element that provides a translational regulatory activity;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR,

wherein the at least one RNA element is a GC-rich RNA element comprising guanine (G) and cytosine (C) nucleobases and, optionally, adenine (A) and uracil (U) nucleobases, or derivatives or analogs thereof, wherein the GC-rich RNA element is at least 50% or greater cytosine (C) nucleobases and is at least 6 nucleotides in length, wherein the GC-rich RNA element is located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the initiation codon in the 5' UTR, and wherein the translational regulatory activity is selected from the group consisting of:

(a) inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome;

(b) increases an amount of a polypeptide translated from the full open reading frame;

(c) increases initiation of polypeptide synthesis at or from the initiation codon;

(d) inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon;

(e) inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame;

(f) inhibits or reduces translation of truncated or aberrant translation products from the mRNA; and

(g) a combination of any of (a)-(f).

In some embodiments, the GC-rich RNA element is 6 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 7 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 8 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 9 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 10 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 11 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 12 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 13 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 14 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 15 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 16 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 17 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 18 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 19 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 20 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 21 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 22 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 23 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 24 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 25 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 26 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 27 nucleotides upstream of the initiation codon in the 5' UTR. In some embodiments, the GC-rich RNA element is 28

nucleotides upstream of the initiation codon in the 5' UTR in the 5' UTR. In some embodiments, the GC-rich RNA element is 29 nucleotides upstream of the initiation codon. In some embodiments, the GC-rich RNA element is 30 nucleotides upstream of the initiation codon in the 5' UTR.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising 50% cytosine (C) nucleobases. In some embodiments, the GC-rich RNA element is >50% cytosine (C) nucleobases. In some embodiments, the GC-rich RNA element is >60% cytosine (C) nucleobases. In some embodiments, the GC-rich RNA element is >70% cytosine (C) nucleobases. In some embodiments, the GC-rich RNA element is about 50%-55% cytosine, about 55%-60% cytosine, about 60%-65% cytosine, about 65%-70% cytosine, about 70%-75% cytosine, about 75%-80% cytosine. In some embodiments, the GC-rich RNA element is about 50%-55% cytosine. In some embodiments, the GC-rich RNA element is about 55%-60% cytosine. In some embodiments, the GC-rich RNA element is about 60%-65% cytosine. In some embodiments, the GC-rich RNA element is about 65%-70% cytosine. In some embodiments, the GC-rich RNA element is about 70%-75% cytosine. In some embodiments, the GC-rich RNA element is about 75%-80% cytosine. In some embodiments, the GC-rich RNA element is >80% cytosine (C) nucleobases. In some embodiments, the GC-rich RNA element is 90% cytosine (C) nucleobases. In some embodiments, the GC-rich RNA element is 100% cytosine (C) nucleobases.

In some embodiments, the GC-rich RNA element comprises a nucleotide sequence of about 6-10 nucleotides in length, about 10-15 nucleotides in length, about 15-20 nucleotides in length, about 20-25 nucleotides in length, about 25-30 nucleotides in length. In some embodiments, the GC-rich RNA element is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length. In some embodiments, the GC-rich RNA element is 6 nucleotides in length. In some embodiments, the GC-rich RNA element is 7 nucleotides in length. In some embodiments, the GC-rich RNA element is 8 nucleotides in length. In some embodiments, the GC-rich RNA element is 9 nucleotides in length. In some embodiments, the GC-rich RNA element is 10 nucleotides in length. In some embodiments, the GC-rich RNA element is 11 nucleotides in length. In some embodiments, the GC-rich RNA element is 12 nucleotides in length. In some embodiments, the GC-rich RNA element is 13 nucleotides in length. In some embodiments, the GC-rich RNA element is 14 nucleotides in length. In some embodiments, the GC-rich RNA element is 15 nucleotides in length. In some embodiments, the GC-rich RNA element is 16 nucleotides in length. In some embodiments, the GC-rich RNA element is 17 nucleotides in length. In some embodiments, the GC-rich RNA element is 18 nucleotides in length. In some embodiments, the GC-rich RNA element is 19 nucleotides in length. In some embodiments, the GC-rich RNA element is 20 nucleotides in length. In some embodiments, the GC-rich RNA element is 21 nucleotides in length. In some embodiments, the GC-rich RNA element is 22 nucleotides in length. In some embodiments, the GC-rich RNA element is 23 nucleotides in length. In some embodiments, the GC-rich RNA element is 24 nucleotides in length. In some embodiments, the GC-rich RNA element is 25 nucleotides in length. In some embodiments, the GC-rich RNA element is 26 nucleotides in length. In some embodiments, the GC-rich RNA element is 27 nucleotides in length. In some embodiments, the GC-rich RNA element is 28 nucleotides in length. In some embodiments, the GC-rich RNA element is 29 nucleotides in length. In some embodiments, the GC-rich RNA element is 30 nucleotides in length.

In some embodiments, the GC-rich RNA element does not comprise adenine (A) or uracil (U) or both A and U (or T). In some embodiments, the GC-rich RNA element does not comprise adenine (A). In some embodiments, the GC-rich RNA element does not comprise uracil (U).

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising a nucleotide sequence 6 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 6 nucleotides in length, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising a nucleotide sequence 7 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 7 nucleotides in length, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising a nucleotide sequence 8 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 8 nucleotides in length, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising a nucleotide sequence 9 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 9 nucleotides in length, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising a nucleotide sequence 10 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 10 nucleotides in length, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases.

In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 11 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 12 nucleotides in length, or derivatives or analogs thereof,

linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 13 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 14 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 15 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 16 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 17 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 18 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 19 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 20 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising a nucleotide sequence 20 nucleotides in length, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 21 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 22 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 23 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 24 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 25 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 26 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 27 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 28 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 29 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 30 nucleotides in length, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising a nucleotide sequence of about 6-30 guanine (G) and cytosine (C) nucleotides, or derivatives or analogues thereof, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases, and wherein the GC-rich RNA element comprises a repeating sequence motif. In some embodiments, the repeating sequence motif is $[CCG]_n$, wherein n=2 to 10, 2 to 5, 4, 3 or 2. In some embodiments, the repeating sequence motif is $[CCG]_n$, wherein n=2 to 10. In some embodiments, the repeating sequence motif is $[CCG]_n$, where n=2 to 5. In some embodiments, the repeating sequence motif is $[CCG]_n$, where n=4. In some embodiments, the repeating sequence motif is $[CCG]_n$, where n=3. In some embodiments, the repeating sequence motif is $[CCG]_n$, where n=2. In some embodiments, the repeating sequence motif is $[GCC]_n$, where n=2 to 10, 2 to 5, 4, 3 or 2. In some embodiments, the repeating sequence motif is $[GCC]_n$, where n=2 to 10. In some embodiments, the repeating sequence motif is $[GCC]_n$, where n=2 to 5. In some embodiments, the repeating sequence motif is $[GCC]_n$, where n=4. In some embodiments, the repeating sequence motif is $[GCC]_n$, where n=3. In some embodiments, the repeating sequence motif is $[GCC]_n$, where n=2. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 7 and SEQ ID NO: 8.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the at least one RNA element is a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 2. In some embodiments, the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 3. In some embodiments, the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 4. In some embodiments, the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 5.

In some aspects, the disclosure provides an mRNA comprising a 5' UTR, wherein the 5' UTR comprises the nucleotide sequence 5'-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC-3' set forth in SEQ ID NO: 33, wherein the 5' UTR comprises a GC-rich RNA element of the disclosure located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33. In some embodiments, the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33.

In some embodiments, the disclosure provides an mRNA comprising:

(i) a 5' untranslated region (UTR) comprising a GC-rich RNA element that provides a translational regulatory activity;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR,

wherein the 5' UTR comprises the nucleotide sequence 5'-GG-

GAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAA UAUAAGAGCCACC-3' set forth in SEQ ID NO: 33, wherein the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 2, and wherein the 5' UTR comprises the GC-rich RNA element located about 20-30, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33. In some embodiments, the GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 2 is located about 6 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33

In some aspects, the disclosure provides an mRNA comprising:

(i) a 5' untranslated region (UTR) comprising a GC-rich RNA element that provides a translational regulatory activity;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR,

wherein the 5' UTR comprises the nucleotide sequence 5'-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAG AA AUAUAAGAGCCACC-3' set forth in SEQ ID NO: 33, wherein the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 3, and wherein the GC-rich RNA element is located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33. In some embodiments, the GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 3 is located about 6 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33.

In some embodiments, the disclosure provides an mRNA comprising:

(i) a 5' untranslated region (UTR) comprising a GC-rich RNA element that provides a translational regulatory activity;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR,

wherein the 5' UTR comprises the nucleotide sequence 5'-GGGAAAUAAGAGAGAAAAGAAGAGUAAGAA GA AAUAUAAGAGCCACC-3' set forth in SEQ ID NO: 33, wherein the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 4, and wherein the GC-rich RNA element is located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33. In some embodiments, the GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 4 is located about 6 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33.

In some embodiments, the disclosure provides an mRNA comprising

(i) a 5' untranslated region (UTR) comprising the nucleotide sequence set forth in SEQ ID NO: 34;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR.

In some embodiments, the disclosure provides an mRNA comprising

(i) a 5' untranslated region (UTR) comprising the nucleotide sequence set forth in SEQ ID NO: 54;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR.

In some embodiments, the disclosure provides an mRNA comprising

(i) a 5' untranslated region (UTR) comprising the nucleotide sequence set forth in SEQ ID NO: 73;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR.

In some aspects, the disclosure provides messenger RNA (mRNA) comprising a second RNA element that provides a translational regulatory activity, wherein the second RNA element comprises a stable RNA secondary structure, and wherein the translational regulatory activity is selected from the group consisting of:

(a) inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome;

(b) increases an amount of a polypeptide translated from the full open reading frame;

(c) increases initiation of polypeptide synthesis at or from the initiation codon;

(d) inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon;

(e) inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame;

(f) inhibits or reduces translation of truncated or aberrant translation products from the mRNA; and

(g) a combination of any of (a)-(f).

In some embodiments, the stable RNA secondary structure located downstream of the initiation codon in the full open reading frame. In some embodiments, the stable RNA secondary structure is located about 30, about 25, about 20, about 15, about 10, or about 5 nucleotides downstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located about 20, about 15, about 10 or about 5 nucleotides downstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located about 5, about 4, about 3, about 2, about 1 nucleotide downstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides downstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located about 25-30, about 20-25, about 15-20, about 10-15, about 5-10, or about 1-5 nucleotide(s) downstream of the initiation codon in the full open reading frame. In some embodiments, the stable RNA secondary structure is located 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 nucleotide(s) downstream of the initiation codon in the full open reading frame. In some embodiments, the stable RNA secondary structure is located 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 nucleotides downstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located 15 nucleotides downstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located 14 nucleotides downstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located 13 nucleotides downstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located 12 nucleotides downstream of the initiation codon.

In some embodiments, stable RNA secondary structure located upstream of the initiation codon in the 5' UTR. In some embodiments, the stable RNA secondary structure is located about 25-30, about 20-25, about 15-20, about 10-15, about 5-10, or about 1-5 nucleotide(s) upstream of the initiation codon in the 5' UTR. In some embodiments, the

stable RNA secondary structure is located 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 nucleotide(s) upstream of the initiation codon in the 5' UTR. In some embodiments, the stable RNA secondary structure is located about 40, about 35, about 30, about 25, about 20, about 15, about 10, or about 5 nucleotides upstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located about 20, about 15, about 10 or about 5 nucleotides upstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located about 5, about 4, about 3, about 2, about 1 nucleotide upstream of the initiation codon. In some embodiments, the stable RNA secondary structure is located about 15-40, about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides upstream of the initiation codon.

In some embodiments, the stable RNA secondary structure comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32. In some embodiments, the stable RNA secondary structure comprises a nucleotide sequence set forth in SEQ ID NO: 28. In some embodiments, the stable RNA secondary structure comprises a nucleotide sequence set forth in SEQ ID NO: 29. In some embodiments, the stable RNA secondary structure comprises a nucleotide sequence set forth in SEQ ID NO: 30. In some embodiments, the stable RNA secondary structure comprises a nucleotide sequence set forth in SEQ ID NO: 31. In some embodiments, the stable RNA secondary structure comprises a nucleotide sequence set forth in SEQ ID NO: 32.

In some embodiments, the stable RNA secondary structure is a hairpin or a stem-loop.

In some embodiments, the stable RNA secondary structure has a deltaG of about −30 kcal/mol, about −20 to −30 kcal/mol, about −20 kcal/mol, about −10 to −20 kcal/mol, about −10 kcal/mol, about −5 to ~10 kcal/mol.

In some embodiments, the disclosure provides mRNA comprising a 5' UTR comprising at least one RNA element that provides a translational regulatory activity, wherein the initiation codon comprises at least one modified nucleotide, and wherein the at least one modified nucleotide increases binding affinity with the initiator Met-tRNA$_i^{Met}$. In some embodiments, the at least one modified nucleotide is selected from the group consisting of 2-thiouridine, 2'-O-methyl-2-thiouridine, 2-selenouridine, 2'-O-methyl ribose, a modified nucleotide in which the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon, inosine, 2-methylguanosine, 6-methyl-adenosine, a deoxyribonucleotide.

In some embodiments, the disclosure provides an mRNA comprising:

(i) a first polynucleotide, wherein the first polynucleotide is chemically synthesized, wherein the first polynucleotide comprises a 5' UTR; and

(ii) a second polynucleotide, wherein the second polynucleotide is synthesized by in vitro transcription, and wherein the second polynucleotide comprises a full open reading frame encoding a polypeptide, and a 3' UTR. In some embodiments, (i) and (ii) are chemically cross-linked or enzymatically ligated. In some embodiments, the first polynucleotide and the second polynucleotide are operably linked.

In any one of the aforementioned embodiments, the RNA element provides a translational regulatory activity which increases or enhances potency of the mRNA relative to an mRNA without the RNA element.

In any one of the aforementioned embodiments, the mRNA comprises a poly A tail (e.g., a poly A tail of about 100 nucleotides). In any one of the aforementioned embodiments, the mRNA comprises a 5' Cap 1 structure.

In any one of the aforementioned embodiments, the mRNA comprises at least one chemical modification. In some embodiments, the chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methyl cytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, and 2'-O-methyl uridine. In some embodiments, the chemical modification is selected from the group consisting of pseudouridine or a pseudouridine analog. In some embodiments, the chemical modification is N1-methylpseudouridine. In some embodiments, the mRNA is fully modified with N1-methylpseudouridine.

In some aspects, the disclosure provides a composition comprising any one of the aforementioned mRNAs and a pharmaceutically acceptable carrier.

In some embodiments, the disclosure provides a lipid nanoparticle comprising any one of the aforementioned mRNAs.

In some embodiments, the disclosure provides a pharmaceutical composition comprising a lipid nanoparticle comprising any one of the aforementioned mRNAs, and a pharmaceutically acceptable carrier.

In some embodiments, the disclosure provides a method of inhibiting or reducing leaky scanning of an mRNA by a PIC or ribosome, the method comprising: contacting a cell with any one of the aforementioned mRNAs, any one of the aforementioned compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical compositions.

In some aspects, the disclosure provides a method of increasing an amount of a polypeptide translated from a full open reading frame comprising an mRNA, the method comprising:

contacting a cell with any one of the aforementioned mRNAs, any one of the aforementioned compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical compositions.

In some aspects, the disclosure provides a method of increasing potency of a polypeptide translated from an mRNA, the method comprising: contacting a cell with any one of the aforementioned mRNAs, any one of the aforementioned compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical compositions.

In some aspects, the disclosure provides a method of increasing initiation of polypeptide synthesis at or from an initiation codon comprising an mRNA, the method comprising: contacting a cell with any one of the aforementioned mRNAs, any one of the aforementioned compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical compositions.

In some aspects, the disclosure provides a method of inhibiting or reducing initiation of polypeptide synthesis at any codon within an mRNA other than an initiation codon, the method comprising: contacting a cell with any one of the aforementioned mRNAs, any one of the aforementioned

compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical compositions.

In some aspects, the disclosure provides a method of inhibiting or reducing an amount of polypeptide translated from any open reading frame within an mRNA other than a full open reading frame, the method comprising: contacting a cell with any one of the aforementioned mRNAs, any one of the aforementioned compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical compositions.

In some aspects, the disclosure provides a method of inhibiting or reducing translation of truncated or aberrant translation products from an mRNA, the method comprising: contacting a cell with any one of the aforementioned mRNAs, any one of the aforementioned compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical compositions.

In some aspects, the disclosure provides a method of treating a disease, the method comprising: administering any one of the aforementioned mRNAs, any one of the aforementioned compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical compositions, wherein treatment results in the translation of the mRNA, wherein the translation results in the formation of a polypeptide that alleviates the disease or that does not cause or contribute to the disease.

In some aspects, the disclosure provides a kit comprising a container comprising any one of the aforementioned mRNAs, any one of the aforementioned compositions, any one of the aforementioned lipid nanoparticles, or any one of the aforementioned pharmaceutical composition and a package insert comprising instructions for use.

In some embodiments, the disclosure provides a method of identifying an RNA element that provides a translational regulatory activity, the method comprising: (i) synthesizing a 1^{st} control mRNA comprising: (a) a polynucleotide sequence comprising an open reading frame encoding a reporter polypeptide, an 1^{st} AUG codon upstream of, in-frame, and operably linked to the open reading frame encoding the reporter polypeptide; a coding sequence for a first epitope tag upstream of, in-frame, and operably linked to the 1^{st} AUG codon; a 2^{nd} AUG codon upstream of, in-frame, and operably linked to the coding sequence for the first epitope tag; a coding sequence for a second epitope tag upstream of, in-frame, and operably linked to the 2^{nd} AUG codon; a 3^{rd} AUG codon upstream of, in-frame, and operably linked to the coding sequence for the second epitope tag, a 5' UTR and a 3' UTR; and, (ii) synthesizing a 2^{nd} test mRNA comprising: (b)a polynucleotide sequence comprising an open reading frame encoding a reporter polypeptide, an 1^{st} AUG codon upstream of, in-frame, and operably linked to the open reading frame encoding the reporter polypeptide; a coding sequence for a first epitope tag upstream of, in-frame, and operably linked to the 1^{st} AUG codon; a 2^{nd} AUG codon upstream of, in-frame, and operably linked to the coding sequence for the first epitope tag; a coding sequence for a second epitope tag upstream of, in-frame, and operably linked to the 2^{nd} AUG codon; a 3^{rd} AUG codon upstream of, in-frame, and operably linked to the coding sequence for the second epitope tag, a 5' UTR and a 3' UTR, wherein the 5' UTR comprises a test RNA element; and (iii) introducing the 1st control mRNA and 2^{nd} test mRNA to conditions suitable for translation of the polynucleotide sequence encoding the reporter polypeptide; measuring the effect of the RNA element on the initiation of translation of the polynucleotide sequence encoding the reporter polypeptide from each of the three AUG codons.

In some embodiments, the reporter polypeptide is eGFP. In some embodiments, the epitope tag is selected from the group consisting of: a FLAG tag, a 3×FLAG tag, a Myc tag, a V5 tag, a hemagglutinin A (HA) tag, a histidine tag (e.g. a 6×His tag), an HSV tag, a VSV-G tag, an NE tag, an AviTag, a Calmodulin tag, an E tag, an S tag, an SBP tag, a Softag 1, a Softag 3, a Strep tag, a Ty tag, or an Xpress tag.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1**A depicts a schematic representation of reporter mRNA.

FIG. **1**B is a depiction of representative 5' UTR sequences. Sequences in order are set forth in SEQ ID NOs: 536-539 respectively.

FIG. **2**A depicts an SDS-PAGE/Western Blot of lysates derived from HeLa cells or murine embryonic fibroblasts (MEFs) that were transfected with reporter mRNAs containing 5' UTRs varying in length and/or base composition. Full-length and truncated translation products were detected using an eGFP-specific antibody.

FIG. **2**B depicts an SDS-PAGE/Western Blot of lysates derived from mouse livers from mice that were administered reporter mRNAs containing 5' UTRs varying in length and/or base composition. Full-length and truncated translation products were detected using an eGFP-specific antibody.

FIGS. **2**C and **2**D depict graphs representing the results of quantitative analysis of formation of truncated protein from experiments described in (A) and (B), respectively.

FIG. **3**A provides a schematic representation of reporter mRNA containing a 5' UTR consists of 1×, 2×, 3×, or 4× copies of the standard 5' UTR depicted in FIG. **1**B.

FIG. **3**B depicts an SDS-PAGE/Western Blot of lysates derived from HeLa cells that were administered reporter mRNA contain 5' UTRs consisting of 1×, 2×, 3×, or 4× copies of the standard 5' UTR as depicted in FIG. **3**A.

FIG. **3**C provides a graph representing the results of a quantitative analysis of formation of truncated protein from experiments shown in FIG. **3**B.

FIG. **3**D provides a graph representing the results of at quantitative analysis of formation of total full-length protein from experiments shown in FIG. **3**B.

FIG. **4**A provides a graph representing the results of small ribosome subunit footprinting analysis, wherein sequencing reads were mapped to a human transcriptome and the number of reads overlapping with each AUG in each mRNA was counted. The number of reads overlapping with each AUG was then normalized to the first AUG.

FIG. **4**B provides a graph representing the results of small ribosomal footprinting analysis, wherein the frequency of leaky scanning for each mRNA in primary human hepatocytes was estimated by dividing the mean small subunit read density in the first 500 nt of the coding sequence by the mean small subunit read density in the 5' UTR. This metric was plotted against length of 5' UTR. Each point represents an individual mRNA with at least 100 mapped reads. Black line represents a moving average.

FIG. **5**A provides a schematic representation of reporter mRNA containing GC-rich elements in the 5' UTR.

FIG. **5**B provides a picture and graph representing the results of experiments, wherein HeLa cells or murine embryonic fibroblasts (MEFs) were transfected with reporter mRNAs containing 5' UTRs with GC-rich RNA elements as indicated in FIG. **5**A. Full-length and truncated translation

products were visualized by SDS-PAGE/Western blot analysis using an eGFP-specific antibody. Quantitative analysis of formation of truncated protein is shown below Western blots.

FIGS. **6**A and **6**B provides graphs representing the results of experiments, wherein HeLa cells or human hepatocytes, as indicated, were transfected with reporter mRNAs for human Erythropoietin (Epo) containing 5' UTRs with GC-rich RNA elements depicted in FIG. **5**A and the amount of Epo was quantified.

FIGS. **6**C and **6**D provides a graphs representing the results of experiments, wherein HeLa cells or human hepatocytes, as indicated, were transfected with reporter mRNAs for luciferase (Luc) containing 5' UTRs with GC-rich RNA elements depicted in FIG. **5**A and the amount of Luc was quantified.

FIG. **7**A provides a graph depicting leaky scanning efficiency of 254 different 5' UTRs from natural and synthetic sources, varying in base composition and length, that were tested in HeLa cells with the eGFP reporter depicted in FIG. **3**, as measured by quantitative analysis of immunoblots.

FIG. **7**B provides a graph representing the results of small ribosome subunit footprinting analysis, wherein the frequency of leaky scanning for each mRNA in primary human hepatocytes was quantified and plotted against number of G and C bases in the final 20 nt of the 5' UTR. Each point represents an individual mRNA with at least 100 mapped reads. Black line represents a moving average.

FIG. **8**A is a table depicting the sequence of 5' UTRs tested in the reporter construct depicted in FIG. **8**B. 5' UTR sequences in order are set forth in SEQ ID NOs: 540-545 respectively.

FIG. **8**B is a diagram depicting the reporter construct and system used to test the effect of various 5' UTRs comprising GC-rich RNA elements, as shown in FIG. **8**A.

FIG. **9**A depicts an SDS-PAGE/Western Blot of lysates derived from hepatocytes that were administered reporter mRNA contain 5' UTRs as indicated 5' UTR as depicted in FIG. **8**A.

FIG. **9**B provides a graph representing the results of a quantitative analysis of formation of truncated protein from experiments shown in FIG. **9**A.

FIG. **10** provides a graph depicting the results of whole body imaging analysis of mice administered mRNAs comprising various 5' UTRs, as indicated, and encoding luciferase. Luminescence signal is given in total flux (p/s).

FIGS. **11**A, **11**B, and **11**C provides graphs depicting the results of fluorescence imaging analysis of cells administered mRNAs comprising V1-UTR and encoding eGFP in various cell types as indicated.

FIG. **12**A provides a graph representing the results of small ribosome subunit footprinting analysis using HeLa cells, wherein sequencing reads were mapped to a human transcriptome and the number of reads overlapping with each AUG in each mRNA was counted. The number of reads overlapping with each AUG was then normalized to the first AUG.

FIG. **12**B provides a graph representing the results of small ribosome subunit footprinting analysis using mouse spleen cells, wherein sequencing reads were mapped to a mouse transcriptome and the number of reads overlapping with each AUG in each mRNA was counted. The number of reads overlapping with each AUG was then normalized to the first AUG.

Modified Polynucleotides Comprising Functional RNA Elements

The present disclosure provides synthetic polynucleotides (e.g., mRNAs) comprising a modification (e.g., an RNA element), wherein the modification provides a desired translational regulatory activity. In some embodiments, the disclosure provides a polynucleotide comprising a 5' untranslated region (UTR), an initiation codon, a full open reading frame encoding a polypeptide, a 3' UTR, and at least one modification, wherein the at least one modification provides a desired translational regulatory activity, for example, a modification that promotes and/or enhances the translational fidelity of mRNA translation. In some embodiments, the desired translational regulatory activity is a cis-acting regulatory activity. In some embodiments, the desired translational regulatory activity is an increase in the residence time of the 43 S pre-initiation complex (PIC) or ribosome at, or proximal to, the initiation codon. In some embodiments, the desired translational regulatory activity is an increase in the initiation of polypeptide synthesis at or from the initiation codon. In some embodiments, the desired translational regulatory activity is an increase in the amount of polypeptide translated from the full open reading frame. In some embodiments, the desired translational regulatory activity is an increase in the fidelity of initiation codon decoding by the PIC or ribosome. In some embodiments, the desired translational regulatory activity is inhibition or reduction of leaky scanning by the PIC or ribosome. In some embodiments, the desired translational regulatory activity is a decrease in the rate of decoding the initiation codon by the PIC or ribosome. In some embodiments, the desired translational regulatory activity is inhibition or reduction in the initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon. In some embodiments, the desired translational regulatory activity is inhibition or reduction of the amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame. In some embodiments, the desired translational regulatory activity is inhibition or reduction in the production of aberrant translation products. In some embodiments, the desired translational regulatory activity is a combination of one or more of the foregoing translational regulatory activities.

Accordingly, the present disclosure provides a polynucleotide, e.g., an mRNA, comprising an RNA element that comprises a sequence and/or an RNA secondary structure(s) that provides a desired translational regulatory activity as described herein. In some aspects, the mRNA comprises an RNA element that comprises a sequence and/or an RNA secondary structure(s) that promotes and/or enhances the translational fidelity of mRNA translation. In some aspects, the mRNA comprises an RNA element that comprises a sequence and/or an RNA secondary structure(s) that provides a desired translational regulatory activity, such as inhibiting and/or reducing leaky scanning. In some aspects, the disclosure provides an mRNA that comprises an RNA element that comprises a sequence and/or an RNA secondary structure(s) that inhibits and/or reduces leaky scanning thereby promoting the translational fidelity of the mRNA.

RNA Elements

In some embodiments, the disclosure provides mRNAs comprising RNA elements that provide one or more translational regulatory activities. In some embodiments, the disclosure provides mRNAs comprising RNA elements that provide one or more translational regulatory activities which improve potency of an mRNA having the RNA element (e.g., a G C-rich RNA element located in the 5' UTR), relative to an mRNA without the RNA element. An RNA element is a portion, fragment or segment of an RNA

molecule that has biological significance (e.g., provides a biological function or activity such as a translational regulatory activity). In some embodiments, an RNA element comprises a GC-rich RNA element. In some embodiments, an RNA element comprises a stable RNA secondary structure. In some embodiments, the RNA element provides one or more translational regulatory activities.

GC-Rich RNA Elements

In some embodiments, the disclosure provides mRNAs with 5' UTRs comprising an RNA element that is a GC-rich RNA element that provides a translational regulatory activity. In some embodiments, the disclosure provides mRNAs with 5' UTRs comprising an RNA element that is a GC-rich RNA element that provides a translational regulatory activity which improves potency of the mRNA having the RNA element relative to an mRNA without the element. In some embodiments, the translational regulatory activity is selected from the group consisting of:

(a) inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome;

(b) increases an amount of a polypeptide translated from the full open reading frame;

(c) increases initiation of polypeptide synthesis at or from the initiation codon;

(d) inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon;

(e) inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame;

inhibits or reduces translation of truncated or aberrant translation products from the mRNA; and

(g) a combination of any of (a)-(g).

In some embodiments, the GC-rich RNA element inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome. In some embodiments, the GC-rich RNA element inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the GC-rich RNA element increases an amount of a polypeptide translated from the full open reading frame. In some embodiments, the GC-rich RNA element increases an amount of a polypeptide translated from the full open reading frame and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the GC-rich RNA element increases potency of a polypeptide translated from the mRNA. In some embodiments, the GC-rich RNA element increases potency of a polypeptide translated from the mRNA and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the GC-rich RNA element increases initiation of polypeptide synthesis at or from the initiation codon. In some embodiments, the GC-rich RNA element increases initiation of polypeptide synthesis at or from the initiation codon and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the GC-rich RNA element inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon. In some embodiments, the GC-rich RNA element inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the GC-rich RNA element inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame. In some embodiments, the GC-rich RNA element inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the GC-rich RNA element inhibits or reduces translation of truncated or aberrant translation products from the mRNA. In some embodiments, the GC-rich RNA element inhibits or reduces translation of truncated or aberrant translation products from the mRNA and improves (e.g., increases or enhances) potency of the mRNA.

In some embodiments, the GC-rich RNA element comprises guanine (G) and cytosine (C) nucleobases, or derivatives or analogues thereof and, optionally, adenine (A) and uracil (U) nucleobases, or derivatives or analogues thereof. In some embodiments, the GC-rich RNA element does not comprise adenine (A) nucleobases. In some embodiments, the GC-rich RNA element does not comprise uracil (U) nucleobases. In some embodiments, the GC-rich RNA element does not comprise adenine (A) or uracil (U) nucleobases.

In some embodiments, the GC-rich RNA element is at least 50% or greater cytosine (C) nucleobases. In some embodiments, the GC-rich RNA element is about 50%-55% cytosine, about 55%-60% cytosine, about 60%-65% cytosine, about 65%-70% cytosine, about 70%-75% cytosine or about 75%-80% cytosine. In some embodiments, the GC-rich RNA element is >50% cytosine, >60% cytosine or >70% cytosine nucleobases. In some embodiments, the GC-rich RNA element is >50% cytosine. In some embodiments, the GC-rich RNA element is >60% cytosine. In some embodiments, the GC-rich RNA element is >70% cytosine.

In some embodiments, the GC-rich RNA element is at least 6 nucleotides in length. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence of about 6-10 nucleotides in length, about 10-15 nucleotides in length, about 15-20 nucleotides in length, about 20-25 nucleotides in length or about 25-30 nucleotides in length. In some embodiments, the GC-rich RNA element is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length.

In some embodiments, the GC-rich RNA The mRNA of any one of claims **1-5**, wherein the GC-rich RNA element comprises a nucleotide sequence 6 nucleotides in length and comprises >50% cytosine, >60% cytosine or >70% cytosine nucleobases. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 7 nucleotides in length and comprises >50% cytosine, >60% cytosine or >70% cytosine nucleobases. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 8 nucleotides in length and comprises >50% cytosine, >60% cytosine or >70% cytosine nucleobases. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 9 nucleotides in length and >50% cytosine, >60% cytosine or >70% cytosine nucleobases. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 10 nucleotides in length and comprises >50% cytosine, >60% cytosine or >70% cytosine nucleobases. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence 20 nucleotides in length, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases. In some embodiments, the GC-rich RNA element comprises a nucleotide sequence of about 6-30 guanine (G) and cytosine (C) nucleotides, or derivatives or analogues thereof, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases, and wherein the sequence comprises a repeating sequence motif.

In any of the foregoing or related aspects, the disclosure provides a GC-rich RNA element which comprises a

sequence of 3-30, 5-25, 10-20, 15-20, about 20, about 15, about 12, about 10, about 7, about 6 or about 3 nucleotides, derivatives or analogs thereof, linked in any order, wherein the sequence composition is 70-80% cytosine, 60-70% cytosine, 50%-60% cytosine, 40-50% cytosine, 30-40% cytosine bases. In any of the foregoing or related aspects, the disclosure provides a GC-rich RNA element which comprises a sequence of 3-30, 5-25, 10-20, 15-20, about 20, about 15, about 12, about 10, about 7, about 6 or about 3 nucleotides, derivatives or analogs thereof, linked in any order, wherein the sequence composition is about 80% cytosine, about 70% cytosine, about 60% cytosine, about 50% cytosine, about 40% cytosine, or about 30% cytosine.

In any of the foregoing or related aspects, the disclosure provides a GC-rich RNA element which comprises a sequence of 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, or 3 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence composition is 70-80% cytosine, 60-70% cytosine, 50%-60% cytosine, 40-50% cytosine, or 30-40% cytosine. In any of the foregoing or related aspects, the disclosure provides a GC-rich RNA element which comprises a sequence of 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, or 3 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence composition is about 80% cytosine, about 70% cytosine, about 60% cytosine, about 50% cytosine, about 40% cytosine, or about 30% cytosine.

In some embodiments, the disclosure provides an mRNA comprising a GC-rich RNA element, wherein the GC-rich RNA element is located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of an initiation codon and within a 5' UTR. In some embodiments, the GC-rich RNA element is located 6 nucleotides upstream of an initiation codon and within a 5' UTR. In some embodiments, the GC-rich RNA element is located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR. In some embodiments, the GC-rich RNA element upstream of a Kozak sequence in a 5' UTR. In some embodiments, the GC-rich RNA element is upstream of a Kozak consensus sequence in a 5' UTR. In some embodiments, the GC-rich RNA element is upstream of a Kozak-like sequence in a 5' UTR.

In some embodiments, the disclosure provides a modified mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising a sequence of linked nucleotides, or derivatives or analogs thereof, preceding a Kozak consensus sequence in a 5' UTR of the mRNA, wherein the GC-rich RNA element is located about 30, about 25, about 20, about 15, about 10, about 5, about 4, about 3, about 2, or about 1 nucleotide(s) upstream of a Kozak consensus sequence in the 5' UTR of the mRNA, and wherein the GC-rich RNA element comprises a sequence of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence composition is >50% cytosine. In some embodiments, the sequence composition is >55% cytosine, >60% cytosine, >65% cytosine, >70% cytosine, >75% cytosine, >80% cytosine, >85% cytosine, or >90% cytosine.

In other aspects, the disclosure provides an mRNA comprising a GC-rich RNA element, wherein the GC-rich RNA element comprises a repeating sequence motif. In some embodiments the repeating sequence motif is $[CCG]_n$, wherein n=2 to 10, 2 to 5, 4, 3 or 2. In some embodiments, the repeating sequence motif is $[GCC]_n$, where n=2 to 10, 2 to 5, 4, 3 or 2. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 12. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 13. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 14. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 15. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 16. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 17. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 18. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 19. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 20. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 21. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 22. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 23. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 24. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 25. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 26. In some embodiments, a GC-rich RNA element comprising a repeating sequence motif comprises the nucleotide sequence set forth in SEQ ID NO: 27.

In other aspects, the disclosure provides an mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising a sequence of linked nucleotides, or derivatives or analogs thereof, preceding a Kozak consensus sequence in a 5' UTR of the mRNA, wherein the GC-rich RNA element is located about 30, about 25, about 20, about 15, about 10, about 5, about 4, about 3, about 2, or about 1 nucleotide(s) upstream of a Kozak consensus sequence in the 5' UTR of the mRNA, and wherein the GC-rich RNA element comprises a sequence of about 3-30, 5-25, 10-20, 15-20 or about 20, about 15, about 12, about 10, about 6 or about 3 nucleotides, or derivatives or analogues thereof, wherein the sequence comprises a repeating GC-motif, wherein the repeating GC-motif is $[CCG]_n$, wherein n=1 to 10, n=2 to 8, n=3 to 6, or n=4 to 5. In some embodiments, the sequence comprises a repeating GC-motif $[CCG]_n$, wherein n=1, 2, 3, 4 or 5. In some embodiments, the sequence comprises a repeating GC-motif $[CCG]_n$, wherein n=1, 2, or 3. In some embodiments, the sequence comprises a repeating GC-motif $[CCG]_n$, wherein n=1. In some embodiments, the sequence comprises a repeating GC-motif $[CCG]_n$, wherein n=2. In some embodiments, the sequence comprises a repeating GC-motif $[CCG]_n$, wherein n=3. In some embodiments, the sequence comprises a repeating GC-motif $[CCG]_n$, wherein

n=4. In some embodiments, the sequence comprises a repeating GC-motif $[CCG]_n$, wherein n=5.

In another aspect, the disclosure provides mRNAs comprising a GC-rich RNA element, wherein the GC-rich RNA element is located in the 5' UTR upstream of the 3' end of the 5' UTR, and wherein the GC-rich RNA element comprises any one of the GC-rich RNA elements comprising a nucleotide sequence set forth in SEQ ID NO: 2 to SEQ ID NO: 27. In one embodiment, the disclosure provides mRNAs comprising a GC-rich RNA element, wherein the GC-rich RNA element is located in the 5' UTR upstream of the 3' end of the 5' UTR, wherein the GC-rich RNA element comprises any one of the GC-rich RNA elements comprising a nucleotide sequence set forth in SEQ ID NO: 2 to SEQ ID NO: 27, and wherein the GC-rich RNA element is located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR.

In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element, wherein the GC-rich RNA element is located in the 5' UTR upstream of the 3' end of the 5' UTR, wherein the GC-rich RNA element comprises any one of the GC-rich RNA elements comprising a nucleotide sequence set forth in SEQ ID NO: 2 to SEQ ID NO: 27, and wherein the GC-rich RNA element is located about 20-30 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element, wherein the GC-rich RNA element is located in the 5' UTR upstream of the 3' end of the 5' UTR, wherein the GC-rich RNA element comprises any one of the GC-rich RNA elements comprising a nucleotide sequence set forth in SEQ ID NO: 2 to SEQ ID NO: 27, and wherein the GC-rich RNA element is located about 10-20 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element, wherein the GC-rich RNA element is located in the 5' UTR upstream of the 3' end of the 5' UTR, wherein the GC-rich RNA element comprises any one of the GC-rich RNA elements comprising a nucleotide sequence set forth in SEQ ID NO: 2 to SEQ ID NO: 27, and wherein the GC-rich RNA element is located about 6-10 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element, wherein the GC-rich RNA element is located in the 5' UTR upstream of the 3' end of the 5' UTR, wherein the GC-rich RNA element comprises any one of the GC-rich RNA elements comprising a nucleotide sequence set forth in SEQ ID NO: 2 to SEQ ID NO: 27, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR.

In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 2, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising any one of the GC-rich RNA elements set forth in SEQ ID NO: 3 to SEQ ID NO: 27, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 4, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 5, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 6, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element, wherein the GC-rich RNA element is located in the 5' UTR upstream of the 3' end of the 5' UTR, wherein the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 7, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element, wherein the GC-rich RNA comprises the nucleotide sequence set forth in SEQ ID NO: 8, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 9, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 10, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR.

In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 11, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 12, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 13, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 14, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 15, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 16, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 17, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides an mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 18, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides an mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 19, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one

embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 20, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 21, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 22, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 23, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 24, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 25, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 26, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR. In one embodiment, the disclosure provides a mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 27, and wherein the GC-rich RNA element is located about 6 nucleotides upstream of the 3' end of the 5' UTR.

In another aspect, the disclosure provides an mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising a sequence of linked nucleotides, or derivatives or analogs thereof, preceding a Kozak consensus sequence in a 5' UTR of the mRNA, wherein the GC-rich RNA element comprises any one of the sequences set forth in SEQ ID NO: 2 to SEQ ID NO: 27.

In one embodiment, the GC-rich RNA element is located about 30, about 25, about 20, about 15, about 10, about 5, about 4, about 3, about 2, or about 1 nucleotide(s) upstream of a Kozak consensus sequence in the 5' UTR of the mRNA. In another embodiment, the GC-rich RNA element is located about 15-30, 15-20, 15-25, 10-15, or 5-10 nucleotides upstream of a Kozak consensus sequence. In another embodiment, the GC-rich RNA element is located immediately adjacent to a Kozak consensus sequence in the 5' UTR of the mRNA.

In another aspect, the disclosure provides an mRNA comprising a GC-rich RNA element comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 7 and SEQ ID NO: 8. In some embodiments, the mRNA provided by the disclosure comprises a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 2. In some embodiments, the mRNA provided by the disclosure comprises a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 3. In some embodiments, the disclosure provides an mRNA comprising a GC-rich RNA element comprising the nucleotide sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 5.

In other aspects, the disclosure provides a modified mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising the nucleotide sequence V1 [CCCCGGCGCC] (SEQ ID NO: 2) as set forth in Table 1, or derivatives or analogs thereof, preceding a Kozak consensus sequence in the 5' UTR of the mRNA. In some embodiments, the GC-rich element comprises the nucleotide sequence V1 [CCCCGGCGCC] (SEQ ID NO: 2) as set forth in Table 1 located immediately adjacent to and upstream of the Kozak consensus sequence in the 5' UTR of the mRNA. In some embodiments, the GC-rich element comprises the nucleotide sequence V1 [CCCCGGCGCC] (SEQ ID NO: 2) as set forth in Table 1 located 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 bases upstream of the Kozak consensus sequence in the 5' UTR of the mRNA. In other embodiments, the GC-rich element comprises the nucleotide sequence V1 [CCCCGGCGCC] (SEQ ID NO: 2) as set forth in Table 1 located 1-3, 3-5, 5-7, 7-9, 9-12, or 12-15 bases upstream of the Kozak consensus sequence in the 5' UTR of the mRNA.

In other aspects, the disclosure provides a modified mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising the nucleotide sequence V2 [CCCCGGC] (SEQ ID NO: 3) as set forth in Table 1, or derivatives or analogs thereof, preceding a Kozak consensus sequence in the 5' UTR of the mRNA. In some embodiments, the GC-rich element comprises the nucleotide sequence V2 [CCCCGGC] (SEQ ID NO: 3) as set forth in Table 1 located immediately adjacent to and upstream of the Kozak consensus sequence in the 5' UTR of the mRNA. In some embodiments, the GC-rich element comprises the nucleotide sequence V2 [CCCCGGC] (SEQ ID NO: 3) as set forth in Table 1 located 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 bases upstream of the Kozak consensus sequence in the 5' UTR of the mRNA. In other embodiments, the GC-rich element comprises the nucleotide sequence V2 [CCCCGGC] (SEQ ID NO: 3) as set forth in Table 1 located 1-3, 3-5, 5-7, 7-9, 9-12, or 12-15 bases upstream of the Kozak consensus sequence in the 5' UTR of the mRNA.

In other aspects, the disclosure provides a modified mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising the sequence EK2 [GCCGCC] (SEQ ID NO: 10) as set forth in Table 1, or derivatives or analogs thereof, preceding a Kozak consensus sequence in the 5' UTR of the mRNA. In some embodiments, the GC-rich element comprises the sequence EK2 [GCCGCC] (SEQ ID NO: 10) as set forth in Table 1 located immediately adjacent to and upstream of the Kozak consensus sequence in the 5' UTR of the mRNA. In some embodiments, the GC-rich element comprises the sequence EK2 [GCCGCC] (SEQ ID NO: 10) as set forth in Table 1 located 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 bases upstream of the Kozak consensus sequence in the 5' UTR of the mRNA. In other embodiments, the GC-rich element comprises the sequence EK2 [GCCGCC] (SEQ ID NO: 10) as set forth in Table 1 located 1-3, 3-5, 5-7, 7-9, 9-12, or 12-15 bases upstream of the Kozak consensus sequence in the 5' UTR of the mRNA.

In yet other aspects, the disclosure provides a modified mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising the sequence V1 [CCCCGGCGCC] (SEQ ID NO: 2) as set forth in Table 1, or derivatives or analogs thereof, preceding a Kozak consensus sequence in the 5' UTR of the mRNA, wherein the 5' UTR comprises the following sequence shown in Table 1:

(SEQ ID NO: 33)
GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC.

In some embodiments, the GC-rich element comprises the sequence V1 (SEQ ID NO: 2) as set forth in Table 1 located immediately adjacent to and upstream of the Kozak consensus sequence in the 5' UTR sequence shown in Table 1. In some embodiments, the GC-rich element comprises the sequence V1 (SEQ ID NO: 2) as set forth in Table 1 located 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 bases upstream of the Kozak consensus sequence in the 5' UTR of the mRNA, wherein the 5' UTR comprises the following sequence shown in Table 1:

(SEQ ID NO: 33)
GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC.

In other embodiments, the GC-rich element comprises the sequence V1 (SEQ ID NO: 2) as set forth in Table 1 located 1-3, 3-5, 5-7, 7-9, 9-12, or 12-15 bases upstream of the Kozak consensus sequence in the 5' UTR of the mRNA, wherein the 5' UTR comprises the following sequence shown in Table 1:

(SEQ ID NO: 33)
GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC.

In some embodiments, the 5' UTR comprises the following sequence set forth in Table 1:

(SEQ ID NO: 33)
GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGACCCCGGCGC

CGCCACC.

In some embodiments, the disclosure provides an mRNA comprising a 5' UTR, wherein the 5' UTR comprises the nucleotide sequence 5 '-GGGAAAUAAGAGAGAAA AGAAGAGUAAGAAGAAAUAUAAGAGCCACC-3' set forth in SEQ ID NO: 33, wherein the 5' UTR comprises a GC-rich RNA element located about 20-30, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33. In some embodiments, the disclosure provides an mRNA comprising: (i) a 5' untranslated region (UTR) comprising a GC-rich RNA element that provides a translational regulatory activity described herein; (ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and (iii) a 3' UTR, wherein the 5' UTR comprises the nucleotide sequence 5'-GGGAAAUAAGAGAGAAAAGAAGAGUA AG AAG AAAUAUAAGAGCCACC-3' set forth in SEQ ID NO: 33, wherein the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 2, and wherein the 5' UTR comprises the GC-rich RNA element located about 20-30, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33. In some embodiments, the disclosure provides an mRNA comprising: (i) a 5' untranslated region (UTR) comprising a GC-rich RNA element that provides a translational regulatory activity described herein; (ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and (iii) a 3' UTR, wherein the 5' UTR comprises the nucleotide sequence 5'-GG-GAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAA UAUAAGAGCCACC-3' set forth in SEQ ID NO: 33, wherein the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 3, and wherein the GC-rich RNA element is located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33. In some embodiments, the disclosure provides an mRNA comprising: (i)a 5' untranslated region (UTR) comprising a GC-rich RNA element that provides a translational regulatory activity described herein; (ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and (iii) a 3' UTR, wherein the 5' UTR comprises the nucleotide sequence 5'-GGGAAAUAAGAGAGAAAAG AAGAGUAAGAAGAAAUAUAAGAGCCACC-3' set forth in SEQ ID NO: 33, wherein the GC-rich RNA element comprises the nucleotide sequence set forth in SEQ ID NO: 4, and wherein the GC-rich RNA element is located about 20-30 nucleotides, about 10-20 nucleotides, or about 6-10 nucleotides upstream of the 3' end of the 5' UTR sequence set forth in SEQ ID NO: 33.

In some embodiments, the disclosure provides an mRNA comprising (i) a 5' untranslated region (UTR) comprising the nucleotide sequence set forth in SEQ ID NO: 34; (ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and (iii) a 3' UTR.

In some embodiments, the disclosure provides an mRNA comprising (i) a 5' untranslated region (UTR) comprising the nucleotide sequence set forth in SEQ ID NO: 54; (ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and (iii) a 3' UTR. An mRNA comprising (i) a 5' untranslated region (UTR) comprising the nucleotide sequence set forth in SEQ ID NO: 73 (CG1-UTR) (ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and (iii) a 3' UTR.

Stable RNA Secondary Structures

In some embodiments, the disclosure provides mRNAs comprising RNA elements that provide one or more translational regulatory activities arising from the formation of a secondary structure. Without being bound by theory, it is thought that an RNA element that provides a function (e.g, a translational regulatory activity) by the formation of a secondary structure (e.g. a stable RNA secondary structure) is distinguished from an RNA element that provide a translational regulatory activity provided by the RNA element's primary structure or sequence (e.g., a GC-rich RNA element). Typical examples of stable RNA secondary structures include duplexes, hairpins, and stem-loops.

Accordingly, in some embodiments, the disclosure provides mRNAs comprising an RNA element that comprises a stable RNA secondary structure that provides a translational regulatory activity. In some embodiments, the translational regulatory activity is selected from the group consisting of:

(a) inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome;

(b) increases an amount of a polypeptide translated from the full open reading frame;

(c) increases initiation of polypeptide synthesis at or from the initiation codon;

(d) inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon;

(e) inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame;

(f) inhibits or reduces translation of truncated or aberrant translation products from the mRNA; and

(g) a combination of any of (a)-(f).

In some embodiments, the stable RNA secondary structure inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome. In some embodiments, the stable RNA secondary structure inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the Stable RNA secondary structure increases an amount of a polypeptide translated from the full open reading frame. In

some embodiments, the stable RNA secondary structure increases an amount of a polypeptide translated from the full open reading frame and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the stable RNA secondary structure increases potency of a polypeptide translated from the mRNA. In some embodiments, the stable RNA secondary structure increases potency of a polypeptide translated from the mRNA and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the stable RNA secondary structure increases initiation of polypeptide synthesis at or from the initiation codon. In some embodiments, the stable RNA secondary structure increases initiation of polypeptide synthesis at or from the initiation codon and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the stable RNA secondary structure inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon. In some embodiments, the stable RNA secondary structure inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the stable RNA secondary structure inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame. In some embodiments, the stable RNA secondary structure inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the stable RNA secondary structure inhibits or reduces translation of truncated or aberrant translation products from the mRNA. In some embodiments, the stable RNA secondary structure inhibits or reduces translation of truncated or aberrant translation products from the mRNA and improves (e.g., increases or enhances) potency of the mRNA. In some embodiments, the stable RNA secondary structure is located downstream of the initiation codon in the full open reading frame. In some embodiments, the stable RNA secondary structure is located about 25-30, about 20-25, about 15-20, about 10-15, about 5-10, or about 1-5 nucleotide(s) downstream of the initiation codon in the full open reading frame.

In some embodiments, the stable RNA secondary structure is located 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 nucleotide(s) downstream of the initiation codon in the full open reading frame. In some embodiments, the stable RNA secondary structure is located upstream of the initiation codon in the 5' UTR.

In some embodiments, the stable RNA secondary structure is located about 25-30, about 20-25, about 15-20, about 10-15, about 5-10, or about 1-5 nucleotide(s) upstream of the initiation codon in the 5' UTR. In some embodiments, the stable RNA secondary structure is located 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 nucleotide(s) upstream of the initiation codon in the 5' UTR.

In some embodiments, the stable RNA secondary structure comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

In another aspect, the disclosure provides a modified mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising a stable RNA secondary structure comprising a sequence of nucleotides, or derivatives or analogs thereof, linked in an order which forms a hairpin or a stem-loop. In one embodiment, the stable RNA secondary structure is upstream or downstream of the initiation codon. In another embodiment, the stable RNA secondary structure is located about 30, about 25, about 20, about 15, about 10, or about 5 nucleotides upstream or downstream of the initiation codon. In another embodiment, the stable RNA secondary structure is located about 20, about 15, about 10 or about 5 nucleotides upstream or downstream of the initiation codon. In another embodiment, the stable RNA secondary structure is located about 5, about 4, about 3, about 2, about 1 nucleotides upstream or downstream of the initiation codon. In another embodiment, the stable RNA secondary structure is located about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides upstream or downstream of the initiation codon. In another embodiment, the stable RNA secondary structure is located 12-15 nucleotides upstream and downstream of the initiation codon. In another embodiment, the stable RNA secondary structure comprises the initiation codon. In another embodiment, the stable RNA secondary structure has a deltaG of about −30 kcal/mol, about −20 to −30 kcal/mol, about −20 kcal/mol, about −10 to −20 kcal/mol, about −10 kcal/mol, about −5 to ~10 kcal/mol.

In another embodiment, the modification is operably linked to an open reading frame encoding a polypeptide and wherein the modification and the open reading frame are heterologous.

In another embodiment, the sequence of the GC-rich RNA element is comprised exclusively of guanine (G) and cytosine (C) nucleobases.

In some aspects, the disclosure provides an mRNA having one or more structural modifications that inhibits leaky scanning and/or promotes the translational fidelity of mRNA translation, wherein at least one of the structural modifications is a GC-rich RNA element. In some aspects, the disclosure provides a modified mRNA comprising at least one modification, wherein at least one modification is a GC-rich RNA element comprising a sequence of linked nucleotides, or derivatives or analogs thereof, preceding a Kozak consensus sequence in a 5' UTR of the mRNA. In one embodiment, the GC-rich RNA element is located about 30, about 25, about 20, about 15, about 10, about 5, about 4, about 3, about 2, or about 1 nucleotide(s) upstream of a Kozak consensus sequence in the 5' UTR of the mRNA. In another embodiment, the GC-rich RNA element is located 15-30, 15-20, 15-25, 10-15, or 5-10 nucleotides upstream of a Kozak consensus sequence. In another embodiment, the GC-rich RNA element is located immediately adjacent to a Kozak consensus sequence in the 5' UTR of the mRNA. In some embodiments, the RNA element comprises natural and/or modified nucleotides. In some embodiments, the RNA element comprises of a sequence of linked nucleotides, or derivatives or analogs thereof, that provides a desired translational regulatory activity as described herein. In some embodiments, the RNA element comprises a sequence of linked nucleotides, or derivatives or analogs thereof, that forms or folds into a stable RNA secondary structure, wherein the RNA secondary structure provides a desired translational regulatory activity as described herein. RNA elements can be identified and/or characterized based on the primary sequence of the element (e.g., GC-rich RNA element), by RNA secondary structure formed by the element (e.g. stem-loop), by the location of the element within the RNA molecule (e.g., located within the 5' UTR of an mRNA), by the biological function and/or activity of the element (e.g., "translational enhancer element"), and any combination thereof.

Exemplary 5' UTRs, and modifications including GC-rich elements, and stable RNA secondary structures (e.g. hairpins) provided by the disclosure are set forth in Table 1. These 5' UTRs, and modifications including GC-rich elements, and stable RNA secondary structures, and any combination thereof, are useful in the mRNAs of the disclosure.

TABLE 1

5' UTRs	Sequence
Standard	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATA AGAGCCACC (SEQ ID NO: 33)
V1-UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATA AGACCCCGGCGCCGCCACC (SEQ ID NO: 34)
V2-UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATA AGACCCCGGCGCCACC (SEQ ID NO: 54)
CG1-UTR	GGGAAATAAGAGAGAAAAGAAGAGT/kAG/VAGAAATATA AGAGCGCCCCGCGGCGCCCCGCGGCCACC (SEQ ID NO: 73)
CG2-UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATA AGACCCGCCCGCCCCGCCCCGCCGCCACC (SEQ ID NO: 92)
KT1-UTR	GGGCCCGCCGCCAAC (SEQ ID NO: 472)
KT2-UTR	GGGCCCGCCGCCACC (SEQ ID NO: 473)
KT3-UTR	GGGCCCGCCGCCGAC (SEQ ID NO: 474)
KT4-UTR	GGGCCCGCCGCCGCC (SEQ ID NO: 475)
GC-Rich RNA Elements	**Sequence**
K0 (Traditional Kozak consensus)	[GCCA/GCC]
EK1	[CCCGCC](SEQ ID NO: 9)
EK2	[GCCGCC](SEQ ID NO: 10)
EK3	[CCGCCG](SEQ ID NO: 11)
V1	[CCCCGGCGCC] (SEQ ID NO: 2)
V2	[CCCCGGC] (SEQ ID NO: 3)
CG1	[GCGCCCCGCGGCGCCCCGCG] (SEQ ID NO: 4)
CG2	[CCCGCCCGCCCCGCCCCGCC] (SEQ ID NO: 5)
$(CCG)_n$, n = 1-10	$[CCG]_n$
$(GCC)_n$, n = 1-10	$[GCC]_n$
Stable RNA Secondary Structures	**Sequence**
SL1	CCGCGGCGCCCCGCGG (−−9.90 kcal/mol) (SEQ ID NO: 28)
SL2	GCGCGCAUAUAGCGCGC (−10.90 kcal/mol) (SEQ ID NO: 29)
SL3	CATGGTGGCGGCCCGCCGCCACCATG (−22.10 kcal/mol) (SEQ ID NO: 30)
SL4	CATGGTGGCCCGCCGCCACCATG (−14.90 kcal/mol) (SEQ ID NO: 31)
SL5	CATGGTGCCCGCCGCCACCATG (−8.00 kcal/mol) (SEQ ID NO: 32)

Methods to Identify and Characterize the Function of RNA Elements

In one aspect, the disclosure provides methods to identify and/or characterize RNA elements that provide a desired translational regulatory activity of the disclosure, including those that modulate (e.g., reduce) leaking scanning to polynucleotides (e.g., mRNA).

Ribosome Profiling

In one aspect, RNA elements that provide a desired translational regulatory activity, including modulation of leaking scanning, to polynucleotides e.g., mRNA, are identified and/or characterized by ribosome profiling.

Ribosome profiling is a technique that allows the determination of the number and position of ribosomes bound to mRNAs (see e.g., Ingolia et al., (2009) Science 324(5924): 218-23, incorporated herein by reference). The technique is based on protection by the ribosome of a region or segment of mRNA from ribonuclease digestion, which region or segment is subsequently assayed. In this approach, a cell lysate is treated with ribonucleases, leading to generation of

80S ribosomes with fragments of mRNA to which they are bound. The 80S ribosomes are then purified by techniques known in the art (e.g., density gradient centrifugation), and mRNA fragments that are protected by the ribosomes are isolated. Protection results in the generation of a 30-bp fragment of RNA termed a 'footprint'. The number and sequence of RNA footprints can be analyzed by methods known in the art (e.g., Ribo-seq, RNA-seq). The footprint is roughly centered on the A-site of the ribosome. During translation, a ribosome may dwell at a particular position or location along an mRNA (e.g., at an initiation codon). Footprints generated at these dwell positions are more abundant than footprints generated at positions along the mRNA where the ribosome is more processive. Studies have shown that more footprints are generated at positions where the ribosome exhibits decreased processivity (dwell positions) and fewer footprints where the ribosome exhibits increased processivity (Gardin et al., (2014) eLife 3:e03735). High-throughput sequencing of these footprints provides information on the mRNA locations (sequence of footprints) of ribosomes and generates a quantitative measure of ribosome density (number of footprints comprising a particular sequence) along an mRNA. Accordingly, ribosome profiling data provides information that can be used to identify and/or characterize RNA elements that provide a desired translational regulatory activity of the disclosure, including those that reduce leaky scanning, to polynucleotides as described herein e.g., mRNA.

Ribosome profiling can also be used to determine the extent of ribosome density (aka "ribosome loading") on an mRNA. It is known that dissociated ribosomal subunits initiate translation at the initiation codon within the 5'-terminal region of mRNA. Upon initiation, the translating ribosome moves along the mRNA chain toward the 3'-end of mRNA, thus vacating the initiation site for loading the next ribosome on the mRNA. In this way a group of ribosomes moving one after another and translating the same mRNA chain is formed. Such a group is referred to as a "polyribosome" or "polysome" (Warner et al., (1963) Proc Natl Acad Sci USA 49:122-129). The number of different mRNA fragments protected by ribosomes per mRNA, per region of an mRNA (e.g., a 5' UTR), or per location in an mRNA (e.g., an initiation codon) indicates an extent of ribosome density. In general, an increase in the number of ribosomes bound to an mRNA (i.e. ribosome density) is associated with increased levels of protein synthesis.

Accordingly, in some embodiments, an increase in ribosome density of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, an increase in ribosome density of a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by ribosome density.

Ribosome profiling is also used to determine the time, extent, rate and/or fidelity of ribosome decoding of a particular codon of an mRNA (and by extension the expected number of corresponding RNA-seq reads in a library of isolated footprints), which in turn is determined by the amount of time a ribosome spends at a particular codon (dwell time). The latter is referred to as a "codon elongation rate" or a "codon decoding rate". Relative dwell time of ribosomes between two locations in an mRNA, instead of the actual or absolute dwell time at a single location, can also be determined by the comparing the number of sequencing reads of protected mRNA fragments at each location (e.g., a codon) (O'Connor et al., (2016) Nature Commun 7:12915). For example, initiation of polypeptide synthesis at or from an initiation codon can be determined from an observed increase in dwell time of ribosomes at the initiation codon relative to dwell time of ribosomes at a downstream alternate or alternative initiation codon in an mRNA. Accordingly, initiation of polypeptide synthesis at or from an initiation codon in a polynucleotide (e.g., an mRNA) comprising one or more modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, can be determined from an observed increase in the dwell time of ribosomes at the initiation codon relative to the dwell time of ribosomes at a downstream alternate or alternative initiation codon in each polynucleotide (e.g., mRNA).

In some embodiments, an increase in residence time or the time of occupancy (dwell time) of a ribosome at a discrete position or location (e.g., an initiation codon) along a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some aspects, an increase in residence time or the time of occupancy of a ribosome at an initiation codon in a polynucleotide (e.g., mRNA) comprising a GC-rich element of the disclosure relative to a polynucleotide (e.g., mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

In other aspects, an increase in the initiation of polypeptide synthesis at or from the initiation codon in polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, an increase in the initiation of polypeptide synthesis at or from the initiation codon in a polynucleotide (e.g., mRNA) comprising a GC-rich element of the disclosure relative to a polynucleotide (e.g., mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

In some embodiments, an increase in fidelity of initiation codon decoding by the ribosome of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, an increase in fidelity of initiation codon decoding by the ribosome of a polynucleotide (e.g., mRNA) comprising a GC-rich element of the disclosure relative to a polynucleotide (e.g., mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

In some embodiments, an increase in fidelity of initiation codon decoding by the ribosome of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, an increase in fidelity of initiation codon decoding by the ribosome in a polynucleotide (e.g., mRNA) comprising a GC-rich element of the

disclosure relative to a polynucleotide (e.g., mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

In some embodiments, a decrease in a rate of decoding an initiation codon by the ribosome of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, a decrease in a rate of decoding an initiation codon by the ribosome of a polynucleotide (e.g., mRNA) comprising a GC-rich element of the disclosure relative to a polynucleotide (e.g., mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

Small Ribosomal Subunit Mapping

In some aspects, RNA elements that provide a desired translational regulatory activity, including modulation of leaking scanning, to polynucleotides e.g., mRNA, are identified and/or characterized by small ribosomal subunit mapping.

Small ribosomal subunit (SSU) mapping is a technique similar to ribosome profiling that allows the determination of the number and position of small 40S ribosomal subunits or pre-initiation complexes (PICs) comprising small 40S ribosomal subunits bound to mRNAs. Similar to the technique of ribosome profiling described herein, small ribosomal subunit mapping involves analysis of a region or segment of mRNA protected by the 40S subunit from ribonuclease digestion, resulting in a 'footprint', the number and sequence of which can be analyzed by methods known in the art (e.g., RNA-seq). As described herein, the current model of mRNA translation initiation postulates that the pre-initiation complex (alternatively "43S pre-initiation complex"; abbreviated as "PIC") translocates from the site of recruitment on the mRNA (typically the 5' cap) to the initiation codon by scanning nucleotides in a 5' to 3' direction until the first AUG codon that resides within a specific translation-promotive nucleotide context (the Kozak sequence) is encountered (Kozak (1989) J Cell Biol 108: 229-241). "Leaky scanning" by the PIC, whereby the PIC bypasses the initiation codon of an mRNA and instead continues scanning downstream until an alternate or alternative initiation codon is recognized, can occur and result in a decrease in translation efficiency and/or the production of an undesired, aberrant translation product. Thus, analysis of the number of SSUs positioned, or mapped, over AUGs downstream of the first AUG in an mRNA allows for the determination of the extent or frequency at which leaky scanning occurs. SSU mapping provides information that can be used to identify or determine a characteristic (e.g., a translational regulatory activity) of a modification or RNA element of the disclosure, that affects the activity of a small 40S ribosomal subunit (SSU or a PIC comprising the SSU.

Accordingly, an inhibition or reduction of leaky scanning by an SSU or a PIC comprising an SSU of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by small ribosomal subunit mapping. In some aspects, an inhibition or reduction of leaky scanning by an SSU or a PIC comprising an SSU of a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by small ribosomal subunit mapping.

In some embodiments, an increase in residence time or the time of occupancy (dwell time) of an SSU or a PIC comprising an SSU at a discrete position or location (e.g., an initiation codon) along a polynucleotide (e.g. an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, an increase in residence time or the time of occupancy of an SSU or a PIC comprising an SSU at an initiation codon in a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

In some embodiments, an increase in the initiation of polypeptide synthesis at or from the initiation codon in polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, an increase in the initiation of polypeptide synthesis at or from the initiation codon in a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

In some embodiments, an increase in fidelity of initiation codon decoding by an SSU or a PIC comprising an SSU of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, an increase in fidelity of initiation codon decoding by an SSU or a PIC comprising an SSU of a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

In some embodiments, an increase in fidelity of initiation codon decoding by an SSU or a PIC comprising an SSU of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, an increase in fidelity of initiation codon decoding by an SSU or a PIC comprising an SSU of a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

In some embodiments, a decrease in a rate of decoding an initiation codon comprising a polynucleotide (e.g., an mRNA) comprising any one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by ribosome profiling. In some embodiments, a decrease in a rate of decoding an initiation codon decoding by the ribosome of a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by ribosome profiling.

RiboFrame-Seq

In some aspects, RNA elements that provide a desired translational regulatory activity, including modulation of

leaking scanning, to polynucleotides e.g., mRNA, are identified and/or characterized by RiboFrame-seq.

RiboFrame-seq is an assay that allows for the high-throughput measurement of leaky scanning for many different 5'-UTR sequences. A population of mRNAs is generated with a library of different 5' UTR sequences, each of which contains a 5' cap and a coding sequence that encodes a polypeptide comprising two to three different epitope tags, each in a different frame and preceded by an AUG. The mRNA population is transfected into cells and allowed to be translated. Cells are then lysed and immunoprecipitations performed against each of the encoded epitope tags. Each of these immunoprecipitations is designed to isolate a nascent polypeptide chain encoding the particular epitope, as well as the active ribosome performing its synthesis, and the mRNA that encodes it. The complement of 5'-UTRs present in each immunoprecipitate is then analyzed by methods known in the art (e.g., RNA-seq). The 5'-UTRs comprising sequences (e.g. RNA elements) that correlate with reduced, inhibited or low leaky scanning are characterized by being abundant in the immunoprecipitate corresponding to the first epitope tag relative to the other immunoprecipitates.

Accordingly, in some embodiments, a modification or RNA element having a translational regulatory activity of the disclosure is identified or characterized by RiboFrame-seq. In some aspects, a modification or RNA element having reduced, inhibited or low leaky scanning when located in a 5' UTR of an mRNA are identified or characterized by being abundant in the immunoprecipitate corresponding to the first epitope tag relative to the other immunoprecipitates as determined by RiboFrame-seq.

Western Blot (Imnzunodetection)

In some aspects, the disclosure provides a method of identifying, isolating, and/or characterizing a modification (e.g., an RNA element) that provides a translational regulatory activity by synthesizing a 1st control mRNA comprising a polynucleotide sequence comprising an open reading frame encoding a reporter polypeptide (e.g., eGFP) and a 1st AUG codon upstream of, in-frame, and operably linked to, the open reading frame encoding the reporter polypeptide. The 1st control mRNA also comprises a coding sequence for a first epitope tag (e.g. 3×FLAG) upstream of, in-frame, and operably linked to the 1st AUG codon, a 2nd AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the first epitope tag. Optionally, the 1st control mRNA further comprises a coding sequence for a second epitope tag (e.g. V5) upstream of, in-frame, and operably linked to the 2nd AUG codon, and a 3rd AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the second epitope tag. The 1st control mRNA also comprises a 5' UTR and a 3' UTR. The method further comprises synthesizing a 2nd test mRNA comprising a polynucleotide sequence comprising the 1st control mRNA and further comprising a modification (e.g. an RNA element). The method further comprises introducing the 1st control mRNA and 2nd test mRNA to conditions suitable for translation of the polynucleotide sequence encoding the reporter polypeptide. The method further comprises measuring the effect of the candidate modification on the amount of reporter polypeptide from each of the three AUG codons. Following transfection of this mRNA into cells, the cell lysate is analyzed by Western blot using antibodies that specifically bind to and detect the reporter polypeptide. This analysis generates two or three bands: a higher band that corresponds to protein generated from the first AUG and lower bands derived from protein generated from the second AUG and, optionally, third AUG.

Leaky scanning is calculated as abundance of the lower bands divided by the sum of the abundance of both bands, as determined by methods known in the art (e.g. densitometry). A test mRNA comprising one or more modifications or RNA elements of the disclosure, that correlate with reduced, inhibited or low leaky scanning is characterized by an increase in amount of polypeptide comprising the second epitope tag compared to the amount of polypeptide that does not comprise an epitope tag, optionally, the amount of polypeptide comprising the first epitope tag, translated from the test mRNA, relative to the control mRNA that does not comprise the one or more modifications or RNA elements. Accordingly, in some embodiments, a modification or RNA element having a translational regulatory activity of the disclosure, is identified by Western blot.

In some embodiments, an inhibition or reduction in leaky scanning of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by Western blot. In some embodiments, an inhibition or reduction in leaky scanning of a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by Western blot.

In some embodiments, an increase in the initiation of polypeptide synthesis at or from the initiation codon comprising a polynucleotide (e.g., an mRNA) comprising any one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide that does not comprise the one or more modifications or RNA elements, is determined by Western blot. In some embodiments, an increase in the initiation of polypeptide synthesis at or from the initiation codon comprising a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by Western blot.

In some embodiments, an increase in an amount of polypeptide translated from the full open reading frame comprising a polynucleotide (e.g., an mRNA) comprising any one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by Western blot. In some embodiments, an increase in an amount of polypeptide translated from the full open reading frame comprising a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by Western blot.

In some embodiments, an inhibition or reduction in an amount of polypeptide translated from any open reading frame other than a full open reading frame comprising a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by Western blot. In some embodiments, an inhibition or reduction in an amount of polypeptide translated from any open reading frame other than a full open reading frame comprising a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by Western blot.

In some embodiments, an inhibition or reduction in the production of aberrant translation products translated from a polynucleotide (e.g., an mRNA) comprising any one or more of the modifications or RNA elements of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, is determined by Western blot. In some embodiments, an inhibition or reduction in the production of aberrant translation products translated from a polynucleotide (e.g., an mRNA) comprising a GC-rich element of the disclosure, relative to a polynucleotide (e.g., an mRNA) that does not comprise the GC-rich element, is determined by Western blot.

In some embodiments, leaky scanning by a 43S pre-initiation complex (PIC) or ribosome of a polynucleotide (e.g., an mRNA) comprising one or more of the modifications or RNA elements (e.g., GC-rich element) of the disclosure is decreased by about 80%-100%, about 60%-80%, about 40%-60%, about 20%-40%, about 10%-20%, about 5%-10%, about 1%-5% relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modifications or RNA elements, as determined by SSU mapping and/or ribosome profiling methods, as described herein.

In some embodiments, leaky scanning by a 43S pre-initiation complex (PIC) or ribosome of a polynucleotide (e.g., an mRNA) comprising any one or more of the modifications or RNA elements of the disclosure is decreased by about 80%-100%, about 60%-80%, about 40%-60%, about 20%-40%, about 10%-20%, about 5%-10%, about 1%-5% and an amount of a polypeptide translated from a full reading frame is increased by about 80%-100%, about 60%-80%, about 40%-60%, about 20%-40%, about 10%-20%, about 5%-10%, about 1%-5% relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modification or RNA elements, as determined by SSU mapping and Western blot, respectively, as described herein.

In some embodiments, leaky scanning by the 43S pre-initiation complex (PIC) or ribosome of a polynucleotide (e.g., an mRNA) comprising any one or more of the modifications or RNA elements (e.g., GC-rich element) of the disclosure is decreased by about 80%-100%, about 60%-80%, about 40%-60%, about 20%-40%, about 10%-20%, about 5%-10%, about 1%-5%, an amount of a polypeptide translated from a full open reading frame is increased by about 80%-100%, about 60%-80%, about 40%-60%, about 20%-40%, about 10%-20%, about 5%-10%, about 1%-5%, and potency of the polypeptide is increased by about 80%-100%, about 60%-80%, about 40%-60%, about 20%-40%, about 10%-20%, about 5%-10%, about 1%-5%, relative to a polynucleotide (e.g., an mRNA) that does not comprise the one or more modification or RNA elements, as determined by SSU mapping and Western blot.

Another RNA element known to regulate translation of mRNA is the five-prime cap (5' cap), which is a specially altered nucleotide the 5' end of natural mRNA co-transcriptionally. This process, known as mRNA capping, is highly regulated and is vital in the creation of stable and mature messenger RNA able to undergo translation. In eukaryotes, the structure of the 5' cap consists of a guanine nucleotide connected to 5' end of an mRNA via an unusual 5' to 5' triphosphate linkage. This guanosine is methylated on the 7 position directly after capping in vivo by a methyltransferase, and as such, is sometimes referred to as a 7-methylguanylate cap, and abbreviated m7G. A 5' cap structure or cap species is a compound including two nucleoside moieties joined by a linker and may be selected from a naturally occurring cap, a non-naturally occurring cap or cap analog, or an anti-reverse cap analog (ARCA). A cap species may include one or more modified nucleosides and/or linker moieties. For example, a natural mRNA cap may include a guanine nucleotide and a guanine (G) nucleotide methylated at the 7 position joined by a triphosphate linkage at their 5' positions, e.g., m7G(5')ppp(5')G, commonly written as m7GpppG. A cap species may also be an anti-reverse cap analog. A non-limiting list of possible cap species includes m7GpppG, m7Gpppm7G, m73'dGpppG, m27, O3'GpppG, m27, O3'GppppG, m27, O2'GppppG, m7Gpppm7G, m73'dGpppG, m27, O3'GpppG, m27, O3'GppppG, and m27, O2'GppppG. Accordingly, in some embodiments, the mRNAs disclosed herein comprise a 5' cap, or derivative, analog, or modification thereof.

An early event in translation initiation involves the formation of the 43S pre-initiation complex (PIC) composed of the small 40S ribosomal subunit, the initiator transfer RNA (Met-tRNAiMet), and several various eIFs. Following recruitment to the mRNA, the PIC biochemically interrogates or "scans" the sequence of the mRNA molecule in search of an initiation codon. In some embodiments of the mRNAs disclosed herein, the mRNAs comprise at least one initiation codon. In some embodiments, the initiation codon is an AUG codon. In some embodiments, the initiation codon comprises one or more modified nucleotides.

Similar to polypeptides, polynucleotides, particularly RNA, can fold into a variety of complex three dimensional structures. The ability of a nucleic acid to form a complex, functional three dimensional structure is exemplified by a transfer RNA molecule (tRNA), which is a single chain of ~70-90 nucleotides in length that folds into an L-shaped 3D structure allowing it to fit into the P and A sites of a ribosome and function as the physical link between the polypeptide coding sequence of mRNA and the amino acid sequence of the polypeptide. Since base pairing between complementary sequences of nucleobases determines the overall secondary (and ultimately tertiary) structure of nucleic acid molecules, sequences predicted to or known to be able to adopt a particular structure (e.g. a stem-loop) are vital considerations in the design and utility of some types of functional elements or motifs (e.g. RNA elements). Nucleic acid secondary structure is generally divided into duplexes (contiguous base pairs) and various kinds of loops (unpaired nucleotides flanked or surrounded by duplexes). As is known in the art, stable RNA secondary structures, or combinations of them, can be further classified and usefully described as, but not limited to, simple loops, tetraloops, pseudoknots, hairpins, helicies, and stem-loops. Secondary structure can also be usefully depicted as a list of nucleobases which are paired in a nucleic acid molecule.

The function(s) of a nucleic acid secondary structure are emergent from the thermodynamic properties of the secondary structure. For example, the thermodynamic stability of an RNA hairpin/stemloop structure is characterized by its free energy change (deltaG). For a spontaneous process, i.e. the formation of a stable RNA hairpin/stemloop, deltaG is negative. The lower the deltaG value, the more energy is required to reverse the process, i.e. the more energy is required to denature or melt ('unfold') the RNA hairpin/stemloop. The stability of an RNA hairpin/stemloop will contribute to its biological function: e.g. in the context of translation, a more stable RNA structure with a relatively low deltaG can act a physical barrier for the ribosome (Kozak, 1986; Babendure et al., 2006), leading to inhibition of protein synthesis. In contrast, a weaker or moderately stable RNA structure can be beneficial as translational

enhancer, as the translational machinery will recognize it as signal for a temporary pause, but ultimately the structure will open up and allow translation to proceed (Kozal, 1986; Kozak, 1990; Babendure et al., 2006). To assign an absolute number to the deltaG value that defines a stable versus a weak/moderately stable RNA hairpin/stemloop is difficult and is very much driven by its context (sequence and structural context, biological context). In the context of the above mentioned examples by Kozak, 1986, Kozak, 1990 and Babendure et al., 2006, stable hairpins/stemloops are characterized by approximate deltaG values lower than −30 kcal/mol, while weak/moderately stable hairpins are characterized by approximate deltaG values between −10 and −30 kcal/mol.

Accordingly, in some embodiments, an mRNA comprises at least one modification, wherein the at least one modification is a structural modification. In some embodiments, the structural modification is an RNA element. In some embodiments, the structural modification is a GC-rich RNA element. In some embodiments, the structural modification is a viral RNA element. In some embodiments, the structural modification is a protein-binding RNA element. In some embodiments, the structural modification is a translation initiation element. In some embodiments, the structural modification is a translation enhancer element. In some embodiments, the structural modification is a translation fidelity enhancing element. In some embodiments, the structural modification is an mRNA nuclear export element. In some embodiments, the structural modification is a stable RNA secondary structure.

The mRNAs of the present disclosure, or regions thereof, may be codon optimized. Codon optimization methods are known in the art and may be useful for a variety of purposes: matching codon frequencies in host organisms to ensure proper folding, bias GC content to increase mRNA stability or reduce secondary structures, minimize tandem repeat codons or base runs that may impair gene construction or expression, customize transcriptional and translational control regions, insert or remove proteins trafficking sequences, remove/add post translation modification sites in encoded proteins (e.g., glycosylation sites), add, remove or shuffle protein domains, insert or delete restriction sites, modify ribosome binding sites and mRNA degradation sites, adjust translation rates to allow the various domains of the protein to fold properly, or to reduce or eliminate problem secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art; non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park, Calif.) and/or proprietary methods. In one embodiment, the mRNA sequence is optimized using optimization algorithms, e.g., to optimize expression in mammalian cells or enhance mRNA stability. Accordingly in some embodiments, an mmRNA comprises a structural modification, wherein the structural modification is a codon optimized open reading frame. In some embodiments, the structural modification is a modification of base composition.

mRNA Construct Components

An mRNA may be a naturally or non-naturally occurring mRNA. An mRNA may include one or more modified nucleobases, nucleosides, or nucleotides, as described below, in which case it may be referred to as a "modified mRNA" or "mmRNA." As described herein "nucleoside" is defined as a compound containing a sugar molecule (e.g., a pentose or ribose) or derivative thereof in combination with an organic base (e.g., a purine or pyrimidine) or a derivative thereof (also referred to herein as "nucleobase"). As described herein, "nucleotide" is defined as a nucleoside including a phosphate group.

An mRNA may include a 5' untranslated region (5'-UTR), a 3' untranslated region (3'-UTR), and/or a coding region (e.g., an open reading frame). An exemplary 5' UTR for use in the constructs is shown in SEQ ID NO: 33. An mRNA may include any suitable number of base pairs, including tens (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100), hundreds (e.g., 200, 300, 400, 500, 600, 700, 800, or 900) or thousands (e.g., 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000) of base pairs. Any number (e.g., all, some, or none) of nucleobases, nucleosides, or nucleotides may be an analog of a canonical species, substituted, modified, or otherwise non-naturally occurring. In certain embodiments, all of a particular nucleobase type may be modified.

In some embodiments, an mRNA as described herein may include a 5' cap structure, a chain terminating nucleotide, optionally a Kozak sequence (also known as a Kozak consensus sequence), a stem loop, a polyA sequence, and/or a polyadenylation signal.

A 5' cap structure or cap species is a compound including two nucleoside moieties joined by a linker and may be selected from a naturally occurring cap, a non-naturally occurring cap or cap analog, or an anti-reverse cap analog (ARCA). A cap species may include one or more modified nucleosides and/or linker moieties. For example, a natural mRNA cap may include a guanine nucleotide and a guanine (G) nucleotide methylated at the 7 position joined by a triphosphate linkage at their 5' positions, e.g., $m^7G(5')ppp(5')G$, commonly written as m^7GpppG. A cap species may also be an anti-reverse cap analog. A non-limiting list of possible cap species includes m^7GpppG, m^7Gpppm^7G, $m^73'dGpppG$, $m_2^{7,O3'}GpppG$, $m_2^{7,O3'}GppppG$, $m_2^{7,O2'}GppppG$, m^7Gpppm^7G, $m^73'dGpppG$, $m_2^{7,O3'}GpppG$, $m_2^{7,O3'}GppppG$, and $m_2^{7,O2'}GppppG$.

An mRNA may instead or additionally include a chain terminating nucleoside. For example, a chain terminating nucleoside may include those nucleosides deoxygenated at the 2' and/or 3' positions of their sugar group. Such species may include 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, and 2',3'-dideoxynucleosides, such as 2',3'-dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'-dideoxyguanosine, and 2',3'-dideoxythymine. In some embodiments, incorporation of a chain terminating nucleotide into an mRNA, for example at the 3'-terminus, may result in stabilization of the mRNA, as described, for example, in International Patent Publication No. WO 2013/103659.

An mRNA may instead or additionally include a stem loop, such as a histone stem loop. A stem loop may include 2, 3, 4, 5, 6, 7, 8, or more nucleotide base pairs. For example, a stem loop may include 4, 5, 6, 7, or 8 nucleotide base pairs. A stem loop may be located in any region of an mRNA. For example, a stem loop may be located in, before, or after an untranslated region (a 5' untranslated region or a 3' untranslated region), a coding region, or a polyA sequence or tail. In some embodiments, a stem loop may affect one or more function(s) of an mRNA, such as initiation of translation, translation efficiency, and/or transcriptional termination.

An mRNA may instead or additionally include a polyA sequence and/or polyadenylation signal. A polyA sequence may be comprised entirely or mostly of adenine nucleotides or analogs or derivatives thereof. A polyA sequence may be a tail located adjacent to a 3' untranslated region of an

mRNA. In some embodiments, a polyA sequence may affect the nuclear export, translation, and/or stability of an mRNA.

An mRNA may instead or additionally include a microRNA binding site.

In some embodiments, an mRNA is a bicistronic mRNA comprising a first coding region and a second coding region with an intervening sequence comprising an internal ribosome entry site (IRES) sequence that allows for internal translation initiation between the first and second coding regions, or with an intervening sequence encoding a self-cleaving peptide, such as a 2A peptide. IRES sequences and 2A peptides are typically used to enhance expression of multiple proteins from the same vector. A variety of IRES sequences are known and available in the art and may be used, including, e.g., the encephalomyocarditis virus IRES.

5' UTR and Translation Initiation

In certain embodiments, the polynucleotide (e.g., mRNA) encoding a polypeptide of the present disclosure comprises a 5' UTR and/or a translation initiation sequence. Natural 5' UTRs comprise sequences involved in translation initiation. For example, Kozak sequences comprise natural 5' UTRs and are commonly known to be involved in the process by which the ribosome initiates translation of many genes. 5' UTRs also have been known to form secondary structures which are involved in elongation factor binding.

By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and protein production of the polynucleotides of the disclosure. For example, introduction of 5' UTR of mRNA known to be upregulated in cancers, such as c-myc, could be used to enhance expression of a nucleic acid molecule, such as a polynucleotide, in cancer cells. Untranslated regions useful in the design and manufacture of polynucleotides include, but are not limited, to those disclosed in International Patent Publication No. WO 2014/164253 (see also US20160022840).

Shown in Table 2 is a listing of exemplary 5' UTRs. Variants of 5' UTRs can be utilized wherein one or more nucleotides are added or removed to the termini, including A, U, C or G.

TABLE 2

Exemplary 5'-UTRs

5' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
5UTR-001	Upstream UTR	GGGAAAUAAGAGAGAAAAGAA GAGUAAGAAGAAAUAUAAGAG CCACC	476
5UTR-002	Upstream UTR	GGGAGAUCAGAGAGAAAAGAA GAGUAAGAAGAAAUAUAAGAG CCACC	477
5UTR-003	Upstream UTR	GGAAUAAAAGUCUCAACACAA CAUAUACAAAACAAACGAAUC UCAAGCAAUCAAGCAUUCUAC UUCUAUUGCAGCAAUUUAAAU CAUUUCUUUUAAAGCAAAAGC AAUUUUCUGAAAAUUUUCACC AUUUACGAACGAUAGCAAC	478
5UTR-004	Upstream UTR	GGGAGACAAGCUUGGCAUUCC GGUACUGUU GGUAAAGCCAC C	479
5UTR-005	Upstream UTR	GGGAGAUCAGAGAGAAAAGAA GAGUAAGAAGAAAUAUAAGAG CCACC	480

TABLE 2-continued

Exemplary 5'-UTRs

5' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
5UTR-006	Upstream UTR	GGAAUAAAAGUCUCAACACAA CAUAUACAAAACAAACGAAUC UCAAGCAAUCAAGCAUUCUAC UUCUAUUGCAGCAAUUUAAAU CAUUUCUUUUAAAGCAAAAGC AAUUUUCUGAAAAUUUUCACC AUUUACGAACGAUAGCAAC	481
5UTR-007	Upstream UTR	GGGAGACAAGCUUGGCAUUCC GGUACUGUU GGUAAAGCCAC C	482
5UTR-008	Upstream UTR	GGGAAUUAACAGAGAAAAGAA GAGUAAGAAGAAAUAUAAGAG CCACC	483
5UTR-009	Upstream UTR	GGGAAAUUAGACAGAAAAGAA GAGUAAGAAGAAAUAUAAGAG CCACC	484
5UTR-010	Upstream UTR	GGGAAAUAAGAGAGUAAAGAA CAGUAAGAAGAAAUAUAAGAG CCACC	485
5UTR-011	Upstream UTR	GGGAAAAAAGAGAGAAAAGAA GACUAAGAAGAAAUAUAAGAG CCACC	486
5UTR-012	Upstream UTR	GGGAAAUAAGAGAGAAAAGAA GAGUAAGAAGAUAUAUAAGAG CCACC	487
5UTR-013	Upstream UTR	GGGAAAUAAGAGACAAAACAA GAGUAAGAAGAAAUAUAAGAG CCACC	488
5UTR-014	Upstream UTR	GGGAAAUUAGAGAGUAAAGAA CAGUAAGUAGAAUUAAAAGAG CCACC	489
5UTR-015	Upstream UTR	GGGAAAUAAGAGAGAAUAGAA GAGUAAGAAGAAAUAUAAGAG CCACC	490
5UTR-016	Upstream UTR	GGGAAAUAAGAGAGAAAAGAA GAGUAAGAAGAAAAUUAAGAG CCACC	491
5UTR-017	Upstream UTR	GGGAAAUAAGAGAGAAAAGAA GAGUAAGAAGAAAUUUAAGAG CCACC	492
5UTR-018	Upstream UTR	GGGAAAUAAGAGAGAAAAGAA GAGUAAGAAGAAAUAUAAGAG CCACC	493
5UTR-019	Upstream UTR	UCAAGCUUUUGGACCCUCGUA CAGAAGCUAAUACGACUCACU AUAGGGAAAUAAGAGAGAAAA GAAGAGUAAGAAGAAAUAUAA GAGCCACC	494
5UTR-020	Upstream UTR	GGACAGAUCGCCUGGAGACGC CAUCCACGCUGUUUUGACCUC CAUAGAAGACACCGGGACCGA UCCAGCCUCCGCGGCCGGGAA CGGUGCAUUGGAACGCGGAUU CCCCGUGCCAAGAGUGACUCA CCGUCCUUGACACG	495
5UTR-021	Upstream UTR	GGCGCUGCCUACGGAGGUGGC AGCCAUCUCCUUCUCGGCAUC	496

Other non-UTR sequences can also be used as regions or subregions within the polynucleotides. For example, introns or portions of introns sequences can be incorporated into regions of the polynucleotides. Incorporation of intronic sequences can increase protein production as well as polynucleotide levels.

Combinations of features can be included in flanking regions and can be contained within other features. For example, the ORF can be flanked by a 5' UTR which can contain a strong Kozak translational initiation signal and/or a 3' UTR which can include an oligo(dT) sequence for templated addition of a poly-A tail. A 5' UTR can comprise a first polynucleotide fragment and a second polynucleotide fragment from the same and/or different genes such as the 5' UTRs described in U.S. Patent Application Publication No. 2010-0293625.

These UTRs or portions thereof can be placed in the same orientation as in the transcript from which they were selected or can be altered in orientation or location. Hence a 5' or 3' UTR can be inverted, shortened, lengthened, made with one or more other 5' UTRs or 3' UTRs.

In some embodiments, the UTR sequences can be changed in some way in relation to a reference sequence. For example, a 3' or 5' UTR can be altered relative to a wild type or native UTR by the change in orientation or location as taught above or can be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides. Any of these changes producing an "altered" UTR (whether 3' or 5') comprise a variant UTR.

In some embodiments, a double, triple or quadruple UTR such as a 5' or 3' UTR can be used. As used herein, a "double" UTR is one in which two copies of the same UTR are encoded either in series or substantially in series. For example, a double beta-globin 3' UTR can be used as described in U.S. Patent Application Publication No. 2010-0129877.

In some embodiments, flanking regions can be heterologous. In some embodiments, the 5' untranslated region can be derived from a different species than the 3' untranslated region. The untranslated region can also include translation enhancer elements (TEE). As a non-limiting example, the TEE can include those described in U.S. Patent Application Publication No. 2009-0226470.

In some embodiments, the mRNAs provided by the disclosure comprise a 5' UTR comprising a T7 leader sequence at the 5' end of the 5' UTR. In some embodiments, the mRNA of the disclosure comprises a 5' UTR comprising a T7 leader sequence comprising the sequence GGGAGA at the 5' end of the 5' UTR. In some embodiments, the mRNA of the disclosure comprises a 5' UTR comprising a T7 leader sequence comprising the sequence GGGAAA at the 5' end of the 5' UTR. In some embodiments, the mRNA comprises a 5' UTR which does not comprise a T7 leader sequence at the 5' end of the 5' UTR.

In another aspect, the disclosure provides an mRNA comprising a 5' UTR, wherein the nucleotide sequence of the 5' UTR comprises any one of the nucleotide sequences set forth in SEQ ID NO: 1 to SEQ ID NO: 497. In another embodiment, the disclosure provides an mRNA comprising a 5' UTR, wherein the nucleotide sequence of the 5' UTR comprises the nucleotide sequence set forth in SEQ ID NO: 33. In another embodiment, the disclosure provides an mRNA comprising a 5' UTR, wherein the nucleotide sequence of the 5' UTR comprises the nucleotide sequence set forth in SEQ ID NO: 34. In another embodiment, the disclosure provides an mRNA comprising a 5' UTR, wherein the nucleotide sequence of the 5' UTR comprises the nucleotide sequence set forth in SEQ ID NO: 52. In another embodiment, the disclosure provides an mRNA comprising a 5' UTR, wherein the nucleotide sequence of the 5' UTR comprises the nucleotide sequence set forth in SEQ ID NO: 53. In another embodiment, the disclosure provides an mRNA comprising a 5' UTR, wherein the nucleotide sequence of the 5' UTR comprises the nucleotide sequence set forth in SEQ ID NO: 54. In another embodiment, the disclosure provides an mRNA comprising a 5' UTR, wherein the nucleotide sequence of the 5' UTR comprises the nucleotide sequence set forth in SEQ ID NO: 73.

3' UTR and the AU Rich Elements

In certain embodiments, the polynucleotide (e.g., mRNA) encoding a polypeptide further comprises a 3' UTR. 3'-UTR is the section of mRNA that immediately follows the translation termination codon and often contains regulatory regions that post-transcriptionally influence gene expression. Regulatory regions within the 3'-UTR can influence polyadenylation, translation efficiency, localization, and stability of the mRNA. In one embodiment, the 3'-UTR useful for the disclosure comprises a binding site for regulatory proteins or microRNAs. In some embodiments, the 3'-UTR has a silencer region, which binds to repressor proteins and inhibits the expression of the mRNA. In other embodiments, the 3'-UTR comprises an AU-rich element. Proteins bind AREs to affect the stability or decay rate of transcripts in a localized manner or affect translation initiation. In other embodiments, the 3'-UTR comprises the sequence AAUAAA that directs addition of several hundred adenine residues called the poly(A) tail to the end of the mRNA transcript.

Table 3 shows a listing of 3'-untranslated regions useful for the mRNAs encoding a polypeptide. Variants of 3' UTRs can be utilized wherein one or more nucleotides are added or removed to the termini, including A, U, C or G.

TABLE 3

Exemplary 3'-Untranslated Regions

3' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
3UTR-001	Creatine Kinase	GCGCCUGCCCACCUGCCACCGACUGC UGGAACCCAGCCAGUGGGAGGGCCUG GCCCACCAGAGUCCUGCUCCCUCACU CCUCGCCCCGCCCCCUGUCCCAGAGU CCCACCUGGGGGCUCUCUCCACCCUU CUCAGAGUUCCAGUUUCAACCAGAGU UCCAACCAAUGGGCUCCAUCCUCUGG AUUCUGGCCAAUGAAAUAUCUCCCUG GCAGGGUCCUCUUCUUUUCCCAGAGC UCCACCCCAACCAGGAGCUCUAGUUA AUGGAGAGCUCCCAGCACACUCGGAG CUUGUGCUUUGUCUCCACGCAAAGCG AUAAAUAAAAGCAUUGGUGGCCUUUG GUCUUUGAAUAAAGCCUGAGUAGGAA GUCUAGA	497
3UTR-002	Myoglobin	GCCCCUGCCGCUCCCACCCCCACCCA UCUGGGCCCCGGGUUCAAGAGAGAGC GGGGUCUGAUCUCGUGUAGCCAUAUA GAGUUUGCUUCUGAGUGUCUGCUUUG UUUAGUAGAGGUGGGCAGGAGGAGCU GAGGGGCUGGGGCUGGGGUGUUGAAG UUGGCUUUGCAUGCCCAGCGAUGCGC CUCCCUGUGGGAUGUCAUCACCCUGG GAACCGGGAGUGGCCCUUGGCUCACU GUGUUCUGCAUGGUUUGGAUCUGAAU UAAUUGUCCUUUCUUCUAAAUCCCAA CCGAACUUCUUCCAACCUCCAAACUG GCUGUAACCCCAAAUCCAAGCCAUUA	498

TABLE 3-continued

Exemplary 3'-Untranslated Regions

3' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
		ACUACACCUGACAGUAGCAAUUGUCU GAUUAAUCACUGGCCCCUUGAAGACA GCAGAAUGUCCCUUUGCAAUGAGGAG GAGAUCUGGGCUGGGCGGGCCAGCUG GGGAAGCAUUUGACUAUCUGGAACUU GUGUGUGCCUCCUCAGGUAUGGCAGU GACUCACCUGGUUUUAAUAAAACAAC CUGCAACAUCUCAUGGUCUUUGAAUA AAGCCUGAGUAGGAAGUCUAGA	
3UTR-003	α-actin	ACACACUCCACCUCCAGCACGCGACU UCUCAGGACGACGAAUCUUCUCAAUG GGGGGGCGGCUGAGCUCCAGCCACCC CGCAGUCACUUUCUUUGUAACAACUU CCGUUGCUGCCAUCGUAAACUGACAC AGUGUUUAUAACGUGUACAUACAUUA ACUUAUUACCUCAUUUUGUUAUUUUU CGAAACAAAGCCCUGUGGAAGAAAAU GGAAAACUUGAAGAAGCAUUAAAGUC AUUCUGUUAAGCUGCGUAAAUGGUCU UUGAAUAAAGCCUGAGUAGGAAGUCU AGA	499
3UTR-004	Albumin	CAUCACAUUUAAAAGCAUCUCAGCCU ACCAUGAGAAUAAGAGAAAGAAAAUG AAGAUCAAAAGCUUAUUCAUCUGUUU UUCUUUUUCGUUGGUGUAAAGCCAAC ACCCUGUCUAAAAAACAUAAAUUUCU UUAAUCAUUUUGCCUCUUUUCUCUGU GCUUCAAUUAAUAAAAAAUGGAAAGA AUCUAAUAGAGUGGUACAGCACUGUU AUUUUUCAAAGAUGUGUUGCUAUCCU GAAAAUUCUGUAGGUUCUGUGGAAGU UCCAGUGUUCUCUCUUAUUCCACUUC GGUAGAGGAUUUCUAGUUUCUUGUGG GCUAAUUAAAUAAAUCAUUAAUACUC UUCUAAUGGUCUUUGAAUAAAGCCUG AGUAGGAAGUCUAGA	500
3UTR-005	α-globin	GCUGCCUUCUGCGGGGCUUGCCUUCU GGCCAUGCCCUUCUUCUCUCCCUUGC ACCUGUACCUCUUGGUCUUUGAAUAA AGCCUGAGUAGGAAGGCGGCCGCUCG AGCAUGCAUCUAGA	501
3UTR-006	G-CSF	GCCAAGCCCUCCCCAUCCCAUGUAUU UAUCUCUAUUUAAUAUUUAUGUCUAU UUAAGCCUCAUAUUUAAAGACAGGGA AGAGCAGAACGGAGCCCCAGGCCUCU GUGUCCUUCCCUGCAUUUCUGAGUUU CAUUCUCCUGCCUGUAGCAGUGAGAA AAAGCUCCUGUCCUCCCAUCCCCUGG ACUGGGAGGUAGAUAGGUAAAUACCA AGUAUUUAUUACUAUGACUGCUCCCC AGCCCUGGCUCUGCAAUGGGCACUGG GAUGAGCCGCUGUGAGCCCCUGGUCC UGAGGGUCCCCACCUGGGACCCUUGA GAGUAUCAGGUCUCCCACGUGGGAGA CAAGAAAUCCCUGUUUAAUAUUUAAA CAGCAGUGUUCCCCAUCUGGGUCCUU GCACCCCUCACUCUGGCCUCAGCCGA CUGCACAGCGGCCCCUGCAUCCCCUU GGCUGUGAGGCCCCUGGACAAGCAGA GGUGGCCAGAGCUGGGAGGCAUGGCC CUGGGGUCCCACGAAUUUGCUGGGGA AUCUCGUUUUUCUUCUUAAGACUUUU GGGACAUGGUUUGACUCCCGAACAUC ACCGACGCGUCUCCUGUUUUUCUGGG UGGCCUCGGGACACCUGCCCUGCCCC CACGAGGGUCAGGACUGUGACUCUUU UUAGGGCCAGGCAGGUGCCUGGACAU UUGCCUUGCUGGACGGGGACUGGGGA UGUGGGAGGGAGCAGACAGGAGGAAU CAUGUCAGGCCUGUGUGUGAAAGGAA	502
3UTR-007	Col1a2; collagen, type I, alpha 2	GCUCCACUGUCACCCUCCACCUCUUC ACCCCCCACUCACCAGUGUCCCCUCC ACUGUCACAUUGUAACUGAACUUCAG GAUAAUAAAGUGUUUGCCUCCAUGGU CUUUGAAUAAAGCCUGAGUAGGAAGG CGGCCGCUCGAGCAUGCAUCUAGA	
		ACUCAAUCUAAAUUAAAAAAGAAAGA AAUUUGAAAAAACUUUCUCUUUGCCA UUUCUUCUUCUUCUUUUUUAACUGAA AGCUGAAUCCUUCCAUUUCUUCUGCA CAUCUACUUGCUUAAAUUGUGGGCAA AAGAGAAAAAGAAGGAUUGAUCAGAG CAUUGUGCAAUACAGUUUCAUUAACU CCUUCCCCCGCUCCCCCAAAAAUUUG AAUUUUUUUUUCAACACUCUUACACC UGUUAUGGAAAAUGUCAACCUUUGUA AGAAAACCAAAAUAAAAAUUGAAAAA UAAAAACCAUAAACAUUUGCACCACU UGUGGCUUUUGAAUAUCUUCCACAGA GGGAAGUUUAAAACCCAAACUUCCAA AGGUUUAAACUACCUCAAAACACUUU CCCAUGAGUGUGAUCCACAUUGUUAG GUGCUGACCUAGACAGAGAUGAACUG AGGUCCUUGUUUUGUUUUGUUCAUAA UACAAAGGUGCUAAUUAAUAGUAUUU CAGAUACUUGAAGAAUGUUGAUGGUG CUAGAAGAAUUUGAGAAGAAAUACUC CUGUAUUGAGUUGUAUCGUGUGGUGU AUUUUUUAAAAAAUUUGAUUUAGCAU UCAUAUUUUCCAUCUUAUUCCCAAUU AAAAGUAUGCAGAUUAUUUGCCCAAA UCUUCUUCAGAUUCAGCAUUUGUUCU UUGCCAGUCUCAUUUUCAUCUUCUUC CAUGGUUCCACAGAAGCUUUGUUUCU UGGGCAAGCAGAAAAAUUAAAUUGUA CCUAUUUUGUAUAUGUGAGAUGUUUA AAUAAAUUGUGAAAAAAAUGAAAUA AAGCAUGUUUGGUUUUCCAAAAGAAC AUAU	503
3UTR-008	Col6a2; collagen, type VI, alpha 2	CGCCGCCGCCCGGGCCCCGCAGUCGA GGGUCGUGAGCCCACCCCGUCCAUGG UGCUAAGCGGGCCCGGGUCCCACACG GCCAGCACCGCUGCUCACUCGGACGA CGCCCUGGGCCUGCACCUCUCCAGCU CCUCCCACGGGGUCCCCGUAGCCCCG GCCCCCGCCCAGCCCCAGGUCUCCCC AGGCCCUCCCGCAGGCUGCCCGGCCUC CCUCCCCCUGCAGCCAUCCCAAGGCU CCUGACCUACCUGGCCCCUGAGCUCU GGAGCAAGCCCUGACCCAAUAAAGGC UUUGAACCCAU	504
3UTR-009	RPN1; ribophorin I	GGGGCUAGAGCCCUCUCCGCACAGCG UGGAGACGGGGCAAGGAGGGGGGUUA UUAGGAUUGGUGGUUUUGUUUUGCUU UGUUUAAAGCCGUGGGAAAAUGGCAC AACUUUACCUCUGUGGGAGAUGCAAC ACUGAGAGCCAAGGGGUGGGAGUUGG GAUAAUUUUUAUAUAAAAGAAGUUUU UCCACUUUGAAUUGCUAAAAGUGGCA UUUUUCCUAUGUGCAGUCACUCCUCU CAUUUCUAAAAUAGGGACGUGGCCAG GCACGGUGGCUCAUGCCUGUAAUCCC AGCACUUUGGGAGGCCGAGGCAGGCG GCUCACGAGGUCAGGAGAUCGAGACU AUCCUGGCUAACACGGUAAAACCCUG UCUCUACUAAAAGUACAAAAAAUUAG CUGGGCGUGGUGGUGGGCACCUGUAG UCCCAGCUACUCGGGAGGCUGAGGCA GGAGAAAGGCAUGAAUCCAAGAGGCA GAGCUUGCAGUGAGCUGAGAUCACGC CAUUGCACUCCAGCCUGGGCAACAGU	505

TABLE 3-continued

Exemplary 3'-Untranslated Regions

3' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
		GUUAAGACUCUGUCUCAAAUAUAAAU	
		AAAUAAAUAAAUAAAUAAAUAAAUAA	
		AUAAAAAUAAAGCGAGAUGUUGCCCU	
		CAAA	
3UTR-010	LRP1; low density lipoprotein receptor-related protein 1	GGCCCUGCCCCGUCGGACUGCCCCCA	506
		GAAAGCCUCCUGCCCCCUGCCAGUGA	
		AGUCCUUCAGUGAGCCCCUCCCCAGC	
		CAGCCCUUCCCUGGCCCCGCCGGAUG	
		UAUAAAUGUAAAAAUGAAGGAAUUAC	
		AUUUUAUAUGUGAGCGAGCAAGCCGG	
		CAAGCGAGCACAGUAUUAUUUCUCCA	
		UCCCCUCCCUGCCUGCUCCUUGGCAC	
		CCCCAUGCUGCCUUCAGGGAGACAGG	
		CAGGGAGGGCUUGGGGCUGCACCUCC	
		UACCCUCCCACCAGAACGCACCCCAC	
		UGGGAGAGCUGGUGGUGCAGCCUUCC	
		CCUCCCUGUAUAAGACACUUUGCCAA	
		GGCUCUCCCCCUCUCGCCCCAUCCCUG	
		CUUGCCCGCUCCCACAGCUUCCUGAG	
		GGCUAAUUCUGGGAAGGGAGAGUUCU	
		UUGCUGCCCCUGUCUGGAAGACGUGG	
		CUCUGGGUGAGGUAGGCGGGAAAGGA	
		UGGAGUGUUUUAGUUCUUGGGGGAGG	
		CCACCCCAAACCCCAGCCCCAACUCC	
		AGGGGCACCUAUGAGAUGGCCAUGCU	
		CAACCCCCCUCCCAGACAGGCCCUCC	
		CUGUCUCCAGGGCCCCCACCGAGGUU	
		CCCAGGGCUGGAGACUUCCUCUGGUA	
		AACAUUCCUCCAGCCUCCCCUCCCCU	
		GGGGACGCCAAGGAGGUGGGCCACAC	
		CCAGGAAGGGAAAGCGGGCAGCCCCG	
		UUUUGGGGACGUGAACGUUUUAAUAA	
		UUUUUGCUGAAUUCCUUUACAACUAA	
		AUAACACAGAUAUUGUUAUAAAUAAA	
		AUUGU	
3UTR-011	Nnt1; cardiotrophin-like cytokine factor 1	AUAUUAAGGAUCAAGCUGUUAGCUAA	507
		UAAUGCCACCUCUGCAGUUUUGGGAA	
		CAGGCAAAUAAAGUAUCAGUAUACAU	
		GGUGAUGUACAUCUGUAGCAAAGCUC	
		UUGGAGAAAAUGAAGACUGAAGAAAG	
		CAAAGCAAAAACUGUAUAGAGAGAUU	
		UUUCAAAAGCAGUAAUCCCUCAAUUU	
		UAAAAAAGGAUUGAAAAUUCUAAAUG	
		UCUUUCUGUGCAUAUUUUUUGUGUUA	
		GGAAUCAAAAGUAUUUUAUAAAAGGA	
		GAAAGAACAGCCUCAUUUUAGAUGUA	
		GUCCUGUUGGAUUUUUUAUGCCUCCU	
		CAGUAACCAGAAAUGUUUUAAAAAAC	
		UAAGUGUUUAGGAUUUCAAGACAACA	
		UUAUACAUGGCUCUGAAAUAUCUGAC	
		ACAAUGUAAACAUUGCAGGCACCUGC	
		AUUUUAUGUUUUUUUUUUCAACAAAU	
		GUGACUAAUUUGAAACUUUUAUGAAC	
		UUCUGAGCUGUCCCCUUGCAAUUCAA	
		CCGCAGUUUGAAUUAAUCAUAUCAAA	
		UCAGUUUUAAUUUUUUAAAUUGUACU	
		UCAGAGUCUAUAUUUCAAGGGCACAU	
		UUUCUCACUACUAUUUUAAUACAUUA	
		AAGGACUAAAUAAUCUUUCAGAGAUG	
		CUGGAAACAAAUCAUUUGCUUUAUAU	
		GUUUCAUUAGAAUACCAAUGAAACAU	
		ACAACUUGAAAAUUAGUAAUAGUAUU	
		UUUGAAGAUCCCAUUUCUAAUUGGAG	
		AUCUCUUUAAUUUCGAUCAACUUAUA	
		AUGUGUAGUACUAUAUUAAGUGCACU	
		UGAGUGGAAUUCAACAUUUGACUAAU	
		AAAAUGAGUUCAUCAUGUUGGCAAGU	
		GAUGUGGCAAUUAUCUCUGGUGACAA	
		AAGAGUAAAAUCAAAUAUUUCUGCCU	
		GUUACAAAUAUCAAGGAAGACCUGCU	
		ACUAUGAAAUAGAUGACAUUAAUCUG	
		UCUUCACUGUUUAUAAUACGGAUGGA	

TABLE 3-continued

Exemplary 3'-Untranslated Regions

3' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
		UUUUUUUUCAAAUCAGUGUGUGUUUU	
		GAGGUCUUAUGUAAUUGAUGACAUUU	
		GAGAGAAAUGGUGGCUUUUUUUAGCU	
		ACCUCUUUGUUCAUUUAAGCACCAGU	
		AAAGAUCAUGUCUUUUUAUAGAAGUG	
		UAGAUUUUCUUUGUGACUUUGCUAUC	
		GUGCCUAAAGCUCUAAAUAUAGGUGA	
		AUGUGUGAUGAAUACUCAGAUUAUUU	
		GUCUCUCUAUAUAAUUAGUUUGGUAC	
		UAAGUUUCUCAAAAAAUUAUUAACAC	
		AUGAAAGACAAUCUCUAAACCAGAAA	
		AAGAAGUAGUACAAAUUUUGUUACUG	
		UAAUGCUCGCGUUUAGUGAGUUUAAA	
		ACACACAGUAUCUUUUGGUUUUAUAA	
		UCAGUUUCUAUUUUGCUGUGCCUGAG	
		AUUAAGAUCUGUGUAUGUGUGUGUGU	
		GUGUGUGUGCGUUUGUGUGUUAAAGC	
		AGAAAAGACUUUUUUAAAAGUUUUAA	
		GUGAUAAAUGCAAUUUGUUAAUUGAU	
		CUUAGAUCACUAGUAAACUCAGGGCU	
		GAAUUAUACCAUGUAUAUUCUAUUAG	
		AAGAAAGUAAACACCAUCUUUAUUCC	
		UGCCCUUUUUCUUCUCUCAAAGUAGU	
		UGUAGUUAUAUCUAGAAAGAAGCAAU	
		UUUGAUUUCUUGAAAAGGUAGUUCCU	
		GCACUCAGUUUAAACUAAAAAUAAUC	
		AUACUUGGAUUUUAUUUAUUUUUGUC	
		AUAGUAAAAAUUUUAAUUUAUAUAUA	
		UUUUUAUUUAGUAUUAUCUUAUUCUU	
		UGCUAUUUGCCAAUCCUUUGUCAUCA	
		AUUGUGUUAAAUGAAUUGAAAAUUCA	
		UGCCCUGUUCAUUUUAUUUUACUUUA	
		UUGGUUAGGAUAUUUAAAGGAUUUUU	
		GUAUAUAUAAUUUCUUAAAUUAAUAU	
		UCCAAAAGGUUAGUGGACUUAGAUUA	
		UAAAUUAUGGCAAAAAUCUAAAAACA	
		ACAAAAAUGAUUUUUAUACAUUCUAU	
		UUCAUUAUUCCUCUUUUUCCAAUAAG	
		UCAUACAAUUGGUAGAUAUGACUUAU	
		UUUAUUUUUGUAUUAUUCACUAUAUC	
		UUUAUGAUAUUUAAGUAUAAAAUAAUU	
		AAAAAAAUUUAUUGUACCUUAUAGUC	
		UGUCACCAAAAAAAAAAAAUUAUCUG	
		UAGGUAGUGAAAUGCUAAUGUUGAUU	
		UGUCUUUAAGGGCUUGUUAACUAUCC	
		UUUAUUUUCUCAUUUGUCUUAAAUUA	
		GGAGUUUGUGUUUAAAUUACUCAUCU	
		AAGCAAAAAAUGUAUAUAAAUCCCAU	
		UACUGGGUAUAUACCCAAAGGAUUAU	
		AAAUCAUGCUGCUAUAAAGACACAUG	
		CACACGUAUGUUUAUUGCAGCACUAU	
		UCACAAUAGCAAAGACUUGGAACCAA	
		CCCAAAUGUCCAUCAAUGAUAGACUU	
		GAUUAAGAAAAUGUGCACAUAUACAC	
		CAUGGAAUACUAUGCAGCCAUAAAAA	
		AGGAUGAGUUCAUGUCCUUUGUAGGG	
		ACAUGGAUAAAGCUGGAAACCAUCAU	
		UCUGAGCAAACUAUUGCAAGGACAGA	
		AAACCAAACACUGCAUGUUCUCACUC	
		AUAGGUGGGAAUUGAACAAUGAGAAC	
		ACUUGGACACAAGGUGGGGAACACCA	
		CACACCAGGGCCUGUCAUGGGGUGGG	
		GGGAGUGGGGAGGGAUAGCAUUAGGA	
		GAUAUACCUAAUGUAAAUGAUGAGUU	
		AAUGGGUGCAGCACACCAACAUGGCA	
		CAUGUAUACAUAUGUAGCAAACCUGC	
		ACGUUGUGCACAUGUACCCUAGAACU	
		UAAAGUAUAAUUAAAAAAAAAAAGAA	
		AACAGAAGCUAUUUAUAAAGAAGUUA	
		UUUGCUGAAAUAAAUGUGAUCUUUCC	
		CAUUAAAAAAAUAAAGAAAUUUUGGG	
		GUAAAAAAACACAAUAUAUUGUAUUC	
		UUGAAAAAUUCUAAGAGAGUGGAUGU	
		GAAGUGUUCUCACCACAAAAGUGAUA	

TABLE 3-continued

Exemplary 3'-Untranslated Regions

3' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
		ACUAAUUGAGGUAAUGCACAUAUUAA UUAGAAAGAUUUUGUCAUUCCACAAU GUAUAUAUACUUAAAAAUAUGUUAUA CACAAUAAAUACAUACAUUAAAAAAU AAGUAAAUGUA	
3UTR-012	Col6a1; collagen, type VI, alpha 1	CCCACCCUGCACGCCGGCACCAAACC CUGUCCUCCCACCCCUCCCCACUCAU CACUAAACAGAGUAAAAUGUGAUGCG AAUUUUCCCGACCAACCUGAUUCGCU AGAUUUUUUUUAAGGAAAAGCUUGGA AAGCCAGGACACAACGCUGCUGCCUG CUUUGUGCAGGGUCCUCCGGGGCUCA GCCCUGAGUUGGCAUCACCUGCGCAG GGCCCUCUGGGGCUCAGCCCUGAGCU AGUGUCACCUGCACAGGGCCCUCUGA GGCUCAGCCCUGAGCUGGCGUCACCU GUGCAGGGCCCUCUGGGGCUCAGCCC UGAGCUGGCCUCACCUGGGUUCCCCA CCCCGGGCUCUCCUGCCCUGCCCUCC UGCCCGCCCUCCCUCCUGCCUGCGCA GCUCCUUCCCUAGGCACCUCUGUGCU GCAUCCCACCAGCCUGAGCAAGACGC CCUCUCGGGGCCUGUGCCGCACUAGC CUCCCUCUCCUCUGUCCCCAUAGCUG GUUUUUCCCACCAAUCCUCACCUAAC AGUUACUUUACAAUUAAACUCAAAGC AAGCUCUUCUCCUCAGCUUGGGGCAG CCAUUGGCCUCUGUCUCGUUUUGGGA AACCAAGGUCAGGAGGCCGUUGCAGA CAUAAAUCUCGGCGACUCGGCCCCGU CUCCUGAGGGUCCUGCUGGUGACCGG CCUGGACCUUGGCCCUACAGCCCUGG AGGCCGCUGCUGACCAGCACUGACCC CGACCUCAGAGAGUACUCGCAGGGGC GCUGGCUGCACUCAAGACCCUCGAGA UUAACGGUGCUAACCCCGUCUGCUCC UCCCUCCCGCAGAGACUGGGGCCUGG ACUGGACAUGAGAGCCCCUUGGUGCC ACAGAGGGCUGUGUCUUACUAGAAAC AACGCAAACCUCUCCUUCCUCAGAAU AGUGAUGUGUUCGACGUUUUAUCAAA GGCCCCCUUUCUAUGUUCAUGUUAGU UUUGCUCCUUCUGUGUUUUUUUCUGA ACCAUAUCCAUGUUGCUGACUUUUCC AAAUAAAGGUUUUCACUCCUCUC	508
3UTR-013	Calr; calreticulin	AGAGGCCUGCCUCCAGGGCUGGACUG AGGCCUGAGCGCUCCUGCCGCAGAGC UGGCCGCGCCAAAUAAUGUCUCUGUG AGACUCGAGAACUUUCAUUUUUUUCC AGGCUGGUUCGGAUUUGGGGUGGAUU UUGGUUUUGUUCCCCUCCUCCACUCU CCCCCACCCCCUCCCCGCCCUUUUUU UUUUUUUUUUUUAAACUGGUAUUUUA UCUUUGAUUCUCCUUCAGCCCUCACC CCUGGUUCUCAUCUUUCUUGAUCAAC ACUUUUUCUUGCCUCUGUCCCCUUCU CUCUUUUCUUGCCUCUGUCCCCUUCU CUCAUCUCUUAGCUCCCCUCCAACCU GGGGGGCAGUGGUGUGGAGAAGCCAC AGGCCUGAGAUUUCAUCUGCUCUCCU UCCUGGAGCCCAGAGGAGGGCAGCAG AAGGGGGUGGUGUCUCCAACCCCCCA GCACUGAGGAAGAACGGGGCUCUUCU CAUUUCACCCCUCCCUUUCUCCCCUG CCCCCAGGACUGGGCCACUUCUGGGU GGGGCAGUGGGUCCCAGAUUGGCUCA CACUGAGAAUGUAAGAACUACAAACA AAAUUUCUAUUAAAUUAAAUUUUGUG UCUCC	509
3UTR-014	Col1a1; collagen, type I, alpha 1	CUCCCUCCAUCCCAACCUGGCUCCCU CCCACCCAACCAACUUUCCCCCCAAC CCGGAAACAGACAAGCAACCCAAACU GAACCCCCUCAAAAGCCAAAAAAUGG GAGACAAUUUCACAUGGACUUUGGAA AAUAUUUUUUUCCUUUGCAUUCAUCU CUCAAACUUAGUUUUUAUCUUUGACC AACCGAACAUGACCAAAAACCAAAAG UGCAUUCAACCUUACCAAAAAAAAAA AAAAAAAAAGAAUAAAUAAAUAACUU UUUAAAAAAGGAAGCUUGGUCCACUU GCUUGAAGACCCAUGCGGGGGUAAGU CCCUUUCUGCCCGUUGGGCUUAUGAA ACCCCAAUGCUGCCCUUUCUGCUCCU UUCUCCACACCCCCCUUGGGGCCUCC CCUCCACUCCUUCCCAAAUCUGUCUC CCCAGAAGACACAGGAAACAAUGUAU UGUCUGCCCAGCAAUCAAAGGCAAUG CUCAAACACCCAAGUGGCCCCCACCC UCAGCCCGCUCCUGCCCGCCCAGCAC CCCCAGGCCCUGGGGGACCUGGGGUU CUCAGACUGCCAAAGAAGCCUUGCCA UCUGGCGCUCCCAUGGCUCUUGCAAC AUCUCCCCUUCGUUUUUGAGGGGGUC AUGCCGGGGGAGCCACCAGCCCCUCA CUGGGUUCGGAGGAGAGUCAGGAAGG GCCACGACAAAGCAGAAACAUCGGAU UUGGGGAACGCGUGUCAAUCCCUUGU GCCGCAGGGCUGGGCGGGAGAGACUG UUCUGUUCCUUGUGUAACUGUGUUGC UGAAAGACUACCUCGUUCUUGUCUUG AUGUGUCACCGGGGCAACUGCCUGGG GGCGGGGAUGGGGGCAGGGUGGAAGC GGCUCCCCAUUUUAUACCAAAGGUGC UACAUCUAUGUGAUGGGUGGGGUGGG GAGGGAAUCACUGGUGCUAUAGAAAU UGAGAUGCCCCCCCAGGCCAGCAAAU GUUCCUUUUUGUUCAAAGUCUAUUUU UAUUCCUUGAUAUUUUUCUUUUUUUU UUUUUUUUUUUGUGGAUGGGGACUUG UGAAUUUUUCUAAAGGUGCUAUUUAA CAUGGGAGGAGAGCGUGUGCGGCUCC AGCCCAGCCCGCUGCUCACUUUCCAC CCUCUCUCCACCUGCCUCUGGCUUCU CAGGCCUCUGCUCUCCGACCUCUCUC CUCUGAAACCCUCCUCCACAGCUGCA GCCCAUCCUCCCGGCUCCCUCCUAGU CUGUCCUGCGUCCUCUGUCCCCGGGU UUCAGAGACAACUUCCCAAAGCACAA AGCAGUUUUUCCCCCUAGGGGUGGGA GGAAGCAAAAGACUCUGUACCUAUUU UGUAUGUGUAUAAUAAUUUGAGAUGU UUUUAAUUAUUUUGAUUGCUGGAAUA AAGCAUGUGGAAAUGACCCAAACAUA AUCCGCAGUGGCCUCCUAAUUUCCUU CUUUGGAGUUGGGGGAGGGGUAGACA UGGGGAAGGGGCUUUGGGGUGAUGGG CUUGCCUUCCAUUCCUGCCCUUUCCC UCCCCACUAUUCUCUUCUAGAUCCCU CCAUAACCCCACUCCCCUUUCUCUCA CCCUUCUUAUACCGCAAACCUUUCUA CUUCCUCUUUCAUUUUCUAUUCUUGC AAUUUCCUUGCACCUUUUCCAAAUCC UCUUCUCCCCUGCAAUACCAUACAGG CAAUCCACGUGCACAACACACACACA CACUCUUCACAUCUGGGGUUGUCCAA ACCUCAUACCCACUCCCCUUCAAGCC CAUCCACUCUCCCACCCCCUGGAUGCC CUGCACUUGGUGGCGGUGGGAUGCUC AUGGAUACUGGGAGGGUGAGGGGAGU GGAACCCGUGAGGAGGACCUGGGGGC CUCUCCUUGAACUGACAUGAAGGGUC AUCUGGCCUCUGCUCCCUUCUCACCC ACGCUGACCUCCUGCCGAAGGAGCAA	510

TABLE 3-continued

Exemplary 3'-Untranslated Regions

3' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
		CGCAACAGGAGAGGGGUCUGCUGAGC CUGGCGAGGGUCUGGGAGGGACCAGG AGGAAGGCGUGCUCCCUGCUCGCUGU CCUGGCCCUGGGGGAGUGAGGGAGAC AGACACCUGGGAGAGCUGUGGGGAAG GCACUCGCACCGUGCUCUUGGGAAGG AAGGAGACCUGGCCCUGCUCACCACG GACUGGGUGCCUCGACCUCCUGAAUC CCCAGAACACAACCCCCCUGGGCUGG GGUGGUCUGGGGAACCAUCGUGCCCC CGCCUCCCGCCUACUCCUUUUUAAGC UU	
3UTR-015	Plod1; procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	UUGGCCAGGCCUGACCCUCUUGGACC UUUCUUCUUUGCCGACAACCACUGCC CAGCAGCCUCUGGGACCUCGGGGUCC CAGGGAACCCAGUCCAGCCUCCUGGC UGUUGACUUCCCAUUGCUCUUGGAGC CACCAAUCAAAGAGAUUCAAAGAGAU UCCUGCAGGCCAGAGGCGGAACACAC CUUUAUGGCUGGGGCUCUCCGUGGUG UUCUGGACCCAGCCCCUGGAGACACC AUUCACUUUUACUGCUUUGUAGUGAC UCGUGCUCUCCAACCUGUCUUCCUGA AAAACCAAGGCCCCCUUCCCCCACCU CUUCCAUGGGGUGAGACUUGAGCAGA ACAGGGGCUUCCCCAAGUUGCCCAGA AAGACUGUCUGGGUGAGAAGCCAUGG CCAGAGCUUCUCCCAGGCACAGGUGU UGCACCAGGGACUUCUGCUUCAAGUU UUGGGGUAAAGACACCUGGAUCAGAC UCCAAGGGCUGCCCUGAGUCUGGGAC UUCUGCCUCCAUGGCUGGUCAUGAGA GCAAACCGUAGUCCCCUGGAGACAGC GACUCCAGAGAACCUCUUGGGAGACA GAAGAGGCAUCUGUGCACAGCUCGAU CUUCUACUUGCCUGUGGGGAGGGGAG UGACAGGUCCACACACCACACUGGGU CACCCUGUCCUGGAUGCCUCUGAAGA GAGGGACAGACCGUCAGAAACUGGAG AGUUUCUAUUAAAGGUCAUUUAAACC A	511
3UTR-016	Nucb1; nucleobindin 1	UCCUCCGGGACCCCAGCCCUCAGGAU UCCUGAUGCUCCAAGGCGACUGAUGG GCGCUGGAUGAAGUGGCACAGUCAGC UUCCCUGGGGGCUGGUGUCAUGUUGG GCUCCUGGGGCGGGGGCACGGCCUGG CAUUUCACGCAUUGCUGCCACCCCAG GUCCACCUGUCUCCACUUUCACAGCC UCCAAGUCUGUGGCUCUUCCCUUCUG UCCUCCGAGGGGCUUGCCUUCUCUCG UGUCCAGUGAGGUGCUCAGUGAUCGG CUUAACUUAGAGAAGCCCGCCCCCUC CCCUUCUCCGUCUGUCCCAAGAGGGU CUGCUCUGAGCCUGCGUUCCUAGGUG GCUCGGCCUCAGCUGCCUGGGUUGUG GCCGCCCUAGCAUCCUGUAUGCCCAC AGCUACUGGAAUCCCCGCUGCUGCUC CGGGCCAAGCUUCUGGUUGAUUAAUG AGGGCAUGGGGUGGUCCCUCAAGACC UUCCCCUACCUUUUGUGGAACCAGUG AUGCCUCAAAGACAGUGUCCCCUCCA CAGCUGGGUGCCAGGGGCAGGGGAUC CUCAGUAUAGCCGGUGAACCCUGAUA CCAGGAGCCUGGGCCUCCCUGAACCC CUGGCUUCCAGCCAUCUCAUCGCCAG CCUCCUCCUGGACCUCUUGGCCCCCA GCCCCUUCCCCACACAGCCCCAGAAG GGUCCCAGAGCUGACCCCACUCCAGG ACCUAGGCCCAGCCCCUCAGCCUCAU CUGGAGCCCCUGAAGACCAGUCCCAC CCACCUUUCUGGCCUCAUCUGACACU	512

TABLE 3-continued

Exemplary 3'-Untranslated Regions

3' UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
		GCUCCGCAUCCUGCUGUGUGUCCUGU UCCAUGUUCCGGUUCCAUCCAAAUAC ACUUUCUGGAACAAA	
3UTR-017	α-globin	GCUGGAGCCUCGGUGGCCAUGCUUCU UGCCCCUUGGGCCUCCCCCCAGCCCC UCCUCCCCUUCCUGCACCCGUACCCC CGUGGUCUUUGAAUAAAGUCUGAGUG GGCGGC	513
3UTR-018	Downstream UTR	UAAUAGGCUGGAGCCUCGGUGGCCAU GCUUCUUGCCCCUUGGGCCUCCCCCC AGCCCCUCCUCCCCUUCCUGCACCCG UACCCCCGUGGUCUUUGAAUAAAGUC UGAGUGGGCGGC	514
3UTR-019	Downstream UTR	UGAUAAUAGGCUGGAGCCUCGGUGGC CAUGCUUCUUGCCCCUUGGGCCUCCC CCCAGCCCCUCCUCCCCUUCCUGCAC CCGUACCCCCUGGUCUUUGAAUAAAG UCUGAGUGGGCGGC	515

In certain embodiments, the 3' UTR sequence useful for the disclosure comprises a nucleotide sequence at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 497-515 and any combination thereof. In a particular embodiment, the 3' UTR sequence further comprises a miRNA binding site, e.g., miR-122 binding site. In other embodiments, a 3'UTR sequence useful for the disclosure comprises 3' UTR-018 (SEQ ID NO: 514).

In certain embodiments, the 3' UTR sequence comprises one or more miRNA binding sites, e.g., miR-122 binding sites, or any other heterologous nucleotide sequences therein, without disrupting the function of the 3' UTR. Some examples of 3' UTR sequences comprising a miRNA binding site are listed in Table 4.

TABLE 4

Exemplary 3' UTR with miRNA Binding Sites

3' UTR Identifier/ miRNA BS	Name/ Description	Sequence	SEQ ID NO.
3UTR-018 + miR-122-5p binding site	Down-stream UTR	UAAUAGGCUGGAGCCUCGGUGGC CAUGCUUCUUGCCCCUUGGGCCUC CCCCCAGCCCCUCCUCCCCUUCCU GCACCCGUACCCCCCAAACACCAU UGUCACACUCCAGUGGUCUUUGA AUAAAGUCUGAGUGGGCGGC	516
3UTR-018 + miR-122-3p binding site	Down-stream UTR	UAAUAGGCUGGAGCCUCGGUGGC CAUGCUUCUUGCCCCUUGGGCCUC CCCCCAGCCCCUCCUCCCCUUCCU GCACCCGUACCCCCUAUUUAGUGU GAUAAUGGCGUUGUGGUCUUUGA AUAAAGUCUGAGUGGGCGGC	517
3UTR-019 + miR-122 binding site	Down-stream UTR	UGAUAAUAGGCUGGAGCCUCGGU GGCCAUGCUUCUUGCCCCUUGGGC CUCCCCCCAGCCCCUCCUCCCCUU CCUGCACCCGUACCCCCCAAACAC	518

TABLE 4-continued

Exemplary 3' UTR with miRNA Binding Sites

3' UTR Identifier/ miRNA BS	Name/ Descrip- tion	Sequence	SEQ ID NO.
		CAUUGUCACACUCCAGUGGUCUU UGAAUAAAGUCUGAGUGGGCGGC	

*miRNA binding site is boxed or underlined.

In certain embodiments, the Y UTR sequence useful for the disclosure comprises a nucleotide sequence at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the sequence set forth as SEQ ID NO: 514 or 515.

Regions Having a 5' Cap

The polynucleotide comprising an mRNA encoding a polypeptide of the present disclosure can further comprise a 5' cap. The 5' cap useful for polypeptide encoding mRNA can bind the mRNA Cap Binding Protein (CBP), thereby increasing mRNA stability. The cap can further assist the removal of 5' proximal introns removal during mRNA splicing.

In some embodiments, the polynucleotide comprising an mRNA encoding a polypeptide of the present disclosure comprises a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5'-ppp-5' phosphorodiester linkages, modified nucleotides can be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, Mass.) can be used with α-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5'-ppp-5' cap. Additional modified guanosine nucleotides can be used such as α-methyl-phosphonate and seleno-phosphate nucleotides.

In certain embodiments, the 5' cap comprises 2'-O-methylation of the ribose sugars of 5'-terminal and/or 5'-anteterminal nucleotides on the 2'-hydroxyl group of the sugar ring. In other embodiments, the caps for the polypeptide-encoding mRNA include cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (i.e. endogenous, wild-type or physiological) 5'-caps in their chemical structure, while retaining cap function. Cap analogs can be chemically (i.e. non-enzymatically) or enzymatically synthesized and/or linked to the polynucleotides of the disclosure.

For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5'-5'-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3'-O-methyl group (i.e., N7,3 '-O-dimethyl-guanosine-5'-triphosphate-5'-guanosine (m^7G-3'mppp-G; which can equivalently be designated 3' O-Me-m7G(5')ppp(5')G). The 3'-O atom of the other, unmodified, guanine becomes linked to the 5'-terminal nucleotide of the capped polynucleotide. The N7- and 3'-O-methlyated guanine provides the terminal moiety of the capped polynucleotide.

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

In some embodiments, the cap is a dinucleotide cap analog. As a non-limiting example, the dinucleotide cap analog can be modified at different phosphate positions with a boranophosphate group or a phophoroselenoate group such as the dinucleotide cap analogs described in U.S. Pat. No. 8,519,110.

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

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

An mRNA of the present disclosure can also be capped post-manufacture (whether IVT or chemical synthesis), using enzymes, in order to generate more authentic 5'-cap structures. As used herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an endogenous, wild-type, natural or physiological cellular function and/or structure as compared to synthetic features or analogs, etc., of the prior art, or which outperforms the corresponding endogenous, wild-type, natural or physiological feature in one or more respects.

Non-limiting examples of more authentic 5' cap structures of the present disclosure are those which, among other things, have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5' endonucleases and/or reduced 5'decapping, as compared to synthetic 5'cap structures known in the art (or to a wild-type, natural or physiological 5'cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2'-O-methyltransferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-terminal nucleotide of a polynucleotide and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5'-terminal nucleotide of the mRNA contains a 2'-O-methyl. Such a structure is termed the Cap 1 structure. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, e.g., to other 5'cap analog structures known in the art. Cap structures include, but are not limited to, 7mG(5')ppp(5')N,pN2p (cap 0), 7mG(5')ppp(5')N1mpNp (cap 1), and 7mG(5')-ppp(5')N1mpN2mp (cap 2).

According to the present disclosure, 5' terminal caps can include endogenous caps or cap analogs. According to the present disclosure, a 5' terminal cap can comprise a guanine analog. Useful guanine analogs include, but are not limited to, inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

Poly-A Tails

In some embodiments, a polynucleotide comprising an mRNA encoding a polypeptide of the present disclosure further comprises a poly A tail. In further embodiments,

terminal groups on the poly-A tail can be incorporated for stabilization. In other embodiments, a poly-A tail comprises des-3' hydroxyl tails. The useful poly-A tails can also include structural moieties or 2'-Omethyl modifications as taught by Li et al. (2005) Current Biology 15:1501-1507.

In one embodiment, the length of a poly-A tail, when present, is greater than 30 nucleotides in length. In another embodiment, the poly-A tail is greater than 35 nucleotides in length (e.g., at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000 nucleotides).

In some embodiments, the polynucleotide or region thereof includes from about 30 to about 3,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 750, from 30 to 1,000, from 30 to 1,500, from 30 to 2,000, from 30 to 2,500, from 50 to 100, from 50 to 250, from 50 to 500, from 50 to 750, from 50 to 1,000, from 50 to 1,500, from 50 to 2,000, from 50 to 2,500, from 50 to 3,000, from 100 to 500, from 100 to 750, from 100 to 1,000, from 100 to 1,500, from 100 to 2,000, from 100 to 2,500, from 100 to 3,000, from 500 to 750, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 2,500, from 500 to 3,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 2,500, from 1,000 to 3,000, from 1,500 to 2,000, from 1,500 to 2,500, from 1,500 to 3,000, from 2,000 to 3,000, from 2,000 to 2,500, and from 2,500 to 3,000).

In some embodiments, the poly-A tail is designed relative to the length of the overall polynucleotide or the length of a particular region of the polynucleotide. This design can be based on the length of a coding region, the length of a particular feature or region or based on the length of the ultimate product expressed from the polynucleotides.

In this context, the poly-A tail can be 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% greater in length than the polynucleotide or feature thereof. The poly-A tail can also be designed as a fraction of the polynucleotides to which it belongs. In this context, the poly-A tail can be 10, 20, 30, 40, 50, 60, 70, 80, or 90% or more of the total length of the construct, a construct region or the total length of the construct minus the poly-A tail. Further, engineered binding sites and conjugation of polynucleotides for Poly-A binding protein can enhance expression.

Additionally, multiple distinct polynucleotides can be linked together via the PABP (Poly-A binding protein) through the 3'-end using modified nucleotides at the 3'-terminus of the poly-A tail. Transfection experiments can be conducted in relevant cell lines at and protein production can be assayed by ELISA at 12 hr, 24 hr, 48 hr, 72 hr and day 7 post-transfection.

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

Start Codon Region

In some embodiments, an mRNA of the present disclosure further comprises regions that are analogous to or function like a start codon region.

In some embodiments, the translation of a polynucleotide initiates on a codon which is not the start codon AUG. Translation of the polynucleotide can initiate on an alternative start codon such as, but not limited to, ACG, AGG, AAG, CTG/CUG, GTG/GUG, ATA/AUA, ATT/AUU, TTG/UUG. See Touriol et al. (2003) Biology of the Cell 95:169-178 and Matsuda and Mauro (2010) PLoS ONE 5:11. As a non-limiting example, the translation of a polynucleotide begins on the alternative start codon ACG. As another non-limiting example, polynucleotide translation begins on the alternative start codon CTG or CUG. As yet another non-limiting example, the translation of a polynucleotide begins on the alternative start codon GTG or GUG.

Nucleotides flanking a codon that initiates translation such as, but not limited to, a start codon or an alternative start codon, are known to affect the translation efficiency, the length and/or the structure of the polynucleotide. See, e.g., Matsuda and Mauro (2010) PLoS ONE 5:11. Masking any of the nucleotides flanking a codon that initiates translation can be used to alter the position of translation initiation, translation efficiency, length and/or structure of a polynucleotide.

In some embodiments, a masking agent is used near the start codon or alternative start codon in order to mask or hide the codon to reduce the probability of translation initiation at the masked start codon or alternative start codon. Non-limiting examples of masking agents include antisense locked nucleic acids (LNA) polynucleotides and exon-junction complexes (EJCs). See, e.g., Matsuda and Mauro (2010) PLoS ONE 5:11, describing masking agents LNA polynucleotides and EJCs.

In another embodiment, a masking agent is used to mask a start codon of a polynucleotide in order to increase the likelihood that translation will initiate on an alternative start codon. In some embodiments, a masking agent is used to mask a first start codon or alternative start codon in order to increase the chance that translation will initiate on a start codon or alternative start codon downstream to the masked start codon or alternative start codon.

In some embodiments, a start codon or alternative start codon is located within a perfect complement for a miR binding site. The perfect complement of a miR binding site can help control the translation, length and/or structure of the polynucleotide similar to a masking agent. As a non-limiting example, the start codon or alternative start codon is located in the middle of a perfect complement for a miR-122 binding site. The start codon or alternative start codon can be located after the first nucleotide, second nucleotide, third nucleotide, fourth nucleotide, fifth nucleotide, sixth nucleotide, seventh nucleotide, eighth nucleotide, ninth nucleotide, tenth nucleotide, eleventh nucleotide, twelfth nucleotide, thirteenth nucleotide, fourteenth nucleotide, fifteenth nucleotide, sixteenth nucleotide, seventeenth nucleotide, eighteenth nucleotide, nineteenth nucleotide, twentieth nucleotide or twenty-first nucleotide.

In another embodiment, the start codon of a polynucleotide is removed from the polynucleotide sequence in order to have the translation of the polynucleotide begin on a codon which is not the start codon. Translation of the polynucleotide can begin on the codon following the removed start codon or on a downstream start codon or an alternative start codon. In a non-limiting example, the start codon ATG or AUG is removed as the first 3 nucleotides of the polynucleotide sequence in order to have translation

initiate on a downstream start codon or alternative start codon. The polynucleotide sequence where the start codon was removed can further comprise at least one masking agent for the downstream start codon and/or alternative start codons in order to control or attempt to control the initiation of translation, the length of the polynucleotide and/or the structure of the polynucleotide.

Stop Codon Region

In some embodiments, mRNA of the present disclosure can further comprise at least one stop codon or at least two stop codons before the 3' untranslated region (UTR). The stop codon can be selected from UGA, UAA, and UAG. In some embodiments, the polynucleotides of the present disclosure include the stop codon UGA and one additional stop codon. In a further embodiment the addition stop codon can be UAA. In another embodiment, the polynucleotides of the present disclosure include three stop codons, four stop codons, or more.

Modified mRNAs

In some embodiments, an mRNA of the disclosure comprises one or more modified nucleobases, nucleosides, or nucleotides (termed "modified mRNAs" or "mmRNAs"). In some embodiments, modified mRNAs may have useful properties, including enhanced stability, intracellular retention, enhanced translation, and/or the lack of a substantial induction of the innate immune response of a cell into which the mRNA is introduced, as compared to a reference unmodified mRNA. Therefore, use of modified mRNAs may enhance the efficiency of protein production, intracellular retention of nucleic acids, as well as possess reduced immunogenicity.

Accordingly, in some embodiments, an mRNA described herein comprises a modification, wherein the modification is the incorporation of one or more chemically modified nucleotides. In some embodiments, one or more chemically modified nucleotides is incorporated into the initiation codon of the mmRNA and functions to increases binding affinity between the initiation codon and the anticodon of the initiator Met-tRNAiMet. In some embodiments, the one or more chemically modified nucleotides is 2-thiouridine. In some embodiments, the one or more chemically modified nucleotides is 2'-O-methyl-2-thiouridine. In some embodiments, the one or more chemically modified nucleotides is 2-selenouridine. In some embodiments, the one or more chemically modified nucleotides is 2'-O-methyl ribose. In some embodiments, the one or more chemically modified nucleotides is selected from a locked nucleic acid, inosine, 2-methylguanosine, or 6-methyl-adenosine. In some embodiments, deoxyribonucleotides are incorporated into mmRNA.

An mmRNA of the disclosure may include any suitable number of base pairs, including tens (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100), hundreds (e.g., 200, 300, 400, 500, 600, 700, 800, or 900) or thousands (e.g., 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000) of base pairs. Any number (e.g., all, some, or none) of nucleobases, nucleosides, or nucleotides may be an analog of a canonical species, substituted, modified, or otherwise non-naturally occurring. In certain embodiments, all of a particular nucleobase type may be modified.

In some embodiments, an mRNA may instead or additionally include a chain terminating nucleoside. For example, a chain terminating nucleoside may include those nucleosides deoxygenated at the 2' and/or 3' positions of their sugar group. Such species may include 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, and 2',3'-dideoxynucleosides, such as 2',3'-dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'-dideoxyguanosine, and 2',3'-dideoxythymine. In some embodiments, incorporation of a chain terminating nucleotide into an mRNA, for example at the 3'-terminus, may result in stabilization of the mRNA, as described, for example, in International Patent Publication No. WO 2013/103659.

An mRNA may instead or additionally include a stem loop, such as a histone stem loop. A stem loop may include 2, 3, 4, 5, 6, 7, 8, or more nucleotide base pairs. For example, a stem loop may include 4, 5, 6, 7, or 8 nucleotide base pairs. A stem loop may be located in any region of an mRNA. For example, a stem loop may be located in, before, or after an untranslated region (a 5' untranslated region or a 3' untranslated region), a coding region, or a polyA sequence or tail. In some embodiments, a stem loop may affect one or more function(s) of an mRNA, such as initiation of translation, translation efficiency, and/or transcriptional termination.

Numerous approaches for the chemical modification of mRNA to improve translation efficiency and reduce immunogenicity are known, including modifications at the 5' cap, 5' and 3'-UTRs, the open reading frame, and the poly(A) tail (Sahin et al., (2014) Nat Rev Drug Discovery 13:759-780). For example, pseudouridine (w) modified mRNA was shown to increased expression of encoded erythropoietin (Kariko et al., (2012) Mol Ther 20:948-953). A combination of 2-thiouridine (s2U) and 5-methylcytidine (5meC) in modified mRNAs was shown to extended the expression of encoded protein (Kormann et al., (2011) Nat Biotechnol 29:154-157). A recent study demonstrated the induction of vascular regeneration using modified (5meC and ψ) mRNA encoding human vascular endothelial growth factor (Zangi et al., (2013) Nat Biotechnol 31:898-907). These studies demonstrate the utility of incorporating chemically modified nucleotides to achieve mRNA structural and functional optimization

In some embodiments, an mRNA includes one or more (e.g., 1, 2, 3 or 4) different modified nucleobases, nucleosides, or nucleotides. In some embodiments, an mRNA includes one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more) different modified nucleobases, nucleosides, or nucleotides. In some embodiments, the modified mRNA may have reduced degradation in a cell into which the mRNA is introduced, relative to a corresponding unmodified mRNA.

In some embodiments, the modified nucleobase is a modified uracil. Exemplary nucleobases and nucleosides having a modified uracil include pseudouridine (ψ), pyridin-4-one ribonucleoside, 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s^2U), 4-thio-uridine (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho^5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridineor 5-bromo-uridine), 3-methyl-uridine (m^3U), 5-methoxy-uridine (mo^5U), uridine 5-oxyacetic acid (cmo^5U), uridine 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uridine (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm^5U), 5-carboxyhydroxymethyl-uridine methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uridine (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm^5s^2U), 5-aminomethyl-2-thio-uridine (nm^5s^2U), 5-methylaminomethyl-uridine (mnm^5U), 5-methylaminomethyl-2-thio-uridine (mnm^5s^2U), 5-methylaminomethyl-2-seleno-uridine (mnm^5se^2U), 5-carbamoylmethyl-uridine (ncm^5U), 5-carboxymethylaminomethyl-uridine ($cmnm^5U$), 5-carboxymethylaminomethyl-2-thio-uridine ($cmnm^5s^2U$), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyl-uridine

(τm^5U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-urdine (τm^5s^2U), 1-taurinomethyl-4-thio-pseudouridine, 5-methyl-uridine (m^5U, i.e., having the nucleobase deoxythymine), 1-methyl-pseudouridine ($m^1\psi$), 5-methyl-2-thio-uridine (m^5s^2U), 1-methyl-4-thio-pseudouridine ($m^1s^4\psi$) 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m^5D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp^3U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\psi$-(isopentenylaminomethyl)uridine (inm^5U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm^5s^2U), α-thio-uridine, 2'-O-methyl-uridine (Um), 5,2'-O-dimethyl-uridine (m^5Um), 2'-O-methyl-pseudouridine (ψm), 2-thio-2'-O-methyl-uridine (s^2Um), 5-methoxycarbonylmethyl-2'-O-methyl-uridine (mcm^5Um), 5-carbamoylmethyl-2'-O-methyl-uridine (ncm^5Um), 5-carboxymethylaminomethyl-2'-O-methyl-uridine ($cmnm^5Um$), 3,2'-O-dimethyl-uridine (m^3Um), and 5-(isopentenylaminomethyl)-2'-O-methyl-uridine (inm^5Um), 1-thio-uridine, deoxythymidine, 2'-F-ara-uridine, 2'-F-uridine, 2'-OH-ara-uridine, 5-(2-carbomethoxyvinyl) uridine, and 5-[3-(1-E-propenylamino)] uridine.

In some embodiments, the modified nucleobase is a modified cytosine. Exemplary nucleobases and nucleosides having a modified cytosine include 5-aza-cytidine, 6-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine (m^3C), N4-acetyl-cytidine (ac^4C), 5-formyl-cytidine (f^5C), N4-methyl-cytidine (m^4C), 5-methyl-cytidine (m^5C), 5-halo-cytidine (e.g., 5-iodo-cytidine), 5-hydroxymethyl-cytidine (hm^5C), 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine (s^2C), 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 1-methyl-1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, 4-methoxy-1-methyl-pseudoisocytidine, lysidine (k_2C), α-thio-cytidine, 2'-O-methyl-cytidine (Cm), 5,2'-O-dimethyl-cytidine (m^5Cm), N4-acetyl-2'-O-methyl-cytidine (ac^4Cm), N4,2'-O-dimethyl-cytidine (m^4Cm), 5-formyl-2'-O-methyl-cytidine (f^5Cm), N4,N4,2'-0-trimethyl-cytidine ($m^4{}_2Cm$), 1-thio-cytidine, 2'-F-ara-cytidine, 2'-F-cytidine, and 2'-OH-ara-cytidine.

In some embodiments, the modified nucleobase is a modified adenine. Exemplary nucleobases and nucleosides having a modified adenine include α-thio-adenosine, 2-amino-purine, 2, 6-diaminopurine, 2-amino-6-halo-purine (e.g., 2-amino-6-chloro-purine), 6-halo-purine (e.g., 6-chloro-purine), 2-amino-6-methyl-purine, 8-azido-adenosine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-amino-purine, 7-deaza-8-aza-2-amino-purine, 7-deaza-2, 6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyl-adenosine (m^1A), 2-methyl-adenine (m^2A), N6-methyl-adenosine (m^6A), 2-methylthio-N6-methyl-adenosine (ms^2 m^6A), N6-isopentenyl-adenosine (i^6A), 2-methylthio-N6-isopentenyl-adenosine (ms^2i^6A), N6-(cis-hydroxyisopentenyl)adenosine (io^6A), 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine (ms^2io^6A), N6-glycinyl-carbamoyl-adenosine (g^6A), N6-threonylcarbamoyl-adenosine (t^6A), N6-methyl-N6-threonyl carbamoyl-adenosine (m^6t^6A), 2-methylthio-N6-threonylcarbamoyl-adenosine (ms^2g^6A), N6,N6-dimethyl-adenosine ($m^6{}_2A$), N6-hydroxynorvalylcarbamoyl-adenosine (hn^6A), 2-methylthio-N6-hydroxynorvalylcarbamoyl-adenosine (ms^2hn^6A), N6-acetyl-adenosine (ac^6A), 7-methyl-adenine, 2-methylthio-adenine, 2-methoxy-adenine, α-thio-adenosine, 2'-O-methyl-adenosine (Am), N6,2'-O-dimethyl-adenosine (m^6Am), N6,N6,2'-O-trimethyl-adenosine ($m^6{}_2Am$), 1,2'-O-dimethyl-adenosine (m^1Am), 2'-O-ribosyladenosine (phosphate) (Ar(p)), 2-amino-N6-methyl-purine, 1-thio-adenosine, 8-azido-adenosine, 2'-F-ara-adenosine, 2'-F-adenosine, 2'-OH-ara-adenosine, and N6-(19-amino-pentaoxanonadecyl)-adenosine.

In some embodiments, the modified nucleobase is a modified guanine. Exemplary nucleobases and nucleosides having a modified guanine include α-thio-guanosine, inosine (I), 1-methyl-inosine (m^1I), wyosine (imG), methyl-wyosine (mimG), 4-demethyl-wyosine (imG-14), isowyosine (imG2), wybutosine (yW), peroxywybutosine (o_2yW), hydroxywybutosine (OhyW), undermodified hydroxywybutosine (OhyW*), 7-deaza-guanosine, queuosine (Q), epoxyqueuosine (oQ), galactosyl-queuosine (gal Q), mannosyl-queuosine (manQ), 7-cyano-7-deaza-guanosine ($preQ_0$), 7-aminomethyl-7-deaza-guanosine ($preQ_1$), archaeosine (G^+), 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine (m^7G), 6-thio-7-methyl-guanosine, 7-methyl-inosine, 6-methoxy-guanosine, 1-methyl-guanosine (m^1G), N2-methyl-guanosine (m^2G), N2,N2-dimethyl-guanosine ($m^2{}_2G$), N2,7-dimethyl-guanosine ($m^{2,7}G$), N2,N2,7-dimethyl-guanosine ($m^{2,7,7}G$) 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, N2,N2-dimethyl-6-thio-guanosine, α-thio-guanosine, 2'-O-methyl-guanosine (Gm), N2-methyl-2'-O-methyl-guanosine (m^2Gm), N2,N2-dimethyl-2'-O-methyl-guanosine ($m^2{}_2Gm$), 1-methyl-2'-O-methyl-guanosine (m^1Gm), N2,7-dimethyl-2'-O-methyl-guanosine ($m^{2,7}Gm$), 2'-O-methyl-inosine (Im), 1,2'-O-dimethyl-inosine (m^1Im), 2'-O-ribosylguanosine (phosphate) (Gr(p)), 1-thio-guanosine, O6-methyl-guanosine, 2'-F-ara-guanosine, and 2'-F-guanosine.

In some embodiments, an mRNA of the disclosure includes a combination of one or more of the aforementioned modified nucleobases (e.g., a combination of 2, 3 or 4 of the aforementioned modified nucleobases.)

In some embodiments, the modified nucleobase is pseudouridine (ψ), N1-methylpseudouridine ($m^1\psi$), 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine, or 2'-O-methyl uridine. In some embodiments, an mRNA of the disclosure includes a combination of one or more of the aforementioned modified nucleobases (e.g., a combination of 2, 3 or 4 of the aforementioned modified nucleobases.)

In some embodiments, the modified nucleobase is a modified cytosine. Exemplary nucleobases and nucleosides having a modified cytosine include N4-acetyl-cytidine (ac^4C), 5-methyl-cytidine (m^5C), 5-halo-cytidine (e.g., 5-iodo-cytidine), 5-hydroxymethyl-cytidine (hm^5C), 1-methyl-pseudoisocytidine, 2-thio-cytidine (s^2C), 2-thio-5-methyl-cytidine. In some embodiments, an mRNA of the disclosure includes a combination of one or more of the

aforementioned modified nucleobases (e.g., a combination of 2, 3 or 4 of the aforementioned modified nucleobases.)

In some embodiments, the modified nucleobase is a modified adenine. Exemplary nucleobases and nucleosides having a modified adenine include 7-deaza-adenine, 1-methyl-adenosine (m^1A), 2-methyl-adenine (m^2A), N6-methyl-adenosine (m^6A). In some embodiments, an mRNA of the disclosure includes a combination of one or more of the aforementioned modified nucleobases (e.g., a combination of 2, 3 or 4 of the aforementioned modified nucleobases.)

In some embodiments, the modified nucleobase is a modified guanine. Exemplary nucleobases and nucleosides having a modified guanine include inosine (I), 1-methyl-inosine (m^1I), wyosine (imG), methylwyosine (mimG), 7-deaza-guanosine, 7-cyano-7-deaza-guanosine ($preQ_0$), 7-aminomethyl-7-deaza-guanosine ($preQ_1$), 7-methyl-guanosine (m^7G), 1-methyl-guanosine (m'G), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine. In some embodiments, an mRNA of the disclosure includes a combination of one or more of the aforementioned modified nucleobases (e.g., a combination of 2, 3 or 4 of the aforementioned modified nucleobases.)

In some embodiments, the modified nucleobase is 1-methyl-pseudouridine ($m^1\psi$), 5-methoxy-uridine (mo^5U), 5-methyl-cytidine (m^5C), pseudouridine (ψ), α-thio-guanosine, or α-thio-adenosine. In some embodiments, an mRNA of the disclosure includes a combination of one or more of the aforementioned modified nucleobases (e.g., a combination of 2, 3 or 4 of the aforementioned modified nucleobases.)

In some embodiments, the mRNA comprises pseudouridine (ψ). In some embodiments, the mRNA comprises pseudouridine (ψ) and 5-methyl-cytidine (m^5C). In some embodiments, the mRNA comprises 1-methyl-pseudouridine ($m^1\psi$). In some embodiments, the mRNA comprises 1-methyl-pseudouridine ($m^1\psi$) and 5-methyl-cytidine (m^5C). In some embodiments, the mRNA comprises 2-thiouridine (s^2U). In some embodiments, the mRNA comprises 2-thiouridine and 5-methyl-cytidine (m^5C). In some embodiments, the mRNA comprises 5-methoxy-uridine (mo^5U).

In some embodiments, the mRNA comprises 5-methoxy-uridine (mo^5U) and 5-methyl-cytidine (m^5C). In some embodiments, the mRNA comprises 2'-O-methyl uridine. In some embodiments, the mRNA comprises 2'-O-methyl uridine and 5-methyl-cytidine (m^5C). In some embodiments, the mRNA comprises N6-methyl-adenosine (m^6A). In some embodiments, the mRNA comprises N6-methyl-adenosine (m^6A) and 5-methyl-cytidine (m^5C).

In certain embodiments, an mRNA of the disclosure is uniformly modified (i.e., fully modified, modified throughout the entire sequence) for a particular modification. For example, an mRNA can be uniformly modified with 5-methyl-cytidine (m^5C), meaning that all cytosine residues in the mRNA sequence are replaced with 5-methyl-cytidine (m^5C). Similarly, mRNAs of the disclosure can be uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified residue such as those set forth above.

In some embodiments, an mRNA of the disclosure may be modified in a coding region (e.g., an open reading frame encoding a polypeptide). In other embodiments, an mRNA may be modified in regions besides a coding region. For example, in some embodiments, a 5'-UTR and/or a 3'-UTR are provided, wherein either or both may independently contain one or more different nucleoside modifications. In such embodiments, nucleoside modifications may also be present in the coding region.

Examples of nucleoside modifications and combinations thereof that may be present in mmRNAs of the present disclosure include, but are not limited to, those described in PCT Patent Application Publications: WO2012045075, WO2014081507, WO2014093924, WO2014164253, and WO2014159813.

The mmRNAs of the disclosure can include a combination of modifications to the sugar, the nucleobase, and/or the internucleoside linkage. These combinations can include any one or more modifications described herein.

Examples of modified nucleosides and modified nucleoside combinations are provided below in Table 5 and Table 6. These combinations of modified nucleotides can be used to form the mmRNAs of the disclosure. In certain embodiments, the modified nucleosides may be partially or completely substituted for the natural nucleotides of the mRNAs of the disclosure. As a non-limiting example, the natural nucleotide uridine may be substituted with a modified nucleoside described herein. In another non-limiting example, the natural nucleoside uridine may be partially substituted (e.g., about 0.1%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99.9% of the natural uridines) with at least one of the modified nucleoside disclosed herein.

TABLE 5

Combinations of Nucleoside Modifications

Modified Nucleotide	Modified Nucleotide Combination
α-thio-cytidine	α-thio-cytidine/5-iodo-uridine
	α-thio-cytidine/N1-methyl-pseudouridine
	α-thio-cytidine/a-thio-uridine
	a-thio-cytidine/5-methyl-uridine
	α-thio-cytidine/pseudo-uridine
	about 50% of the cytosines are α-thio-cytidine
pseudoisocytidine	pseudoisocytidine/5-iodo-uridine
	pseudoisocytidine/N1-methyl-pseudouridine
	pseudoisocytidine/α-thio-uridine
	pseudoisocytidine/5-methyl-uridine
	pseudoisocytidine/pseudouridine
	about 25% of cytosines are pseudoisocytidine
	pseudoisocytidine/about 50% of uridines are N1-methyl-pseudouridine and about 50% of uridines are pseudouridine
	pseudoisocytidine/about 25% of uridines are N1-methyl-pseudouridine and about 25% of uridines are pseudouridine
pyrrolo-cytidine	pyrrolo-cytidine/5-iodo-uridine
	pyrrolo-cytidine/N1-methyl-pseudouridine
	pyrrolo-cytidine/α-thio-uridine
	pyrrolo-cytidine/5-methyl-uridine
	pyrrolo-cytidine/pseudouridine
	about 50% of the cytosines are pyrrolo-cytidine
5-methyl-cytidine	5-methyl-cytidine/5-iodo-uridine
	5-methyl-cytidine/N1-methyl-pseudouridine
	5-methyl-cytidine/α-thio-uridine
	5-methyl-cytidine/5-methyl-uridine
	5-methyl-cytidine/pseudouridine
	about 25% of cytosines are 5-methyl-cytidine
	about 50% of cytosines are 5-methyl-cytidine
	5-methyl-cytidine/5-methoxy-uridine
	5-methyl-cytidine/5-bromo-uridine
	5-methyl-cytidine/2-thio-uridine
	5-methyl-cytidine/about 50% of uridines are 2-thio-uridine
	about 50% of uridines are 5-methyl-cytidine/ about 50% of uridines are 2-thio-uridine
N4-acetyl-cytidine	N4-acetyl-cytidine/5-iodo-uridine
	N4-acetyl-cytidine/N1-methyl-pseudouridine
	N4-acetyl-cytidine/α-thio-uridine

TABLE 5-continued

Combinations of Nucleoside Modifications	
Modified Nucleotide	Modified Nucleotide Combination
	N4-acetyl-cytidine/5-methyl-uridine
	N4-acetyl-cytidine/pseudouridine
	about 50% of cytosines are N4-acetyl-cytidine
	about 25% of cytosines are N4-acetyl-cytidine
	N4-acetyl-cytidine/5-methoxy-uridine
	N4-acetyl-cytidine/5-bromo-uridine
	N4-acetyl-cytidine/2-thio-uridine
	about 50% of cytosines are N4-acetyl-cytidine/ about 50% of uridines are 2-thio-uridine

TABLE 6

Modified Nucleosides and Combinations Thereof
1-(2,2,2-Trifluoroethyl)pseudo-UTP
1-Ethyl-pseudo-UTP
1-Methyl-pseudo-U-alpha-thio-TP
1-methyl-pseudouridine TP, ATP, GTP, CTP
1-methyl-pseudo-UTP/5-methyl-CTP/ATP/GTP
1-methyl-pseudo-UTP/CTP/ATP/GTP
1-Propyl-pseudo-UTP
25% 5-Aminoallyl-CTP + 75 % CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Aminoallyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Bromo-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Bromo-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Bromo-CTP + 75% CTP/1-Methyl-pseudo-UTP
25% 5-Carboxy-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Carboxy-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Ethyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Ethyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Ethynyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Ethynyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Fluoro-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Fluoro-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Formyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Formyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Hydroxymethyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Hydroxymethyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Iodo-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Iodo-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Methoxy-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Methoxy-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Methyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% 1-Methyl-pseudo-UTP
25% 5-Methyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Methyl-CTP + 75% CTP/50% 5-Methoxy-UTP + 50% 1-Methyl-pseudo-UTP
25% 5-Methyl-CTP + 75% CTP/50% 5-Methoxy-UTP + 50% UTP
25% 5-Methyl-CTP + 75% CTP/5-Methoxy-UTP
25% 5-Methyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% 1-Methyl-pseudo-UTP
25% 5-Methyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Phenyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Phenyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Trifluoromethyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% 5-Trifluoromethyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Trifluoromethyl-CTP + 75% CTP/1-Methyl-pseudo-UTP
25% N4—Ac-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% N4—Ac-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% N4-Bz-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% N4-Bz-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% N4-Methyl-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% N4-Methyl-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% Pseudo-iso-CTP + 75% CTP/25% 5-Methoxy-UTP + 75% UTP
25% Pseudo-iso-CTP + 75% CTP/75% 5-Methoxy-UTP + 25% UTP
25% 5-Bromo-CTP/75% CTP/Pseudo-UTP
25% 5-methoxy-UTP/25% 5-methyl-CTP/ATP/GTP
25% 5-methoxy-UTP/5-methyl-CTP/ATP/GTP
25% 5-methoxy-UTP/75% 5-methyl-CTP/ATP/GTP
25% 5-methoxy-UTP/CTP/ATP/GTP
25% 5-metoxy-UTP/50% 5-methyl-CTP/ATP/GTP
2-Amino-ATP
2-Thio-CTP
2-thio-pseudouridine TP, ATP, GTP, CTP
2-Thio-pseudo-UTP
2-Thio-UTP
3-Methyl-CTP
3-Methyl-pseudo-UTP
4-Thio-UTP
50% 5-Bromo-CTP + 50% CTP/1-Methyl-pseudo-UTP
50% 5-Hydroxymethyl-CTP + 50% CTP/1-Methyl-pseudo-UTP
50% 5-methoxy-UTP/5-methyl-CTP/ATP/GTP
50% 5-Methyl-CTP + 50% CTP/25% 5-Methoxy-UTP + 75% 1-Methyl-pseudo-UTP
50% 5-Methyl-CTP + 50% CTP/25% 5-Methoxy-UTP + 75% UTP
50% 5-Methyl-CTP + 50% CTP/50% 5-Methoxy-UTP + 50% 1-Methyl-pseudo-UTP
50% 5-Methyl-CTP + 50% CTP/50% 5-Methoxy-UTP + 50% UTP
50% 5-Methyl-CTP + 50% CTP/5-Methoxy-UTP
50% 5-Methyl-CTP + 50% CTP/75% 5-Methoxy-UTP + 25% 1-Methyl-pseudo-UTP
50% 5-Methyl-CTP + 50% CTP/75% 5-Methoxy-UTP + 25% UTP
50% 5-Trifluoromethyl-CTP + 50% CTP/1-Methyl-pseudo-UTP
50% 5-Bromo-CTP/50% CTP/Pseudo-UTP
50% 5-methoxy-UTP/25% 5-methyl-CTP/ATP/GTP
50% 5-methoxy-UTP/50% 5-methyl-CTP/ATP/GTP
50% 5-methoxy-UTP/75% 5-methyl-CTP/ATP/GTP
50% 5-methoxy-UTP/CTP/ATP/GTP
5-Aminoallyl-CTP
5-Aminoallyl-CTP/5-Methoxy-UTP
5-Aminoallyl-UTP
5-Bromo-CTP
5-Bromo-CTP/5-Methoxy-UTP
5-Bromo-CTP/1-Methyl-pseudo-UTP
5-Bromo-CTP/Pseudo-UTP
5-bromocytidine TP, ATP, GTP, UTP
5-Bromo-UTP
5-Carboxy-CTP/5-Methoxy-UTP
5-Ethyl-CTP/5-Methoxy-UTP
5-Ethynyl-CTP/5-Methoxy-UTP
5-Fluoro-CTP/5-Methoxy-UTP
5-Formyl-CTP/5-Methoxy-UTP
5-Hydroxy-methyl-CTP/5-Methoxy-UTP
5-Hydroxymethyl-CTP
5-Hydroxymethyl-CTP/1-Methyl-pseudo-UTP
5-Hydroxymethyl-CTP/5-Methoxy-UTP
5-hydroxymethyl-cytidine TP, ATP, GTP, UTP
5-Iodo-CTP/5-Methoxy-UTP
5-Me-CTP/5-Methoxy-UTP
5-Methoxy carbonyl methyl-UTP
5-Methoxy-CTP/5-Methoxy-UTP
5-methoxy-uridine TP, ATP, GTP, UTP
5-methoxy-UTP
5-Methoxy-UTP
5-Methoxy-UTP/N6-Isopentenyl-ATP
5-methoxy-UTP/25% 5-methyl-CTP/ATP/GTP
5-methoxy-UTP/5-methyl-CTP/ATP/GTP
5-methoxy-UTP/75% 5-methyl-CTP/ATP/GTP
5-methoxy-UTP/CTP/ATP/GTP
5-Methyl-2-thio-UTP
5-Methylaminomethyl-UTP
5-Methyl-CTP/5-Methoxy-UTP
5-Methyl-CTP/5-Methoxy-UTP(cap 0)
5-Methyl-CTP/5-Methoxy-UTP(No cap)
5-Methyl-CTP/25% 5-Methoxy-UTP + 75% 1-Methyl-pseudo-UTP
5-Methyl-CTP/25% 5-Methoxy-UTP + 75% UTP
5-Methyl-CTP/50% 5-Methoxy-UTP + 50% 1-Methyl-pseudo-UTP
5-Methyl-CTP/50% 5-Methoxy-UTP + 50% UTP
5-Methyl-CTP/5-Methoxy-UTP/N6—Me-ATP
5-Methyl-CTP/75% 5-Methoxy-UTP + 25% 1-Methyl-pseudo-UTP
5-Methyl-CTP/75% 5-Methoxy-UTP + 25% UTP
5-Phenyl-CTP/5-Methoxy-UTP
5-Trifluoro-methyl-CTP/5-Methoxy-UTP
5-Trifluoromethyl-CTP
5-Trifluoromethyl-CTP/5-Methoxy-UTP
5-Trifluoromethyl-CTP/1-Methyl-pseudo-UTP
5-Trifluoromethyl-CTP/Pseudo-UTP

TABLE 6-continued

Modified Nucleosides and Combinations Thereof
5-Trifluoromethyl-UTP
5-trifluromethylcytidine TP, ATP, GTP, UTP
75% 5-Aminoallyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Aminoallyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Bromo-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Bromo-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Carboxy-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Carboxy-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Ethyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Ethyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Ethynyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Ethynyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Fluoro-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Fluoro-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Formyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Formyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Hydroxymethyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Hydroxymethyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Iodo-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Iodo-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Methoxy-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Methoxy-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-methoxy-UTP/5-methyl-CTP/ATP/GTP
75% 5-Methyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% 1-Methyl-pseudo-UTP
75% 5-Methyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Methyl-CTP + 25% CTP/50% 5-Methoxy-UTP + 50% 1-Methyl-pseudo-UTP
75% 5-Methyl-CTP + 25% CTP/50% 5-Methoxy-UTP + 50% UTP
75% 5-Methyl-CTP + 25% CTP/5-Methoxy-UTP
75% 5-Methyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% 1-Methyl-pseudo-UTP
75% 5-Methyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Phenyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Phenyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Trifluoromethyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% 5-Trifluoromethyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Trifluoromethyl-CTP + 25% CTP/1-Methyl-pseudo-UTP
75% N4—Ac-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% N4—Ac-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% N4-Bz-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% N4-Bz-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% N4-Methyl-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% N4-Methyl-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% Pseudo-iso-CTP + 25% CTP/25% 5-Methoxy-UTP + 75% UTP
75% Pseudo-iso-CTP + 25% CTP/75% 5-Methoxy-UTP + 25% UTP
75% 5-Bromo-CTP/25% CTP/1-Methyl-pseudo-UTP
75% 5-Bromo-CTP/25% CTP/Pseudo-UTP
75% 5-methoxy-UTP/25% 5-methyl-CTP/ATP/GTP
75% 5-methoxy-UTP/50% 5-methyl-CTP/ATP/GTP
75% 5-methoxy-UTP/75% 5-methyl-CTP/ATP/GTP
75% 5-methoxy-UTP/CTP/ATP/GTP
8-Aza-ATP
Alpha-thio-CTP
CTP/25% 5-Methoxy-UTP + 75% 1-Methyl-pseudo-UTP
CTP/25% 5-Methoxy-UTP + 75% UTP
CTP/50% 5-Methoxy-UTP + 50% 1-Methyl-pseudo-UTP
CTP/50% 5-Methoxy-UTP + 50% UTP
CTP/5-Methoxy-UTP
CTP/5-Methoxy-UTP (cap 0)
CTP/5-Methoxy-UTP(No cap)
CTP/75% 5-Methoxy-UTP + 25% 1-Methyl-pseudo-UTP
CTP/75% 5-Methoxy-UTP + 25% UTP
CTP/UTP(No cap)
N1—Me-GTP
N4—Ac-CTP
N4Ac-CTP/1-Methyl-pseudo-UTP
N4Ac-CTP/5-Methoxy-UTP
N4-acetyl-cytidine TP, ATP, GTP, UTP
N4-Bz-CTP/5-Methoxy-UTP
N4-methyl CTP
N4-Methyl-CTP/5-Methoxy-UTP
Pseudo-iso-CTP/5-Methoxy-UTP
PseudoU-alpha-thio-TP

TABLE 6-continued

Modified Nucleosides and Combinations Thereof
pseudouridine TP, ATP, GTP, CTP
pseudo-UTP/5-methyl-CTP/ATP/GTP
UTP-5-oxyacetic acid Me ester
Xanthosine

According to the disclosure, polynucleotides of the disclosure may be synthesized to comprise the combinations or single modifications of Table 5 or Table 6.

Where a single modification is listed, the listed nucleoside or nucleotide represents 100 percent of that A, U, G or C nucleotide or nucleoside having been modified. Where percentages are listed, these represent the percentage of that particular A, U, G or C nucleobase triphosphate of the total amount of A, U, G, or C triphosphate present. For example, the combination: 25% 5-Aminoallyl-CTP+75 CTP/25% 5-Methoxy-UTP+75 UTP refers to a polynucleotide where 25% of the cytosine triphosphates are 5-Aminoallyl-CTP while 75% of the cytosines are CTP; whereas 25% of the uracils are 5-methoxy UTP while 75% of the uracils are UTP. Where no modified UTP is listed then the naturally occurring ATP, UTP, GTP and/or CTP is used at 100% of the sites of those nucleotides found in the polynucleotide. In this example all of the GTP and ATP nucleotides are left unmodified.

The mRNAs of the present disclosure, or regions thereof, may be codon optimized. Codon optimization methods are known in the art and may be useful for a variety of purposes: matching codon frequencies in host organisms to ensure proper folding, bias GC content to increase mRNA stability or reduce secondary structures, minimize tandem repeat codons or base runs that may impair gene construction or expression, customize transcriptional and translational control regions, insert or remove proteins trafficking sequences, remove/add post translation modification sites in encoded proteins (e.g., glycosylation sites), add, remove or shuffle protein domains, insert or delete restriction sites, modify ribosome binding sites and mRNA degradation sites, adjust translation rates to allow the various domains of the protein to fold properly, or to reduce or eliminate problem secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art; non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park, Calif.) and/or proprietary methods. In one embodiment, the mRNA sequence is optimized using optimization algorithms, e.g., to optimize expression in mammalian cells or enhance mRNA stability.

In certain embodiments, the present disclosure includes polynucleotides having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to any of the polynucleotide sequences described herein.

mRNAs of the present disclosure may be produced by means available in the art, including but not limited to in vitro transcription (IVT) and synthetic methods. Enzymatic (IVT), solid-phase, liquid-phase, combined synthetic methods, small region synthesis, and ligation methods may be utilized. In one embodiment, mRNAs are made using IVT enzymatic synthesis methods. Methods of making polynucleotides by IVT are known in the art and are described in International Application PCT/US2013/30062, the contents of which are incorporated herein by reference in their entirety. Accordingly, the present disclosure also includes

polynucleotides, e.g., DNA, constructs and vectors that may be used to in vitro transcribe an mRNA described herein.

Non-natural modified nucleobases may be introduced into polynucleotides, e.g., mRNA, during synthesis or post-synthesis. In certain embodiments, modifications may be on internucleoside linkages, purine or pyrimidine bases, or sugar. In particular embodiments, the modification may be introduced at the terminal of a polynucleotide chain or anywhere else in the polynucleotide chain; with chemical synthesis or with a polymerase enzyme. Examples of modified nucleic acids and their synthesis are disclosed in PCT application No. PCT/US2012/058519. Synthesis of modified polynucleotides is also described in Verma and Eckstein, Annual Review of Biochemistry, vol. 76, 99-134 (1998).

Either enzymatic or chemical ligation methods may be used to conjugate polynucleotides or their regions with different functional moieties, such as targeting or delivery agents, fluorescent labels, liquids, nanoparticles, etc. Conjugates of polynucleotides and modified polynucleotides are reviewed in Goodchild, Bioconjugate Chemistry, vol. 1(3), 165-187 (1990).

MicroRNA (miRNA) Binding Sites

Polynucleotides of the disclosure can include regulatory elements, for example, microRNA (miRNA) binding sites, transcription factor binding sites, structured mRNA sequences and/or motifs, artificial binding sites engineered to act as pseudo-receptors for endogenous nucleic acid binding molecules, and combinations thereof. In some embodiments, polynucleotides including such regulatory elements are referred to as including "sensor sequences." Non-limiting examples of sensor sequences are described in U.S. Publication 2014/0200261, the contents of which are incorporated herein by reference in their entirety.

In some embodiments, a polynucleotide (e.g., a ribonucleic acid (RNA), e.g., a messenger RNA (mRNA)) of the disclosure comprises an open reading frame (ORF) encoding a polypeptide of interest and further comprises one or more miRNA binding site(s). Inclusion or incorporation of miRNA binding site(s) provides for regulation of polynucleotides of the disclosure, and in turn, of the polypeptides encoded therefrom, based on tissue-specific and/or cell-type specific expression of naturally-occurring miRNAs.

A miRNA, e.g., a natural-occurring miRNA, is a 19-25 nucleotide long noncoding RNA that binds to a polynucleotide and down-regulates gene expression either by reducing stability or by inhibiting translation of the polynucleotide. A miRNA sequence comprises a "seed" region, i.e., a sequence in the region of positions 2-8 of the mature miRNA. A miRNA seed can comprise positions 2-8 or 2-7 of the mature miRNA. In some embodiments, a miRNA seed can comprise 7 nucleotides (e.g., nucleotides 2-8 of the mature miRNA), wherein the seed-complementary site in the corresponding miRNA binding site is flanked by an adenosine (A) opposed to miRNA position 1. In some embodiments, a miRNA seed can comprise 6 nucleotides (e.g., nucleotides 2-7 of the mature miRNA), wherein the seed-complementary site in the corresponding miRNA binding site is flanked by an adenosine (A) opposed to miRNA position 1. See, for example, Grimson A, Farh K K, Johnston W K, Garrett-Engele P, Lim L P, Bartel D P; Mol Cell. 2007 Jul. 6; 27(1):91-105. miRNA profiling of the target cells or tissues can be conducted to determine the presence or absence of miRNA in the cells or tissues. In some embodiments, a polynucleotide (e.g., a ribonucleic acid (RNA), e.g., a messenger RNA (mRNA)) of the disclosure comprises one or more microRNA binding sites, microRNA target sequences, microRNA complementary sequences, or microRNA seed complementary sequences. Such sequences can correspond to, e.g., have complementarity to, any known microRNA such as those taught in US Publication US2005/0261218 and US Publication US2005/0059005, the contents of each of which are incorporated herein by reference in their entirety.

As used herein, the term "microRNA (miRNA or miR) binding site" refers to a sequence within a polynucleotide, e.g., within a DNA or within an RNA transcript, including in the 5'UTR and/or 3'UTR, that has sufficient complementarity to all or a region of a miRNA to interact with, associate with or bind to the miRNA. In some embodiments, a polynucleotide of the disclosure comprising an ORF encoding a polypeptide of interest and further comprises one or more miRNA binding site(s). In exemplary embodiments, a 5'UTR and/or 3'UTR of the polynucleotide (e.g., a ribonucleic acid (RNA), e.g., a messenger RNA (mRNA)) comprises the one or more miRNA binding site(s).

A miRNA binding site having sufficient complementarity to a miRNA refers to a degree of complementarity sufficient to facilitate miRNA-mediated regulation of a polynucleotide, e.g., miRNA-mediated translational repression or degradation of the polynucleotide. In exemplary aspects of the disclosure, a miRNA binding site having sufficient complementarity to the miRNA refers to a degree of complementarity sufficient to facilitate miRNA-mediated degradation of the polynucleotide, e.g., miRNA-guided RNA-induced silencing complex (RISC)-mediated cleavage of mRNA. The miRNA binding site can have complementarity to, for example, a 19-25 nucleotide miRNA sequence, to a 19-23 nucleotide miRNA sequence, or to a 22 nucleotide miRNA sequence. A miRNA binding site can be complementary to only a portion of a miRNA, e.g., to a portion less than 1, 2, 3, or 4 nucleotides of the full length of a naturally-occurring miRNA sequence. Full or complete complementarity (e.g., full complementarity or complete complementarity over all or a significant portion of the length of a naturally-occurring miRNA) is preferred when the desired regulation is mRNA degradation.

In some embodiments, a miRNA binding site includes a sequence that has complementarity (e.g., partial or complete complementarity) with a miRNA seed sequence. In some embodiments, the miRNA binding site includes a sequence that has complete complementarity with a miRNA seed sequence. In some embodiments, a miRNA binding site includes a sequence that has complementarity (e.g., partial or complete complementarity) with an miRNA sequence. In some embodiments, the miRNA binding site includes a sequence that has complete complementarity with a miRNA sequence. In some embodiments, a miRNA binding site has complete complementarity with a miRNA sequence but for 1, 2, or 3 nucleotide substitutions, terminal additions, and/or truncations.

In some embodiments, the miRNA binding site is the same length as the corresponding miRNA. In other embodiments, the miRNA binding site is one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve nucleotide(s) shorter than the corresponding miRNA at the 5' terminus, the 3' terminus, or both. In still other embodiments, the microRNA binding site is two nucleotides shorter than the corresponding microRNA at the 5' terminus, the 3' terminus, or both. The miRNA binding sites that are shorter than the corresponding miRNAs are still capable of degrading the mRNA incorporating one or more of the miRNA binding sites or preventing the mRNA from translation.

In some embodiments, the miRNA binding site binds the corresponding mature miRNA that is part of an active RISC

containing Dicer. In another embodiment, binding of the miRNA binding site to the corresponding miRNA in RISC degrades the mRNA containing the miRNA binding site or prevents the mRNA from being translated. In some embodiments, the miRNA binding site has sufficient complementarity to miRNA so that a RISC complex comprising the miRNA cleaves the polynucleotide comprising the miRNA binding site. In other embodiments, the miRNA binding site has imperfect complementarity so that a RISC complex comprising the miRNA induces instability in the polynucleotide comprising the miRNA binding site. In another embodiment, the miRNA binding site has imperfect complementarity so that a RISC complex comprising the miRNA represses transcription of the polynucleotide comprising the miRNA binding site.

In some embodiments, the miRNA binding site has one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve mismatch(es) from the corresponding miRNA.

In some embodiments, the miRNA binding site has at least about ten, at least about eleven, at least about twelve, at least about thirteen, at least about fourteen, at least about fifteen, at least about sixteen, at least about seventeen, at least about eighteen, at least about nineteen, at least about twenty, or at least about twenty-one contiguous nucleotides complementary to at least about ten, at least about eleven, at least about twelve, at least about thirteen, at least about fourteen, at least about fifteen, at least about sixteen, at least about seventeen, at least about eighteen, at least about nineteen, at least about twenty, or at least about twenty-one, respectively, contiguous nucleotides of the corresponding miRNA.

By engineering one or more miRNA binding sites into a polynucleotide of the disclosure, the polynucleotide can be targeted for degradation or reduced translation, provided the miRNA in question is available. This can reduce off-target effects upon delivery of the polynucleotide. For example, if a polynucleotide of the disclosure is not intended to be delivered to a tissue or cell but ends up is said tissue or cell, then a miRNA abundant in the tissue or cell can inhibit the expression of the gene of interest if one or multiple binding sites of the miRNA are engineered into the 5'UTR and/or 3'UTR of the polynucleotide.

Conversely, miRNA binding sites can be removed from polynucleotide sequences in which they naturally occur in order to increase protein expression in specific tissues. For example, a binding site for a specific miRNA can be removed from a polynucleotide to improve protein expression in tissues or cells containing the miRNA.

In one embodiment, a polynucleotide of the disclosure can include at least one miRNA-binding site in the 5'UTR and/or 3'UTR in order to regulate cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells. In another embodiment, a polynucleotide of the disclosure can include two, three, four, five, six, seven, eight, nine, ten, or more miRNA-binding sites in the 5'-UTR and/or 3'-UTR in order to regulate cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells.

Regulation of expression in multiple tissues can be accomplished through introduction or removal of one or more miRNA binding sites, e.g., one or more distinct miRNA binding sites. The decision whether to remove or insert a miRNA binding site can be made based on miRNA expression patterns and/or their profilings in tissues and/or cells in development and/or disease. Identification of miRNAs, miRNA binding sites, and their expression patterns and role in biology have been reported (e.g., Bonauer et al., Curr Drug Targets 2010 11:943-949; Anand and Cheresh Curr Opin Hematol 2011 18:171-176; Contreras and Rao Leukemia 2012 26:404-413 (2011 December 20. doi: 10.1038/leu.2011.356); Bartel Cell 2009 136:215-233; Landgraf et al, Cell, 2007 129:1401-1414; Gentner and Naldini, Tissue Antigens. 2012 80:393-403 and all references therein; each of which is incorporated herein by reference in its entirety).

miRNAs and miRNA binding sites can correspond to any known sequence, including non-limiting examples described in U.S. Publication Nos. 2014/0200261, 2005/0261218, and 2005/0059005, each of which are incorporated herein by reference in their entirety. Exemplary representative microRNAs and microRNA binding sites are shown in Table 7.

TABLE 7

Representative microRNAs and microRNA binding sites

SEQ ID NO.	Description	Sequence
519	miR-142	GACAGUGCAGUCACCCAUAAAGUAGAAAGCAC UACUAACAGCACUGGAGGGUGUAGUGUUUCC UACUUUAUGGAUGAGUGUACUGUG
520	miR-142-3p	UGUAGUGUUUCCUACUUUAUGGA
521	miR-142-3p binding site	UCCAUAAAGUAGGAAACACUACA
522	miR-142-5p	CAUAAAGUAGAAAGCACUACU
523	miR-142-5p binding site	AGUAGUGCUUUCUACUUUAUG
524	miR-122	CCUUAGCAGAGCUGUGGAGUGUGACAAUGGU GUUUGUGUCUAAACUAUCAAACGCCAUUAUCA CACUAAAUAGCUACUGCUAGGC
525	miR-122-3p	AACGCCAUUAUCACACUAAAUA
526	miR-122-3p binding site	UAUUUAGUGUGAUAAUGGCGUU
527	miR-122-5p	UGGAGUGUGACAAUGGUGUUUG
528	miR-122-5p binding site	CAAACACCAUUGUCACACUCCA

Examples of tissues where miRNA are known to regulate mRNA, and thereby protein expression, include, but are not limited to, liver (miR-122), muscle (miR-133, miR-206, miR-208), endothelial cells (miR-17-92, miR-126), myeloid cells (miR-142-3p, miR-142-5p, miR-16, miR-21, miR-223, miR-24, miR-27), adipose tissue (let-7, miR-30c), heart (miR-1d, miR-149), kidney (miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126).

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

topoietic cells (immune cells). For example, miR-142 and miR-146 are exclusively expressed in immune cells, particularly abundant in myeloid dendritic cells. It has been demonstrated that the immune response to a polynucleotide can be shut-off by adding miR-142 binding sites to the 3'-UTR of the polynucleotide, enabling more stable gene transfer in tissues and cells. miR-142 efficiently degrades exogenous polynucleotides in antigen presenting cells and suppresses cytotoxic elimination of transduced cells (e.g., Annoni A et al., blood, 2009, 114, 5152-5161; Brown B D, et al., Nat med. 2006, 12(5), 585-591; Brown B D, et al., blood, 2007, 110(13): 4144-4152, each of which is incorporated herein by reference in its entirety).

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

Introducing a miR-142 binding site into the 5'UTR and/or 3'UTR of a polynucleotide of the disclosure can selectively repress gene expression in antigen presenting cells through miR-142 mediated degradation, limiting antigen presentation in antigen presenting cells (e.g., dendritic cells) and thereby preventing antigen-mediated immune response after the delivery of the polynucleotide. The polynucleotide is then stably expressed in target tissues or cells without triggering cytotoxic elimination.

In one embodiment, binding sites for miRNAs that are known to be expressed in immune cells, in particular, antigen presenting cells, can be engineered into a polynucleotide of the disclosure to suppress the expression of the polynucleotide in antigen presenting cells through miRNA mediated RNA degradation, subduing the antigen-mediated immune response. Expression of the polynucleotide is maintained in non-immune cells where the immune cell specific miRNAs are not expressed. For example, in some embodiments, to prevent an immunogenic reaction against a liver specific protein, any miR-122 binding site can be removed and a miR-142 (and/or mirR-146) binding site can be engineered into the 5'UTR and/or 3'UTR of a polynucleotide of the disclosure.

To further drive the selective degradation and suppression in APCs and macrophage, a polynucleotide of the disclosure can include a further negative regulatory element in the 5'UTR and/or 3'UTR, either alone or in combination with miR-142 and/or miR-146 binding sites. As a non-limiting example, the further negative regulatory element is a Constitutive Decay Element (CDE).

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

Many miRNA expression studies are conducted to profile the differential expression of miRNAs in various cancer cells/tissues and other diseases. Some miRNAs are abnormally over-expressed in certain cancer cells and others are under-expressed.

As a non-limiting example, miRNA binding sites for miRNAs that are over-expressed in certain cancer and/or tumor cells can be removed from the 3'UTR of a polynucleotide of the disclosure, restoring the expression suppressed by the over-expressed miRNAs in cancer cells, thus ameliorating the corresponsive biological function, for instance, transcription stimulation and/or repression, cell cycle arrest, apoptosis and cell death. Normal cells and tissues, wherein miRNAs expression is not up-regulated, will remain unaffected.

miRNA can also regulate complex biological processes such as angiogenesis (e.g., miR-132) (Anand and Cheresh Curr Opin Hematol 2011 18:171-176). In the polynucleotides of the disclosure, miRNA binding sites that are involved in such processes can be removed or introduced, in order to tailor the expression of the polynucleotides to biologically relevant cell types or relevant biological processes. In this context, the polynucleotides of the disclosure are defined as auxotrophic polynucleotides.

In some embodiments, the therapeutic window and/or differential expression (e.g., tissue-specific expression) of a polypeptide of the disclosure may be altered by incorporation of a miRNA binding site into an mRNA encoding the polypeptide. In one example, an mRNA may include one or more miRNA binding sites that are bound by miRNAs that have higher expression in one tissue type as compared to another. In another example, an mRNA may include one or more miRNA binding sites that are bound by miRNAs that have lower expression in a cancer cell as compared to a non-cancerous cell of the same tissue of origin. When present in a cancer cell that expresses low levels of such an miRNA, the polypeptide encoded by the mRNA typically will show increased expression.

Liver cancer cells (e.g., hepatocellular carcinoma cells) typically express low levels of miR-122 as compared to normal liver cells. Therefore, an mRNA encoding a polypeptide that includes at least one miR-122 binding site (e.g., in the 3'-UTR of the mRNA) will typically express comparatively low levels of the polypeptide in normal liver cells and comparatively high levels of the polypeptide in liver cancer cells.

In some embodiments, a miRNA binding site is inserted in the polynucleotide of the disclosure in any position of the polynucleotide (e.g., the 5'UTR and/or 3'UTR). In some embodiments, the 5'UTR comprises a miRNA binding site. In some embodiments, the 3'UTR comprises a miRNA binding site. In some embodiments, the 5'UTR and the 3'UTR comprise a miRNA binding site. The insertion site in the polynucleotide can be anywhere in the polynucleotide as long as the insertion of the miRNA binding site in the polynucleotide does not interfere with the translation of a functional polypeptide in the absence of the corresponding miRNA; and in the presence of the miRNA, the insertion of the miRNA binding site in the polynucleotide and the binding of the miRNA binding site to the corresponding miRNA are capable of degrading the polynucleotide or preventing the translation of the polynucleotide.

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

In one embodiment, other regulatory elements and/or structural elements of the 5'UTR can influence miRNA

mediated gene regulation. One example of a regulatory element and/or structural element is a structured IRES (Internal Ribosome Entry Site) in the 5'UTR, which is necessary for the binding of translational elongation factors to initiate protein translation. EIF4A2 binding to this secondarily structured element in the 5'-UTR is necessary for miRNA mediated gene expression (Meijer H A et al., Science, 2013, 340, 82-85, incorporated herein by reference in its entirety). The polynucleotides of the disclosure can further include this structured 5'UTR in order to enhance microRNA mediated gene regulation.

At least one miRNA binding site can be engineered into the 3'UTR of a polynucleotide of the disclosure. In this context, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or more miRNA binding sites can be engineered into a 3'UTR of a polynucleotide of the disclosure. For example, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 2, or 1 miRNA binding sites can be engineered into the 3'UTR of a polynucleotide of the disclosure. In one embodiment, miRNA binding sites incorporated into a polynucleotide of the disclosure can be the same or can be different miRNA sites. A combination of different miRNA binding sites incorporated into a polynucleotide of the disclosure can include combinations in which more than one copy of any of the different miRNA sites are incorporated. In another embodiment, miRNA binding sites incorporated into a polynucleotide of the disclosure can target the same or different tissues in the body. As a non-limiting example, through the introduction of tissue-, cell-type-, or disease-specific miRNA binding sites in the 3'-UTR of a polynucleotide of the disclosure, the degree of expression in specific cell types (e.g., hepatocytes, myeloid cells, endothelial cells, cancer cells, etc.) can be reduced.

In one embodiment, a miRNA binding site can be engineered near the 5' terminus of the 3'UTR, about halfway between the 5' terminus and 3' terminus of the 3'UTR and/or near the 3' terminus of the 3'UTR in a polynucleotide of the disclosure. As a non-limiting example, a miRNA binding site can be engineered near the 5' terminus of the 3'UTR and about halfway between the 5' terminus and 3' terminus of the 3'UTR. As another non-limiting example, a miRNA binding site can be engineered near the 3' terminus of the 3'UTR and about halfway between the 5' terminus and 3' terminus of the 3'UTR. As yet another non-limiting example, a miRNA binding site can be engineered near the 5' terminus of the 3'UTR and near the 3' terminus of the 3'UTR.

In another embodiment, a 3'UTR can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 miRNA binding sites. The miRNA binding sites can be complementary to a miRNA, miRNA seed sequence, and/or miRNA sequences flanking the seed sequence.

In one embodiment, a polynucleotide of the disclosure can be engineered to include more than one miRNA site expressed in different tissues or different cell types of a subject. As a non-limiting example, a polynucleotide of the disclosure can be engineered to include miR-192 and miR-122 to regulate expression of the polynucleotide in the liver and kidneys of a subject. In another embodiment, a polynucleotide of the disclosure can be engineered to include more than one miRNA site for the same tissue.

In some embodiments, the therapeutic window and or differential expression associated with the polypeptide encoded by a polynucleotide of the disclosure can be altered with a miRNA binding site. For example, a polynucleotide encoding a polypeptide that provides a death signal can be designed to be more highly expressed in cancer cells by virtue of the miRNA signature of those cells. Where a cancer cell expresses a lower level of a particular miRNA, the polynucleotide encoding the binding site for that miRNA (or miRNAs) would be more highly expressed. Hence, the polypeptide that provides a death signal triggers or induces cell death in the cancer cell. Neighboring noncancer cells, harboring a higher expression of the same miRNA would be less affected by the encoded death signal as the polynucleotide would be expressed at a lower level due to the effects of the miRNA binding to the binding site or "sensor" encoded in the 3'UTR. Conversely, cell survival or cytoprotective signals can be delivered to tissues containing cancer and non-cancerous cells where a miRNA has a higher expression in the cancer cells—the result being a lower survival signal to the cancer cell and a larger survival signal to the normal cell. Multiple polynucleotides can be designed and administered having different signals based on the use of miRNA binding sites as described herein.

In some embodiments, the expression of a polynucleotide of the disclosure can be controlled by incorporating at least one sensor sequence in the polynucleotide and formulating the polynucleotide for administration. As a non-limiting example, a polynucleotide of the disclosure can be targeted to a tissue or cell by incorporating a miRNA binding site and formulating the polynucleotide in a lipid nanoparticle comprising a cationic lipid, including any of the lipids described herein.

A polynucleotide of the disclosure can be engineered for more targeted expression in specific tissues, cell types, or biological conditions based on the expression patterns of miRNAs in the different tissues, cell types, or biological conditions. Through introduction of tissue-specific miRNA binding sites, a polynucleotide of the disclosure can be designed for optimal protein expression in a tissue or cell, or in the context of a biological condition.

In some embodiments, a polynucleotide of the disclosure can be designed to incorporate miRNA binding sites that either have 100% identity to known miRNA seed sequences or have less than 100% identity to miRNA seed sequences. In some embodiments, a polynucleotide of the disclosure can be designed to incorporate miRNA binding sites that have at least: 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to known miRNA seed sequences. The miRNA seed sequence can be partially mutated to decrease miRNA binding affinity and as such result in reduced downmodulation of the polynucleotide. In essence, the degree of match or mis-match between the miRNA binding site and the miRNA seed can act as a rheostat to more finely tune the ability of the miRNA to modulate protein expression. In addition, mutation in the non-seed region of a miRNA binding site can also impact the ability of a miRNA to modulate protein expression.

In one embodiment, a miRNA sequence can be incorporated into the loop of a stem loop.

In another embodiment, a miRNA seed sequence can be incorporated in the loop of a stem loop and a miRNA binding site can be incorporated into the 5' or 3' stem of the stem loop.

In one embodiment, a translation enhancer element (TEE) can be incorporated on the 5'end of the stem of a stem loop and a miRNA seed can be incorporated into the stem of the stem loop. In another embodiment, a TEE can be incorporated on the 5' end of the stem of a stem loop, a miRNA seed can be incorporated into the stem of the stem loop and a miRNA binding site can be incorporated into the 3' end of

the stem or the sequence after the stem loop. The miRNA seed and the miRNA binding site can be for the same and/or different miRNA sequences.

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

In one embodiment, the 5'-UTR of a polynucleotide of the disclosure can comprise at least one miRNA sequence. The miRNA sequence can be, but is not limited to, a 19 or 22 nucleotide sequence and/or a miRNA sequence without the seed.

In one embodiment the miRNA sequence in the 5'UTR can be used to stabilize a polynucleotide of the disclosure described herein.

In another embodiment, a miRNA sequence in the 5'UTR of a polynucleotide of the disclosure can be used to decrease the accessibility of the site of translation initiation such as, but not limited to a start codon. See, e.g., Matsuda et al., PLoS One. 2010 11(5):e15057; incorporated herein by reference in its entirety, which used antisense locked nucleic acid (LNA) oligonucleotides and exon junction complexes (EJCs) around a start codon (−4 to +37 where the A of the AUG codons is +1) in order to decrease the accessibility to the first start codon (AUG). Matsuda showed that altering the sequence around the start codon with an LNA or EJC affected the efficiency, length and structural stability of a polynucleotide. A polynucleotide of the disclosure can comprise a miRNA sequence, instead of the LNA or EJC sequence described by Matsuda et al, near the site of translation initiation in order to decrease the accessibility to the site of translation initiation. The site of translation initiation can be prior to, after or within the miRNA sequence. As a non-limiting example, the site of translation initiation can be located within a miRNA sequence such as a seed sequence or binding site. As another non-limiting example, the site of translation initiation can be located within a miR-122 sequence such as the seed sequence or the mir-122 binding site.

In some embodiments, a polynucleotide of the disclosure can include at least one miRNA in order to dampen the antigen presentation by antigen presenting cells. The miRNA can be the complete miRNA sequence, the miRNA seed sequence, the miRNA sequence without the seed, or a combination thereof. As a non-limiting example, a miRNA incorporated into a polynucleotide of the disclosure can be specific to the hematopoietic system. As another non-limiting example, a miRNA incorporated into a polynucleotide of the disclosure to dampen antigen presentation is miR-142-3p.

In some embodiments, a polynucleotide of the disclosure can include at least one miRNA in order to dampen expression of the encoded polypeptide in a tissue or cell of interest. As a non-limiting example, a polynucleotide of the disclosure can include at least one miR-122 binding site in order to dampen expression of an encoded polypeptide of interest in the liver. As another non-limiting example a polynucleotide of the disclosure can include at least one miR-142-3p binding site, miR-142-3p seed sequence, miR-142-3p binding site without the seed, miR-142-5p binding site, miR-142-5p seed sequence, miR-142-5p binding site without the seed, miR-146 binding site, miR-146 seed sequence and/or miR-146 binding site without the seed sequence.

In some embodiments, a polynucleotide of the disclosure can comprise at least one miRNA binding site in the 3'UTR in order to selectively degrade mRNA therapeutics in the immune cells to subdue unwanted immunogenic reactions caused by therapeutic delivery. As a non-limiting example, the miRNA binding site can make a polynucleotide of the disclosure more unstable in antigen presenting cells. Non-limiting examples of these miRNAs include mir-142-5p, mir-142-3p, mir-146a-5p, and mir-146-3p.

In one embodiment, a polynucleotide of the disclosure comprises at least one miRNA sequence in a region of the polynucleotide that can interact with a RNA binding protein.

In some embodiments, the polynucleotide of the disclosure (e.g., a RNA, e.g., a mRNA) comprising (i) a sequence-optimized nucleotide sequence (e.g., an ORF) and (ii) a miRNA binding site (e.g., a miRNA binding site that binds to miR-142).

In some embodiments, the polynucleotide of the disclosure comprises a uracil-modified sequence encoding a polypeptide disclosed herein and a miRNA binding site disclosed herein, e.g., a miRNA binding site that binds to miR-142 or miR-122. In some embodiments, the uracil-modified sequence encoding a polypeptide comprises at least one chemically modified nucleobase, e.g., 5-methoxyuracil. In some embodiments, at least 95% of a type of nucleobase (e.g., uracil) in a uracil-modified sequence encoding a polypeptide of the disclosure are modified nucleobases. In some embodiments, at least 95% of uricil in a uracil-modified sequence encoding a polypeptide is 5-methoxyuridine. In some embodiments, the polynucleotide comprising a nucleotide sequence encoding a polypeptide disclosed herein and a miRNA binding site is formulated with a delivery agent, e.g., a compound having the Formula (I), e.g., any of Compounds 1-147.

Preparation of High Purity RNA

In order to enhance the purity of synthetically produced RNA, modified in vitro transcription (IVT) processes which produce RNA preparations having vastly different properties from RNA produced using a traditional IVT process may be used. The RNA preparations produced according to these methods have properties that enable the production of qualitatively and quantitatively superior compositions. Even when coupled with extensive purification processes, RNA produced using traditional IVT methods is qualitatively and quantitatively distinct from the RNA preparations produced by the modified IVT processes. For instance, the purified RNA preparations are less immunogenic in comparison to RNA preparations made using traditional IVT. Additionally, increased protein expression levels with higher purity are produced from the purified RNA preparations.

Traditional IVT reactions are performed by incubating a DNA template with an RNA polymerase and equimolar quantities of nucleotide triphosphates, including GTP, ATP, CTP, and UTP in a transcription buffer. An RNA transcript having a 5' terminal guanosine triphosphate is produced from this reaction. These reactions also result in the production of a number of impurities such as double stranded and single stranded RNAs which are immunostimulatory and may have an additive impact. The purity methods described herein prevent formation of reverse complements and thus prevent the innate immune recognition of both species. In some embodiments the modified IVT methods result in the production of RNA having significantly reduced T cell activity than an RNA preparation made using prior art methods with equimolar NTPs. The prior art attempts to remove these undesirable components using a series of subsequent purification steps. Such purification methods are

undesirable because they involve additional time and resources and also result in the incorporation of residual organic solvents in the final product, which is undesirable for a pharmaceutical product. It is labor and capital intensive to scale up processes like reverse phase chromatography (RP): utilizing for instance explosion proof facilities, HPLC columns and purification systems rated for high pressure, high temperature, flammable solvents etc. The scale and throughput for large scale manufacture are limited by these factors. Subsequent purification is also required to remove alkylammonium ion pair utilized in RP process. In contrast the methods described herein even enhance currently utilized methods (eg RP). Lower impurity load leads to higher purification recovery of full length RNA devoid of cytokine inducing contaminants eg. higher quality of materials at the outset.

The modified IVT methods involve the manipulation of one or more of the reaction parameters in the IVT reaction to produce a RNA preparation of highly functional RNA without one or more of the undesirable contaminants produced using the prior art processes. One parameter in the IVT reaction that may be manipulated is the relative amount of a nucleotide or nucleotide analog in comparison to one or more other nucleotides or nucleotide analogs in the reaction mixture (e.g., disparate nucleotide amounts or concentration). For instance, the IVT reaction may include an excess of a nucleotides, e.g., nucleotide monophosphate, nucleotide diphosphate or nucleotide triphosphate and/or an excess of nucleotide analogs and/or nucleoside analogs. The methods produce a high yield product which is significantly more pure than products produced by traditional IVT methods.

Nucleotide analogs are compounds that have the general structure of a nucleotide or are structurally similar to a nucleotide or portion thereof. In particular, nucleotide analogs are nucleotides which contain, for example, an analogue of the nucleic acid portion, sugar portion and/or phosphate groups of the nucleotide. Nucleotides include, for instance, nucleotide monophosphates, nucleotide diphosphates, and nucleotide triphosphates. A nucleotide analog, as used herein is structurally similar to a nucleotide or portion thereof but does not have the typical nucleotide structure (nucleobase-ribose-phosphate). Nucleoside analogs are compounds that have the general structure of a nucleoside or are structurally similar to a nucleoside or portion thereof. In particular, nucleoside analogs are nucleosides which contain, for example, an analogue of the nucleic acid and/or sugar portion of the nucleoside.

The nucleotide analogs useful in the methods are structurally similar to nucleotides or portions thereof but, for example, are not polymerizable by T7. Nucleotide/nucleoside analogs as used herein (including C, T, A, U, G, dC, dT, dA, dU, or dG analogs) include for instance, antiviral nucleotide analogs, phosphate analogs (soluble or immobilized, hydrolyzable or non-hydrolyzable), dinucleotide, trinucleotide, tetranucleotide, e.g., a cap analog, or a precursor/substrate for enzymatic capping (vaccinia, or ligase), a nucleotide labelled with a functional group to facilitate ligation/conjugation of cap or 5' moiety (IRES), a nucleotide labelled with a 5' PO4 to facilitate ligation of cap or 5' moiety, or a nucleotide labelled with a functional group/protecting group that can be chemically or enzymatically cleavable. Antiviral nucleotide/nucleoside analogs include but are not limited to Ganciclovir, Entecavir, Telbivudine, Vidarabine and Cidofovir.

The IVT reaction typically includes the following: an RNA polymerase, e.g., a T7 RNA polymerase at a final concentration of, e.g., 1000-12000 U/mL, e.g., 7000 U/mL; the DNA template at a final concentration of, e.g., 10-70 nM, e.g., 40 nM; nucleotides (NTPs) at a final concentration of e.g., 0.5-10 mM, e.g., 7.5 mM each; magnesium at a final concentration of, e.g., 12-60 mM, e.g., magnesium acetate at 40 mM; a buffer such as, e.g., HEPES or Tris at a pH of, e.g., 7-8.5, e.g. 40 mM Tris HCl, pH 8. In some embodiments 5 mM dithiothreitol (DTT) and/or 1 mM spermidine may be included. In some embodiments, an RNase inhibitor is included in the IVT reaction to ensure no RNase induced degradation during the transcription reaction. For example, murine RNase inhibitor can be utilized at a final concentration of 1000 U/mL. In some embodiments a pyrophosphatase is included in the IVT reaction to cleave the inorganic pyrophosphate generated following each nucleotide incorporation into two units of inorganic phosphate. This ensures that magnesium remains in solution and does not precipitate as magnesium pyrophosphate. For example, an *E. coli* inorganic pyrophosphatase can be utilized at a final concentration of 1 U/mL.

Similar to traditional methods, the modified method may also be produced by forming a reaction mixture comprising a DNA template, and one or more NTPs such as ATP, CTP, UTP, GTP (or corresponding analog of aforementioned components) and a buffer. The reaction is then incubated under conditions such that the RNA is transcribed. However, the modified methods utilize the presence of an excess amount of one or more nucleotides and/or nucleotide analogs that can have significant impact on the end product. These methods involve a modification in the amount (e.g., molar amount or quantity) of nucleotides and/or nucleotide analogs in the reaction mixture. In some aspects, one or more nucleotides and/or one or more nucleotide analogs may be added in excess to the reaction mixture. An excess of nucleotides and/or nucleotide analogs is any amount greater than the amount of one or more of the other nucleotides such as NTPs in the reaction mixture. For instance, an excess of a nucleotide and/or nucleotide analog may be a greater amount than the amount of each or at least one of the other individual NTPs in the reaction mixture or may refer to an amount greater than equimolar amounts of the other NTPs.

In the embodiment when the nucleotide and/or nucleotide analog that is included in the reaction mixture is an NTP, the NTP may be present in a higher concentration than all three of the other NTPs included in the reaction mixture. The other three NTPs may be in an equimolar concentration to one another. Alternatively one or more of the three other NTPs may be in a different concentration than one or more of the other NTPs.

Thus, in some embodiments the IVT reaction may include an equimolar amount of nucleotide triphosphate relative to at least one of the other nucleotide triphosphates.

In some embodiments the RNA is produced by a process or is preparable by a process comprising

(a) forming a reaction mixture comprising a DNA template and NTPs including adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP), guanosine triphosphate (GTP) and optionally guanosine diphosphate (GDP), and (eg. buffer containing T7 co-factor eg. magnesium).

(b) incubating the reaction mixture under conditions such that the RNA is transcribed, wherein the concentration of at least one of GTP, CTP, ATP, and UTP is at least 2× greater than the concentration of any one or more of ATP, CTP or UTP or the reaction further comprises a nucleotide analog

and wherein the concentration of the nucleotide analog is at least 2× greater than the concentration of any one or more of ATP, CTP or UTP.

In some embodiments the ratio of concentration of GTP to the concentration of any one ATP, CTP or UTP is at least 2:1, at least 3:1, at least 4:1, at least 5:1 or at least 6:1. The ratio of concentration of GTP to concentration of ATP, CTP and UTP is, in some embodiments 2:1, 4:1 and 4:1, respectively. In other embodiments the ratio of concentration of GTP to concentration of ATP, CTP and UTP is 3:1, 6:1 and 6:1, respectively. The reaction mixture may comprise GTP and GDP and wherein the ratio of concentration of GTP plus GDP to the concentration of any one of ATP, CTP or UTP is at least 2:1, at least 3:1, at least 4:1, at least 5:1 or at least 6:1 In some embodiments the ratio of concentration of GTP plus GDP to concentration of ATP, CTP and UTP is 3:1, 6:1 and 6:1, respectively.

In some embodiments the method involves incubating the reaction mixture under conditions such that the RNA is transcribed, wherein the effective concentration of phosphate in the reaction is at least 150 mM phosphate, at least 160 mM, at least 170 mM, at least 180 mM, at least 190 mM, at least 200 mM, at least 210 mM or at least 220 mM. The effective concentration of phosphate in the reaction may be 180 mM. The effective concentration of phosphate in the reaction in some embodiments is 195 mM. In other embodiments the effective concentration of phosphate in the reaction is 225 mM.

In other embodiments the RNA is produced by a process or is preparable by a process comprising wherein a buffer magnesium-containing buffer is used when forming the reaction mixture comprising a DNA template and ATP, CTP, UTP, GTP. In some embodiments the magnesium-containing buffer comprises Mg2+ and wherein the molar ratio of concentration of ATP plus CTP plus UTP pus GTP to concentration of Mg2+ is at least 1.0, at least 1.25, at least 1.5, at least 1.75, at least 1.85, at least 3 or higher. The molar ratio of concentration of ATP plus CTP plus UTP pus GTP to concentration of Mg2+ may be 1.5. The molar ratio of concentration of ATP plus CTP plus UTP pus GTP to concentration of Mg2+ in some embodiments is 1.88. The molar ratio of concentration of ATP plus CTP plus UTP pus GTP to concentration of Mg2+ in some embodiments is 3.

In some embodiments the composition is produced by a process which does not comprise an dsRNase (e.g., RNaseIII) treatment step. In other embodiments the composition is produced by a process which does not comprise a reverse phase (RP) chromatography purification step. In yet other embodiments the composition is produced by a process which does not comprise a high-performance liquid chromatography (HPLC) purification step.

In some embodiments the ratio of concentration of GTP to the concentration of any one ATP, CTP or UTP is at least 2:1, at least 3:1, at least 4:1, at least 5:1 or at least 6:1 to produce the RNA.

The purity of the products may be assessed using known analytical methods and assays. For instance, the amount of reverse complement transcription product or cytokine-inducing RNA contaminant may be determined by high-performance liquid chromatography (such as reverse-phase chromatography, size-exclusion chromatography), Bioanalyzer chip-based electrophoresis system, ELISA, flow cytometry, acrylamide gel, a reconstitution or surrogate type assay. The assays may be performed with or without nuclease treatment (P1, RNase III, RNase H etc.) of the RNA preparation. Electrophoretic/chromatographic/mass spec analysis of nuclease digestion products may also be performed.

In some embodiments the purified RNA preparations comprise contaminant transcripts that have a length less than a full length transcript, such as for instance at least 100, 200, 300, 400, 500, 600, 700, 800, or 900 nucleotides less than the full length. Contaminant transcripts can include reverse or forward transcription products (transcripts) that have a length less than a full length transcript, such as for instance at least 100, 200, 300, 400, 500, 600, 700, 800, or 900 nucleotides less than the full length. Exemplary forward transcripts include, for instance, abortive transcripts. In certain embodiments the composition comprises a tri-phosphate poly-U reverse complement of less than 30 nucleotides. In some embodiments the composition comprises a tri-phosphate poly-U reverse complement of any length hybridized to a full length transcript. In other embodiments the composition comprises a single stranded tri-phosphate forward transcript. In other embodiments the composition comprises a single stranded RNA having a terminal tri-phosphate-G. In other embodiments the composition comprises single or double stranded RNA of less than 12 nucleotides or base pairs (including forward or reverse complement transcripts). In any of these embodiments the composition may include less than 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.5% of any one of or combination of these less than full length transcripts.

Delivery Agents

a. Lipid Compound

The present disclosure provides pharmaceutical compositions with advantageous properties. The lipid compositions described herein may be advantageously used in lipid nanoparticle compositions for the delivery of therapeutic and/or prophylactic agents, e.g., mRNAs, to mammalian cells or organs. For example, the lipids described herein have little or no immunogenicity. For example, the lipid compounds disclosed herein have a lower immunogenicity as compared to a reference lipid (e.g., MC3, KC2, or DLinDMA). For example, a formulation comprising a lipid disclosed herein and a therapeutic or prophylactic agent, e.g., mRNA, has an increased therapeutic index as compared to a corresponding formulation which comprises a reference lipid (e.g., MC3, KC2, or DLinDMA) and the same therapeutic or prophylactic agent.

In certain embodiments, the present application provides pharmaceutical compositions comprising:

(a) a polynucleotide comprising a nucleotide sequence encoding a polypeptide; and

(b) a delivery agent.

Lipid Nanoparticle Formulations

In some embodiments, nucleic acids of the invention (e.g. mRNA) are formulated in a lipid nanoparticle (LNP). Lipid nanoparticles typically comprise ionizable cationic lipid, non-cationic lipid, sterol and PEG lipid components along with the nucleic acid cargo of interest. The lipid nanoparticles of the invention can be generated using components, compositions, and methods as are generally known in the art, see for example PCT/US2016/052352; PCT/US2016/068300; PCT/US2017/037551; PCT/US2015/027400; PCT/US2016/047406; PCT/US2016000129; PCT/US2016/014280; PCT/US2016/014280; PCT/US2017/038426; PCT/US2014/027077; PCT/US2014/055394; PCT/US2016/52117; PCT/US2012/069610; PCT/US2017/027492; PCT/US2016/059575 and PCT/US2016/069491 all of which are incorporated by reference herein in their entirety.

Nucleic acids of the present disclosure (e.g. mRNA) are typically formulated in lipid nanoparticle. In some embodiments, the lipid nanoparticle comprises at least one ionizable cationic lipid, at least one non-cationic lipid, at least one sterol, and/or at least one polyethylene glycol (PEG)-modified lipid.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 20-60% ionizable cationic lipid. For example, the lipid nanoparticle may comprise a molar ratio of 20-50%, 20-40%, 20-30%, 30-60%, 30-50%, 30-40%, 40-60%, 40-50%, or 50-60% ionizable cationic lipid. In some embodiments, the lipid nanoparticle comprises a molar ratio of 20%, 30%, 40%, 50, or 60% ionizable cationic lipid.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 5-25% non-cationic lipid. For example, the lipid nanoparticle may comprise a molar ratio of 5-20%, 5-15%, 5-10%, 10-25%, 10-20%, 10-25%, 15-25%, 15-20%, or 20-25% non-cationic lipid. In some embodiments, the lipid nanoparticle comprises a molar ratio of 5%, 10%, 15%, 20%, or 25% non-cationic lipid.

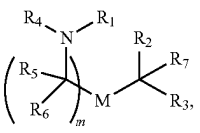
In some embodiments, the lipid nanoparticle comprises a molar ratio of 25-55% sterol. For example, the lipid nanoparticle may comprise a molar ratio of 25-50%, 25-45%, 25-40%, 25-35%, 25-30%, 30-55%, 30-50%, 30-45%, 30-40%, 30-35%, 35-55%, 35-50%, 35-45%, 35-40%, 40-55%, 40-50%, 40-45%, 45-55%, 45-50%, or 50-55% sterol. In some embodiments, the lipid nanoparticle comprises a molar ratio of 25%, 30%, 35%, 40%, 45%, 50%, or 55% sterol.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 0.5-15% PEG-modified lipid. For example, the lipid nanoparticle may comprise a molar ratio of 0.5-10%, 0.5-5%, 1-15%, 1-10%, 1-5%, 2-15%, 2-10%, 2-5%, 5-15%, 5-10%, or 10-15%. In some embodiments, the lipid nanoparticle comprises a molar ratio of 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% PEG-modified lipid.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 20-60% ionizable cationic lipid, 5-25% non-cationic lipid, 25-55% sterol, and 0.5-15% PEG-modified lipid.

Ionizable Lipids

In some aspects, the ionizable lipids of the present disclosure may be one or more of compounds of Formula (I):



(I)

or their N-oxides, or salts or isomers thereof, wherein:

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, —R*YR", —YR", and —R"M'R';

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, —R*YR", —YR", and —R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of hydrogen, a C_{3-6} carbocycle, —$(CH_2)_nQ$, —$(CH_2)_nCHQR$, —CHQR, —$CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a carbocycle, heterocycle, —OR, —$O(CH_2)_nN(R)_2$, —C(O)OR, —OC(O)R, —CX_3, —CX_2H, —CXH_2, —CN, —$N(R)_2$, —$C(O)N(R)_2$, —N(R)C(O)R, —$N(R)S(O)_2R$, —$N(R)C(O)N(R)_2$, —N(R)C(S)N$(R)_2$, —$N(R)R_8$, —$N(R)S(O)_2R_8$, —$O(CH_2)_nOR$, —N(R)C(=NR_9)$N(R)_2$, —N(R)C(=CHR_9)$N(R)_2$, —$OC(O)N(R)_2$, —N(R)C(O)OR, —N(OR)C(O)R, —$N(OR)S(O)_2R$, —N(OR)C(O)OR, —$N(OR)C(O)N(R)_2$, —N(OR)C(S)N$(R)_2$, —N(OR)C(=NR_9)$N(R)_2$, —N(OR)C(=CHR_9)$N(R)_2$, —C(=NR_9)$N(R)_2$, —C(=NR_9)R, —C(O)N(R)OR, and —C(R)$N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from —C(O)O—, —OC(O)—, —OC(O)-M"-C(O)O—, —C(O)N(R')—, —N(R')C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR')O—, —$S(O)_2$—, —S—S—, an aryl group, and a heteroaryl group, in which M" is a bond, C_{1-13} alkyl or C_{2-13} alkenyl;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, —OR, —$S(O)_2R$, —$S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, —R*YR", —YR", and H;

each R" is independently selected from the group consisting of C_{3-15} alkyl and C_{3-15} alkenyl;

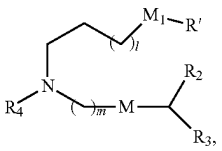
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13; and wherein when R_4 is —$(CH_2)_nQ$, —$(CH_2)_nCHQR$, —CHQR, or —$CQ(R)_2$, then (i) Q is not —$N(R)_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

In certain embodiments, a subset of compounds of Formula (I) includes those of Formula (IA):



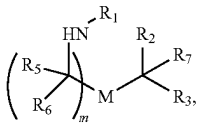
(IA)

or its N-oxide, or a salt or isomer thereof, wherein 1 is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M'; R_4 is hydrogen, unsubstituted C_{1-3} alkyl, or —$(CH_2)_nQ$, in which Q is OH, —NHC(S)$N(R)_2$, —$NHC(O)N(R)_2$, —N(R)C(O)R, —$N(R)S(O)_2R$, —N(R)R_8, —NHC(=NR_9)$N(R)_2$, —NHC(=CHR_9)$N(R)_2$, —OC(O)$N(R)_2$, —N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from —C(O)O—, —OC(O)—, —OC(O)-M"-C(O)O—, —C(O)N(R')—, —P(O)(OR')O—, —S—S—, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl. For

example, m is 5, 7, or 9. For example, Q is OH, —NHC(S)N(R)$_2$, or —NHC(O)N(R)$_2$. For example, Q is —N(R)C(O)R, or —N(R)S(O)$_2$R.

In certain embodiments, a subset of compounds of Formula (I) includes those of Formula (IB):

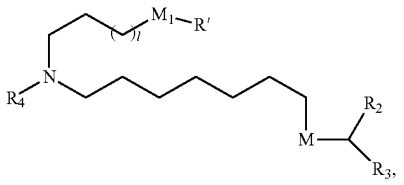
(IB)



or its N-oxide, or a salt or isomer thereof in which all variables are as defined herein. For example, m is selected from 5, 6, 7, 8, and 9; R_4 is hydrogen, unsubstituted C_{1-3} alkyl, or —$(CH_2)_nQ$, in which Q is OH, —NHC(S)N(R)$_2$, —NHC(O)N(R)$_2$, —N(R)C(O)R, —N(R)S(O)$_2$R, —N(R)R_8, —NHC(=NR_9)N(R)$_2$, —NHC(=CHR_9)N(R)$_2$, —OC(O)N(R)$_2$, —N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from —C(O)O—, —OC(O)—, —OC(O)-M"-C(O)O—, —C(O)N(R')—, —P(O)(OR')O—, —S—S—, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl. For example, m is 5, 7, or 9. For example, Q is OH, —NHC(S)N(R)$_2$, or —NHC(O)N(R)$_2$. For example, Q is —N(R)C(O)R, or —N(R)S(O)$_2$R.

In certain embodiments, a subset of compounds of Formula (I) includes those of Formula (II):

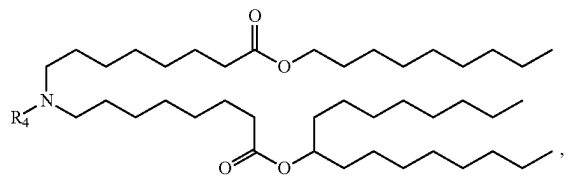
(II)



or its N-oxide, or a salt or isomer thereof, wherein 1 is selected from 1, 2, 3, 4, and 5; M_1 is a bond or M'; R_4 is hydrogen, unsubstituted C_{1-3} alkyl, or —$(CH_2)_nQ$, in which n is 2, 3, or 4, and Q is OH, —NHC(S)N(R)$_2$, —NHC(O)N(R)$_2$, —N(R)C(O)R, —N(R)S(O)$_2$R, —N(R)R_8, —NHC(=NR_9)N(R)$_2$, —NHC(=CHR_9)N(R)$_2$, —OC(O)N(R)$_2$, —N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from —C(O)O—, —OC(O)—, —OC(O)-M"-C(O)O—, —C(O)N(R')—, —P(O)(OR')O—, —S—S—, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

In one embodiment, the compounds of Formula (I) are of Formula (IIa),

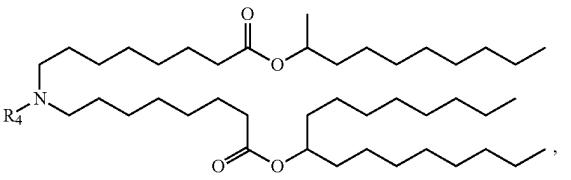
(IIa)



or their N-oxides, or salts or isomers thereof, wherein R_4 is as described herein.

In another embodiment, the compounds of Formula (I) are of Formula (IIb),

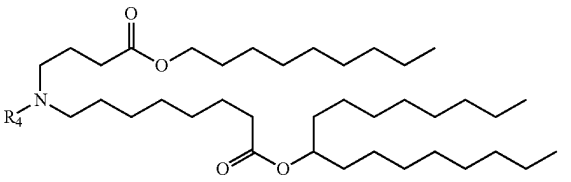
(IIb)



or their N-oxides, or salts or isomers thereof, wherein R_4 is as described herein.

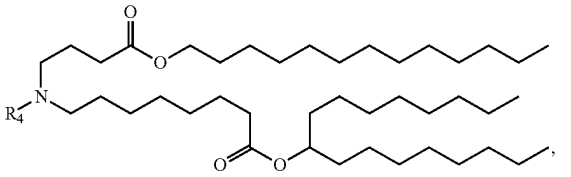
In another embodiment, the compounds of Formula (I) are of Formula (IIc) or (IIe):

(IIc)



or

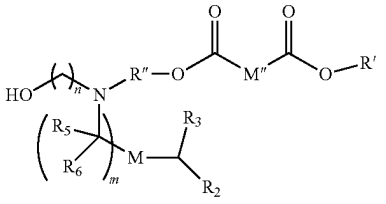
(IIe)



or their N-oxides, or salts or isomers thereof, wherein R_4 is as described herein.

In another embodiment, the compounds of Formula (I) are of Formula

(IIf)

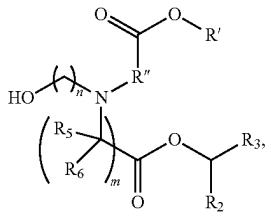


or their N-oxides, or salts or isomers thereof,

wherein M is —C(O)O— or —OC(O)—, M" is C_{1-6} alkyl or C_{2-6} alkenyl, R_2 and R_3 are independently selected from the group consisting of C_{5-14} alkyl and C_{5-14} alkenyl, and n is selected from 2, 3, and 4.

In a further embodiment, the compounds of Formula (I) are of Formula (IId),

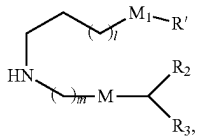
(IId)



or their N-oxides, or salts or isomers thereof, wherein n is 2, 3, or 4; and m, R', R", and R_2 through R_6 are as described herein. For example, each of R_2 and R_3 may be independently selected from the group consisting of C_{5-14} alkyl and C_{5-14} alkenyl.

In a further embodiment, the compounds of Formula (I) are of Formula (IIg),

(IIg)



or their N-oxides, or salts or isomers thereof, wherein 1 is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M'; M and M' are independently selected from

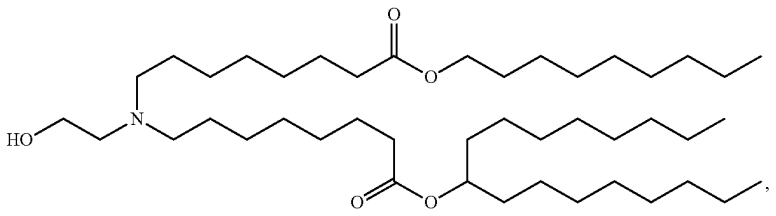
—C(O)O—, —OC(O)—, —OC(O)-M"-C(O)O—, —C(O)N(R')—, —P(O)(OR')O—, —S—S—, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl. For example, M" is C_{1-6} alkyl (e.g., C_{1-4} alkyl) or C_{2-6} alkenyl (e.g. C_{2-4} alkenyl). For example, R_2 and R_3 are independently selected from the group consisting of C_{5-14} alkyl and C_{5-14} alkenyl.

In some embodiments, the ionizable lipids are one or more of the compounds described in U.S. Application Nos. 62/220,091, 62/252,316, 62/253,433, 62/266,460, 62/333,557, 62/382,740, 62/393,940, 62/471,937, 62/471,949, 62/475,140, and 62/475,166, and PCT Application No. PCT/US2016/052352.

In some embodiments, the ionizable lipids are selected from Compounds 1-280 described in U.S. Application No. 62/475,166.

In some embodiments, the ionizable lipid is

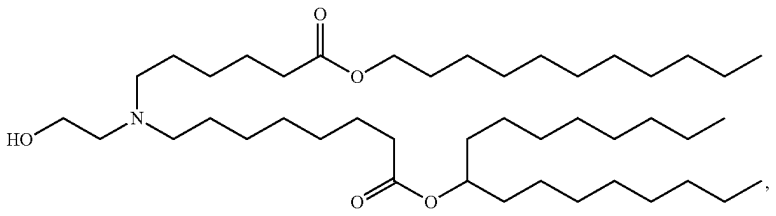
(Compound II)



or a salt thereof.

In some embodiments, the ionizable lipid is

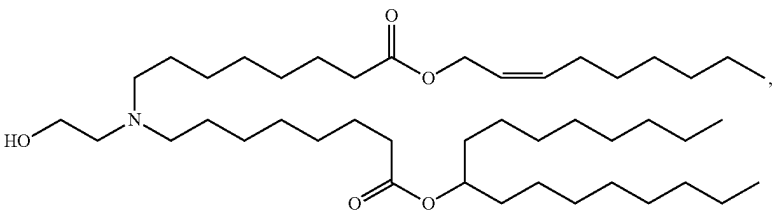
(Compound III)



or a salt thereof.

In some embodiments, the ionizable lipid is

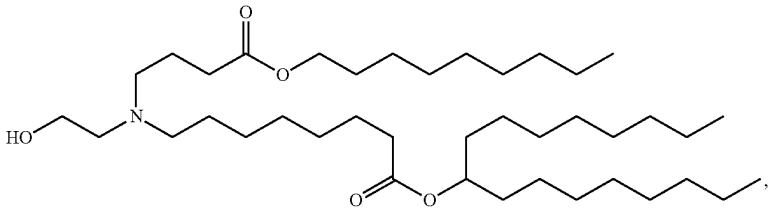
(Compound IV)



or a salt thereof.

In some embodiments, the ionizable lipid is

(Compound V)

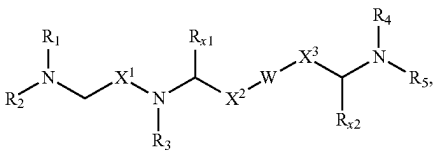


or a salt thereof.

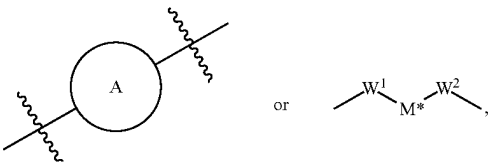
The central amine moiety of a lipid according to Formula (I), (IA), (IB), (II), (IIa), (IIb), (IIc), (IId), (IIe), (IIf), or (IIg) may be protonated at a physiological pH. Thus, a lipid may have a positive or partial positive charge at physiological pH. Such lipids may be referred to as cationic or ionizable (amino)lipids. Lipids may also be zwitterionic, i.e., neutral molecules having both a positive and a negative charge.

In some aspects, the ionizable lipids of the present disclosure may be one or more of compounds of formula (III),

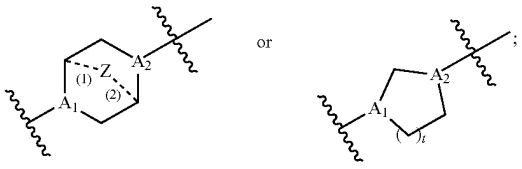
(III)



or salts or isomers thereof, wherein W is



ring A is



t is 1 or 2;

A_1 and A_2 are each independently selected from CH or N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, —R"MR', —R*YR", —YR", and —R*OR";

R_{X1} and R_{X2} are each independently H or C_{1-3} alkyl;

each M is independently selected from the group consisting of —C(O)O—, —OC(O)—, —OC(O)O—, —C(O)N(R')—, —N(R')C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR')O—, —$S(O)_2$—, —C(O)S—, —SC(O)—, an aryl group, and a heteroaryl group;

M* is C_1-C_6 alkyl,

W^1 and W^2 are each independently selected from the group consisting of —O— and —N(R_6)—;

each R_6 is independently selected from the group consisting of H and C_{1-5} alkyl;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, —CH_2—, —$(CH_2)_2$—, —CHR—, —CHY—, —C(O)—, —C(O)O—, —OC(O)—, —$(CH_2)_n$—C(O)—, —C(O)—$(CH_2)_n$—, —$(CH_2)_n$—C(O)O—, —OC(O)—$(CH_2)_n$—, —$(CH_2)_n$—OC(O)—, —C(O)O—$(CH_2)_n$—, —CH(OH)—, —C(S)—, and —CH(SH)—;

each Y is independently a C_{3-6} carbocycle;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

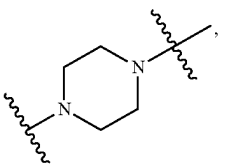
each R is independently selected from the group consisting of C_{1-3} alkyl and a C_{3-6} carbocycle;

each R' is independently selected from the group consisting of C_{1-12} alkyl, C_{2-12} alkenyl, and H;

each R" is independently selected from the group consisting of C_{3-12} alkyl, C_{3-12} alkenyl and —R*MR'; and

n is an integer from 1-6;

when ring A is



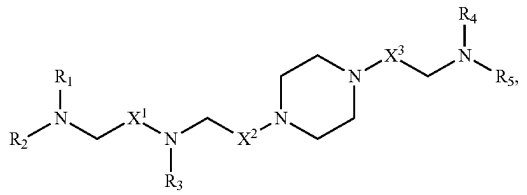
then

i) at least one of X^1, X^2, and X^3 is not —CH_2—; and/or

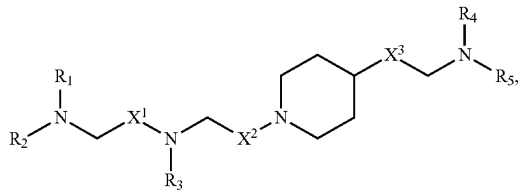
ii) at least one of R_1, R_2, R_3, R_4, and R_5 is —R"MR'.

In some embodiments, the compound is of any of formulae (IIIa1)-(Ma8):

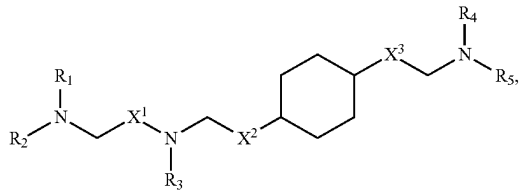
(IIIa1)



(IIIa2)

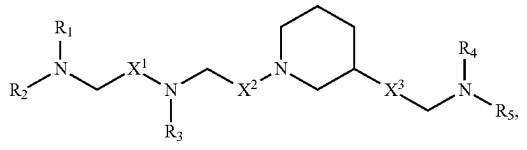


(IIIa3)

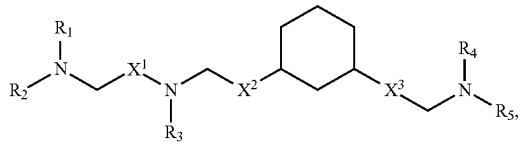


-continued

(IIIa4)



(IIIa5')



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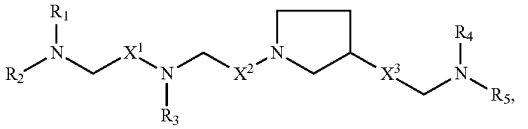
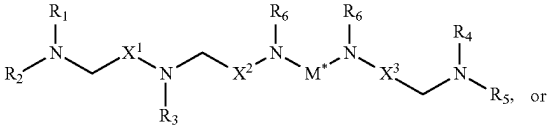
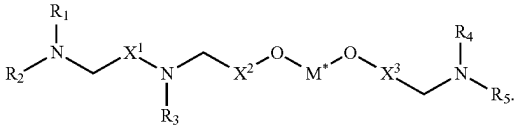
(IIIa6)



(IIIa7)



(IIIa8)



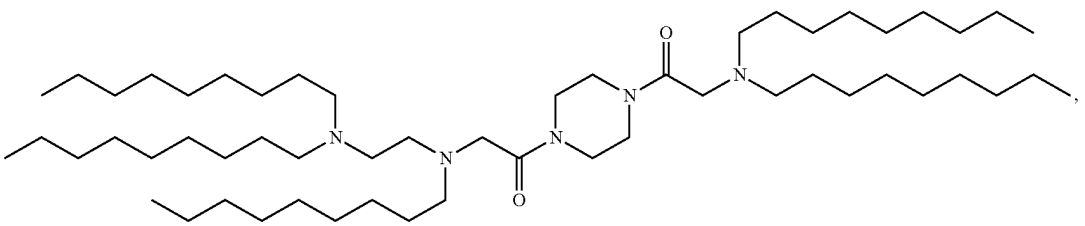
In some embodiments, the ionizable lipids are one or more of the compounds described in U.S. Application Nos. 62/271,146, 62/338,474, 62/413,345, and 62/519,826, and PCT Application No. PCT/US2016/068300.

In some embodiments, the ionizable lipids are selected from Compounds 1-156 described in U.S. Application No. 62/519,826.

In some embodiments, the ionizable lipids are selected from Compounds 1-16, 42-66, 68-76, and 78-156 described in U.S. Application No. 62/519,826.

In some embodiments, the ionizable lipid is

(Compound VI)



or a salt thereof.

The central amine moiety of a lipid according to Formula (III), (IIIa1), (IIIa2), (IIIa3), (IIIa4), (IIIa5), (IIIa6), (111a7), or (IIIa8) may be protonated at a physiological pH. Thus, a lipid may have a positive or partial positive charge at physiological pH. Such lipids may be referred to as cationic or ionizable (amino)lipids. Lipids may also be zwitterionic, i.e., neutral molecules having both a positive and a negative charge.

Phospholipids

The lipid composition of the lipid nanoparticle composition disclosed herein can comprise one or more phospholipids, for example, one or more saturated or (poly)unsaturated phospholipids or a combination thereof. In general, phospholipids comprise a phospholipid moiety and one or more fatty acid moieties.

A phospholipid moiety can be selected, for example, from the non-limiting group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and a sphingomyelin.

A fatty acid moiety can be selected, for example, from the non-limiting group consisting of lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanoic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid.

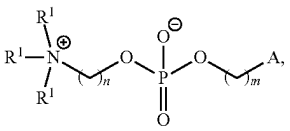
Particular phospholipids can facilitate fusion to a membrane. For example, a cationic phospholipid can interact with one or more negatively charged phospholipids of a membrane (e.g., a cellular or intracellular membrane). Fusion of a phospholipid to a membrane can allow one or more elements (e.g., a therapeutic agent) of a lipid-containing composition (e.g., LNPs) to pass through the membrane permitting, e.g., delivery of the one or more elements to a target tissue.

Non-natural phospholipid species including natural species with modifications and substitutions including branching, oxidation, cyclization, and alkynes are also contemplated. For example, a phospholipid can be functionalized with or cross-linked to one or more alkynes (e.g., an alkenyl group in which one or more double bonds is replaced with a triple bond). Under appropriate reaction conditions, an alkyne group can undergo a copper-catalyzed cycloaddition upon exposure to an azide. Such reactions can be useful in functionalizing a lipid bilayer of a nanoparticle composition to facilitate membrane permeation or cellular recognition or in conjugating a nanoparticle composition to a useful component such as a targeting or imaging moiety (e.g., a dye).

Phospholipids include, but are not limited to, glycerophospholipids such as phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, phosphatidylinositols, phosphatidy glycerols, and phosphatidic acids. Phospholipids also include phosphosphingolipid, such as sphingomyelin.

In some embodiments, a phospholipid of the invention comprises 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-gly cero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2 cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine,1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, and mixtures thereof.

In certain embodiments, a phospholipid useful or potentially useful in the present invention is an analog or variant of DSPC. In certain embodiments, a phospholipid useful or potentially useful in the present invention is a compound of Formula (IV):



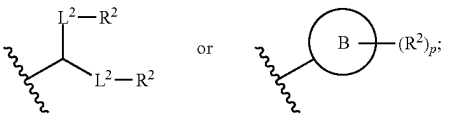
(IV)

or a salt thereof, wherein:

each R^1 is independently optionally substituted alkyl; or optionally two R^1 are joined together with the intervening atoms to form optionally substituted monocyclic carbocyclyl or optionally substituted monocyclic heterocyclyl; or optionally three R^1 are joined together with the intervening atoms to form optionally substituted bicyclic carbocyclyl or optionally substitute bicyclic heterocyclyl;

n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

m is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;



A is of the formula:

each instance of L^2 is independently a bond or optionally substituted C_{1-6} alkylene, wherein one methylene unit of the optionally substituted C_{1-6} alkylene is optionally replaced with O, $N(R^N)$, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, or $NR^NC(O)N(R^N)$;

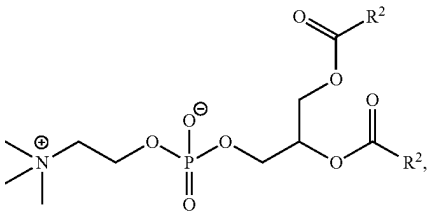
each instance of R^2 is independently optionally substituted C_{1-30} alkyl, optionally substituted C_{1-30} alkenyl, or optionally substituted C_{1-30} alkynyl; optionally wherein one or more methylene units of R^2 are independently replaced with optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, $N(R^N)$, O, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, $NR^NC(O)N(R^N)$, C(O)O, OC(O), —OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, C(O)S, SC(O), $C(=NR^N)$, $C(=NR^N)N(R^N)$, $NR^NC(=NR^N)$, $NR^NC(=NR^N)N(RN)$, C(S), C(S)N(RN), $NR^NC(S)$, $NR^NC(S)N(R^N)$, S(O), OS(O), S(O)O, —OS(O)O, $OS(O)_2$, $S(O)_2O$, $OS(O)_2O$, $N(R^N)S(O)$, $S(O)N(R^N)$, $N(R^N)S(O)N(R^N)$, $OS(O)N(R^N)$, $N(R^N)S(O)O$, $S(O)_2$, $N(R^N)S(O)_2$, $S(O)_2N(R^N)$, $N(R^N)S(O)_2N(R^N)$, $OS(O)_2N(R^N)$, or $—N(R^N)S(O)_2O$;

each instance of R^N is independently hydrogen, optionally substituted alkyl, or a nitrogen protecting group;

Ring B is optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and

p is 1 or 2;

provided that the compound is not of the formula:

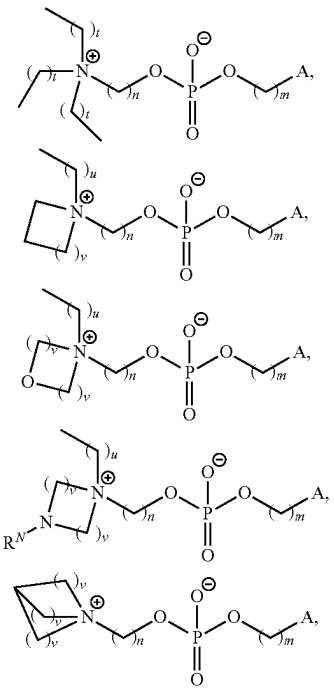


wherein each instance of R^2 is independently unsubstituted alkyl, unsubstituted alkenyl, or unsubstituted alkynyl.

In some embodiments, the phospholipids may be one or more of the phospholipids described in U.S. Application No. 62/520,530.

(i) Phospholipid Head Modifications

In certain embodiments, a phospholipid useful or potentially useful in the present invention comprises a modified phospholipid head (e.g., a modified choline group). In certain embodiments, a phospholipid with a modified head is DSPC, or analog thereof, with a modified quaternary amine. For example, in embodiments of Formula (IV), at least one of R' is not methyl. In certain embodiments, at least one of R' is not hydrogen or methyl. In certain embodiments, the compound of Formula (IV) is of one of the following formulae:



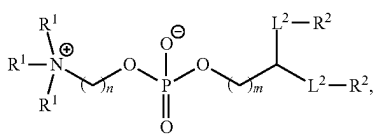
or a salt thereof, wherein:

each t is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

each u is independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; and

each v is independently 1, 2, or 3.

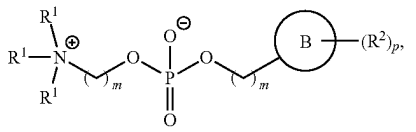
In certain embodiments, a compound of Formula (IV) is of Formula (IV-a):



(IV-a)

or a salt thereof.

In certain embodiments, a phospholipid useful or potentially useful in the present invention comprises a cyclic moiety in place of the glyceride moiety. In certain embodiments, a phospholipid useful in the present invention is DSPC, or analog thereof, with a cyclic moiety in place of the glyceride moiety. In certain embodiments, the compound of Formula (IV) is of Formula (IV-b):



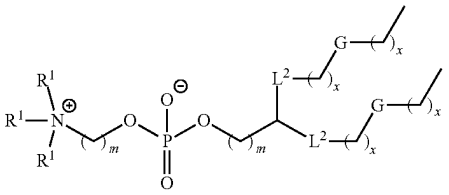
(IV-b)

or a salt thereof.

(ii) Phospholipid Tail Modifications

In certain embodiments, a phospholipid useful or potentially useful in the present invention comprises a modified tail. In certain embodiments, a phospholipid useful or potentially useful in the present invention is DSPC, or analog thereof, with a modified tail. As described herein, a "modified tail" may be a tail with shorter or longer aliphatic chains, aliphatic chains with branching introduced, aliphatic chains with substituents introduced, aliphatic chains wherein one or more methylenes are replaced by cyclic or heteroatom groups, or any combination thereof. For example, in certain embodiments, the compound of (IV) is of Formula (IV-a), or a salt thereof, wherein at least one instance of R^2 is each instance of R^2 is optionally substituted C_{1-30} alkyl, wherein one or more methylene units of R^2 are independently replaced with optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, $N(R^N)$, O, S, C(O), $C(O)N(R^N)$, $—NR^NC(O)$, $NR^NC(O)N(R^N)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, C(O)S, SC(O), $C(=NR^N)$, $C(=NR^N)N(R^N)$, $NRNC(=NR^N)$, $NR^NC(=NR^N)N(R^N)$, C(S), $C(S)N(R^N)$, $NR^NC(S)$, $—NR^NC(S)N(R^N)$, S(O), OS(O), S(O)O, OS(O)O, $OS(O)_2$, $S(O)_2O$, $OS(O)_2O$, $N(R^N)S(O)$, $—S(O)N(R^N)$, $N(R^N)S(O)N(R^N)$, $OS(O)N(R^N)$, $N(R^N)S(O)O$, $S(O)_2$, $N(R^N)S(O)_2$, $S(O)_2N(R^N)$, $—N(R^N)S(O)_2N(R^N)$, $OS(O)_2N(R^N)$, or $N(R^N)S(O)_2O$.

In certain embodiments, the compound of Formula (IV) is of Formula (IV-c):



(IV-c)

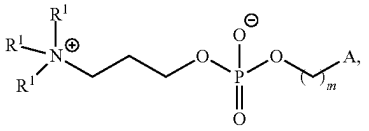
or a salt thereof, wherein:

each x is independently an integer between 0-30, inclusive; and

each instance is G is independently selected from the group consisting of optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, $N(R^N)$, O, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, $NR^NC(O)N(R^N)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, C(O)S, SC(O), $C(=NR^N)$, $C(=NR^N)N(R^N)$, $NR^NC(=NR^N)$, $NR^NC(=NR^N)N(R^N)$, C(S), $C(S)N(R^N)$, $NR^NC(S)$, $NR^NC(S)N(R^N)$, S(O), OS(O), S(O)O, OS(O)O, $OS(O)_2$, $S(O)_2O$,

$OS(O)_2O$, $N(R^N)S(O)$, $S(O)N(R^N)$, $N(R^N)S(O)N(R^N)$, —$OS(O)N(R^N)$, $N(R^N)S(O)O$, $S(O)_2$, $N(R^N)S(O)_2$, $S(O)_2N(R^N)$, $N(R^N)S(O)_2N(R^N)$, $OS(O)_2N(R^N)$, or $N(R^N)S(O)_2O$. Each possibility represents a separate embodiment of the present invention.

In certain embodiments, a phospholipid useful or potentially useful in the present invention comprises a modified phosphocholine moiety, wherein the alkyl chain linking the quaternary amine to the phosphoryl group is not ethylene (e.g., n is not 2). Therefore, in certain embodiments, a phospholipid useful or potentially useful in the present invention is a compound of Formula (IV), wherein n is 1, 3, 4, 5, 6, 7, 8, 9, or 10. For example, in certain embodiments, a compound of Formula (IV) is of one of the following formulae:



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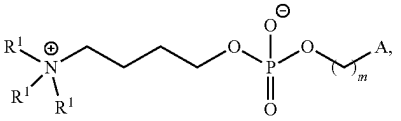


or a salt thereof.

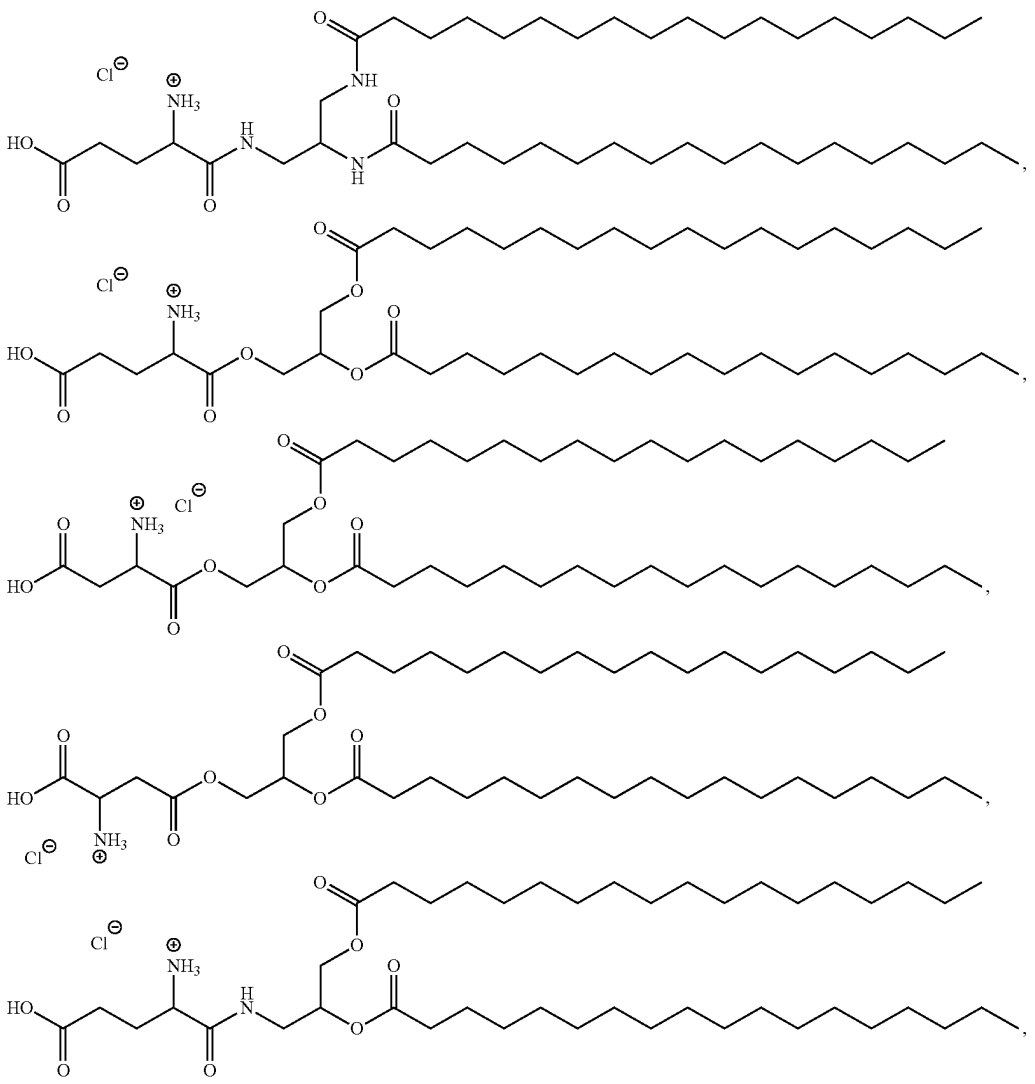
Alternative Lipids

In certain embodiments, a phospholipid useful or potentially useful in the present invention comprises a modified phosphocholine moiety, wherein the alkyl chain linking the quaternary amine to the phosphoryl group is not ethylene (e.g., n is not 2). Therefore, in certain embodiments, a phospholipid useful.

In certain embodiments, an alternative lipid is used in place of a phospholipid of the present disclosure.

In certain embodiments, an alternative lipid of the invention is oleic acid.

In certain embodiments, the alternative lipid is one of the following:



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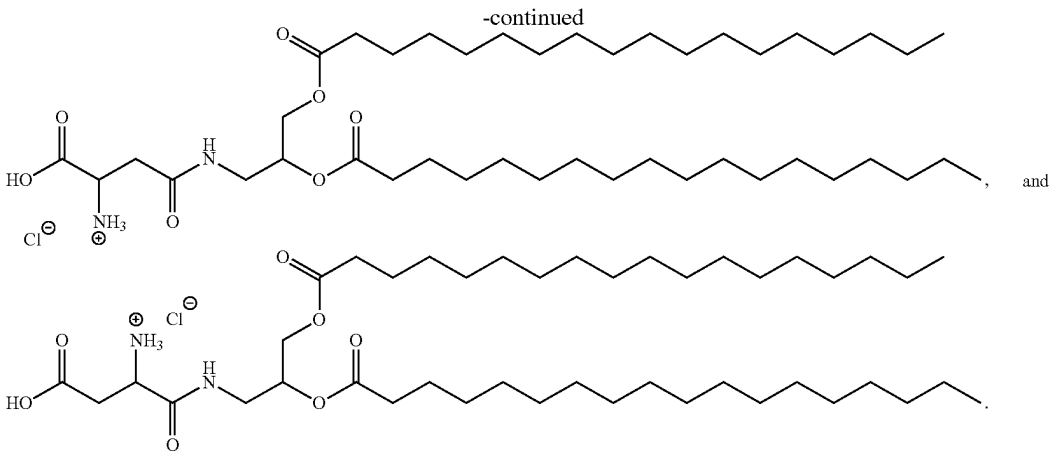


Structural Lipids

The lipid composition of a pharmaceutical composition disclosed herein can comprise one or more structural lipids. As used herein, the term "structural lipid" refers to sterols and also to lipids containing sterol moieties.

Incorporation of structural lipids in the lipid nanoparticle may help mitigate aggregation of other lipids in the particle. Structural lipids can be selected from the group including but not limited to, cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, hopanoids, phytosterols, steroids, and mixtures thereof. In some embodiments, the structural lipid is a sterol. As defined herein, "sterols" are a subgroup of steroids consisting of steroid alcohols. In certain embodiments, the structural lipid is a steroid. In certain embodiments, the structural lipid is cholesterol. In certain embodiments, the structural lipid is an analog of cholesterol. In certain embodiments, the structural lipid is alpha-tocopherol.

In some embodiments, the structural lipids may be one or more of the structural lipids described in U.S. Application No. 62/520,530.

Polyethylene Glycol (PEG)-Lipids

The lipid composition of a pharmaceutical composition disclosed herein can comprise one or more a polyethylene glycol (PEG) lipid.

As used herein, the term "PEG-lipid" refers to polyethylene glycol (PEG)-modified lipids. Non-limiting examples of PEG-lipids include PEG-modified phosphatidylethanolamine and phosphatidic acid, PEG-ceramide conjugates (e.g., PEG-CerC14 or PEG-CerC20), PEG-modified dialkylamines and PEG-modified 1,2-diacyloxypropan-3-amines. Such lipids are also referred to as PEGylated lipids. For example, a PEG lipid can be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, or a PEG-DSPE lipid.

In some embodiments, the PEG-lipid includes, but not limited to 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (PEG-DSPE), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA).

In one embodiment, the PEG-lipid is selected from the group consisting of a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, a PEG-modified dialkylglycerol, and mixtures thereof.

In some embodiments, the lipid moiety of the PEG-lipids includes those having lengths of from about C_{14} to about C_{22}, preferably from about C_{14} to about C_{16}. In some embodiments, a PEG moiety, for example an mPEG-NH_2, has a size of about 1000, 2000, 5000, 10,000, 15,000 or 20,000 daltons. In one embodiment, the PEG-lipid is PEG_{2k}-DMG.

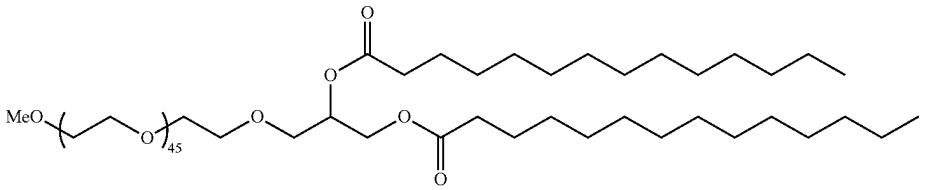
In one embodiment, the lipid nanoparticles described herein can comprise a PEG lipid which is a non-diffusible PEG. Non-limiting examples of non-diffusible PEGs include PEG-DSG and PEG-DSPE.

PEG-lipids are known in the art, such as those described in U.S. Pat. No. 8,158,601 and International Publ. No. WO 2015/130584 A2, which are incorporated herein by reference in their entirety.

In general, some of the other lipid components (e.g., PEG lipids) of various formulae, described herein may be synthesized as described International Patent Application No. PCT/US2016/000129, filed Dec. 10, 2016, entitled "Compositions and Methods for Delivery of Therapeutic Agents," which is incorporated by reference in its entirety.

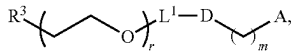
The lipid component of a lipid nanoparticle composition may include one or more molecules comprising polyethylene glycol, such as PEG or PEG-modified lipids. Such species may be alternately referred to as PEGylated lipids. A PEG lipid is a lipid modified with polyethylene glycol. A PEG lipid may be selected from the non-limiting group including PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified di alkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. For example, a PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, or a PEG-DSPE lipid.

In some embodiments the PEG-modified lipids are a modified form of PEG DMG. PEG-DMG has the following structure:



In one embodiment, PEG lipids useful in the present invention can be PEGylated lipids described in International Publication No. WO2012099755, the contents of which is herein incorporated by reference in its entirety. Any of these exemplary PEG lipids described herein may be modified to comprise a hydroxyl group on the PEG chain. In certain embodiments, the PEG lipid is a PEG-OH lipid. As generally defined herein, a "PEG-OH lipid" (also referred to herein as "hydroxy-PEGylated lipid") is a PEGylated lipid having one or more hydroxyl (—OH) groups on the lipid. In certain embodiments, the PEG-OH lipid includes one or more hydroxyl groups on the PEG chain. In certain embodiments, a PEG-OH or hydroxy-PEGylated lipid comprises an —OH group at the terminus of the PEG chain. Each possibility represents a separate embodiment of the present invention.

In certain embodiments, a PEG lipid useful in the present invention is a compound of Formula (V). Provided herein are compounds of Formula (V):



(V)

or salts thereof, wherein:

R^3 is —OR^O;

R^O is hydrogen, optionally substituted alkyl, or an oxygen protecting group;

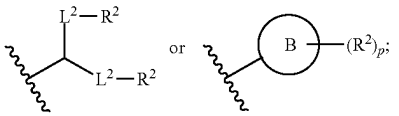
r is an integer between 1 and 100, inclusive;

L^1 is optionally substituted C_{1-10} alkylene, wherein at least one methylene of the optionally substituted C_1-10 alkylene is independently replaced with optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, O, $N(R^N)$, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, or $NR^NC(O)N(R^N)$;

D is a moiety obtained by click chemistry or a moiety cleavable under physiological conditions;

m is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

A is of the formula:



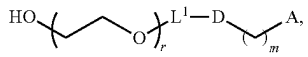
each instance of L^2 is independently a bond or optionally substituted C_{1-6} alkylene, wherein one methylene unit of the optionally substituted C_{1-6} alkylene is optionally replaced with O, $N(R^N)$, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, or $NR^NC(O)N(R^N)$, each instance of R^2 is independently optionally substituted C_{1-30} alkyl, optionally substituted C_{1-30} alkenyl, or optionally substituted C_{1-30} alkynyl; optionally wherein one or more methylene units of R^2 are independently replaced with optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, $N(R^N)$, O, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, $NR^NC(O)N(R^N)$, C(O)O, OC(O), —OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, C(O)S, SC(O), $C(=NR^N)$, $C(=NR^N)N(R^N)$, $NR^NC(=NR^N)$, $NR^NC(=NR^N)N(R^N)$, C(S), $C(S)N(R^N)$, $NR^NC(S)$, $NR^NC(S)N(R^N)$, S(O), OS(O), S(O)O, —OS(O)O, $OS(O)_2$, $S(O)_2O$, $OS(O)_2O$, $N(R^N)S(O)$, $S(O)N(R^N)$, $N(R^N)S(O)N(R^N)$, $OS(O)N(R^N)$, $N(R^N)S(O)O$, $S(O)_2$, $N(R^N)S(O)_2$, $S(O)_2N(R^N)$, $N(R^N)S(O)_2N(R^N)$, $OS(O)_2N(R^N)$, or $N(R^N)S(O)_2O$;

each instance of R^N is independently hydrogen, optionally substituted alkyl, or a nitrogen protecting group;

Ring B is optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and

p is 1 or 2.

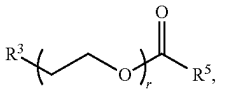
In certain embodiments, the compound of Formula (V) is a PEG-OH lipid (i.e., R^3 is —OR^O, and R^O is hydrogen). In certain embodiments, the compound of Formula (V) is of Formula (V-OH):



(V-OH)

or a salt thereof.

In certain embodiments, a PEG lipid useful in the present invention is a PEGylated fatty acid. In certain embodiments, a PEG lipid useful in the present invention is a compound of Formula (VI). Provided herein are compounds of Formula (VI):



(VI)

or a salts thereof, wherein:

R^3 is —OR^O;

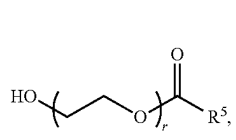
R^O is hydrogen, optionally substituted alkyl or an oxygen protecting group;

r is an integer between 1 and 100, inclusive;

R^5 is optionally substituted C_{10-40} alkyl, optionally substituted C_{10-40} alkenyl, or optionally substituted C_{10-40} alkynyl; and optionally one or more methylene groups of R^5 are replaced with optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, $N(R^N)$, 0, S, C(O), $C(O)N(R^N)$, —$NR^NC(O)$, $NR^NC(O)N(R^N)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, C(O)S, SC(O), $C(=NR^N)$, $C(=NR^N)N(R^N)$, $NR^NC(=NR^N)$, $NR^NC(=NR^N)N(R^N)$, C(S), $C(S)N(R^N)$, $NR^NC(S)$, —$NR^NC(S)N(R^N)$, S(O), OS(O), S(O)O, OS(O)O, $OS(O)_2$, $S(O)_2O$, $OS(O)_2O$, $N(R^N)S(O)$, —$S(O)N(R^N)$, $N(R^N)S(O)N(R^N)$, $OS(O)N(R^N)$, $N(R^N)S(O)O$, $S(O)_2$, $N(R^N)S(O)_2$, $S(O)_2N(R^N)$, —$N(R^N)S(O)_2N(R^N)$, $OS(O)_2N(R^N)$, or $N(R^N)S(O)_2O$; and

each instance of R^N is independently hydrogen, optionally substituted alkyl, or a nitrogen protecting group.

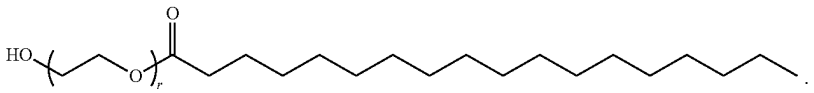
In certain embodiments, the compound of Formula (VI) is of Formula (VI-OH):



(VI-OH)

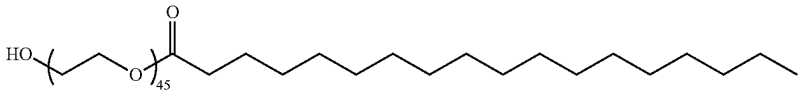
or a salt thereof. In some embodiments, r is 45.

In yet other embodiments the compound of Formula (VI) is:



or a salt thereof.

In one embodiment, the compound of Formula (VI) is



(Compound I).

In some aspects, the lipid composition of the pharmaceutical compositions disclosed herein does not comprise a PEG-lipid.

In some embodiments, the PEG-lipids may be one or more of the PEG lipids described in U.S. Application No. 62/520,530.

In some embodiments, a PEG lipid of the invention comprises a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, a PEG-modified dialkylglycerol, and mixtures thereof. In some embodiments, the PEG-modified lipid is PEG-DMG, PEG-c-DOMG (also referred to as PEG-DOMG), PEG-DSG and/or PEG-DPG.

In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of any of Formula I, II or III, a phospholipid comprising DSPC, a structural lipid, and a PEG lipid comprising PEG-DMG.

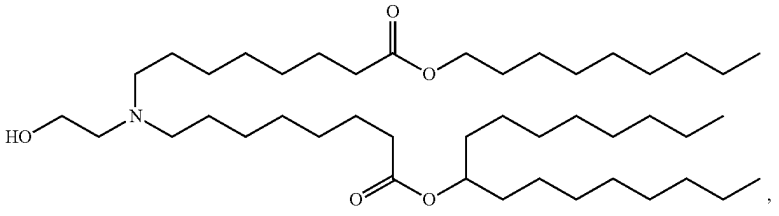
In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of any of Formula I, II or III, a phospholipid comprising DSPC, a structural lipid, and a PEG lipid comprising a compound having Formula VI.

In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of Formula I, II or III, a phospholipid comprising a compound having Formula IV, a structural lipid, and the PEG lipid comprising a compound having Formula V or VI.

In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of Formula I, II or III, a phospholipid comprising a compound having Formula IV, a structural lipid, and the PEG lipid comprising a compound having Formula V or VI.

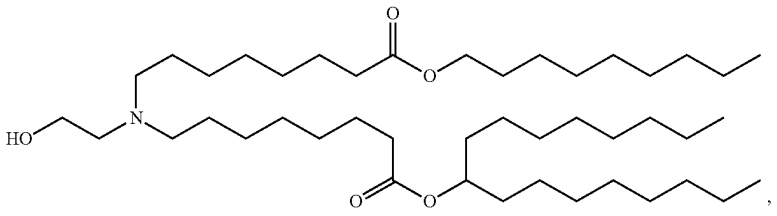
In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of Formula I, II or III, a phospholipid having Formula IV, a structural lipid, and a PEG lipid comprising a compound having Formula VI.

In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of



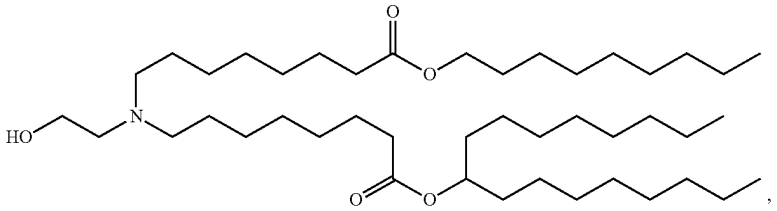
and a PEG lipid comprising Formula VI.

In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of



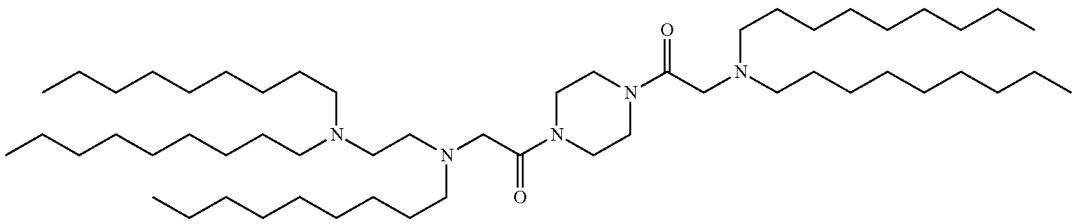
and an alternative lipid comprising oleic acid.

In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of



an alternative lipid comprising oleic acid, a structural lipid comprising cholesterol, and a PEG lipid comprising a compound having Formula VI.

In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of



a phospholipid comprising DOPE, a structural lipid comprising cholesterol, and a PEG lipid comprising a compound having Formula VI.

In some embodiments, a LNP of the invention comprises an ionizable cationic lipid of a phospholipid comprising DOPE, a structural lipid comprising cholesterol, and a PEG lipid comprising a compound having Formula VII.

In some embodiments, a LNP of the invention comprises an N:P ratio of from about 2:1 to about 30:1.

In some embodiments, a LNP of the invention comprises an N:P ratio of about 6:1.

In some embodiments, a LNP of the invention comprises an N:P ratio of about 3:1.

In some embodiments, a LNP of the invention comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of from about 10:1 to about 100:1.

In some embodiments, a LNP of the invention comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of about 20:1.

In some embodiments, a LNP of the invention comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of about 10:1.

In some embodiments, a LNP of the invention has a mean diameter from about 50 nm to about 150 nm.

In some embodiments, a LNP of the invention has a mean diameter from about 70 nm to about 120 nm.

As used herein, the term "alkyl", "alkyl group", or "alkylene" means a linear or branched, saturated hydrocarbon including one or more carbon atoms (e.g., one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more carbon atoms), which is optionally substituted. The notation "C_{1-14} alkyl" means an optionally substituted linear or branched, saturated hydrocarbon including 1 14 carbon atoms. Unless otherwise specified, an alkyl group described herein refers to both unsubstituted and substituted alkyl groups.

As used herein, the term "alkenyl", "alkenyl group", or "alkenylene" means a linear or branched hydrocarbon including two or more carbon atoms (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more carbon atoms) and at least one double bond, which is optionally substituted. The notation "C_{2-14} alkenyl" means an optionally substituted linear or branched hydro-

carbon including 2 14 carbon atoms and at least one carbon-carbon double bond. An alkenyl group may include one, two, three, four, or more carbon-carbon double bonds. For example, C18 alkenyl may include one or more double bonds. A C18 alkenyl group including two double bonds may be a linoleyl group. Unless otherwise specified, an alkenyl group described herein refers to both unsubstituted and substituted alkenyl groups.

As used herein, the term "alkynyl", "alkynyl group", or "alkynylene" means a linear or branched hydrocarbon including two or more carbon atoms (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more carbon atoms) and at least one carbon-carbon triple bond, which is optionally substituted. The notation "C_{2-14} alkynyl" means an optionally substituted linear or branched hydrocarbon including 2 14 carbon atoms and at least one carbon-carbon triple bond. An alkynyl group may include one, two, three, four, or more carbon-carbon triple bonds. For example, C18 alkynyl may include one or more carbon-carbon triple bonds. Unless otherwise specified, an alkynyl group described herein refers to both unsubstituted and substituted alkynyl groups.

As used herein, the term "carbocycle" or "carbocyclic group" means an optionally substituted mono- or multicyclic system including one or more rings of carbon atoms. Rings may be three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty membered rings. The notation "C_{3-6} carbocycle" means a carbocycle including a single ring having 3-6 carbon atoms. Carbocycles may include one or more carbon-carbon double or triple bonds and may be non-aromatic or aromatic (e.g., cycloalkyl or aryl groups). Examples of carbocycles include cyclopropyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, and 1,2 dihydronaphthyl groups. The term "cycloalkyl" as used herein means a non-aromatic carbocycle and may or may not include any double or triple bond. Unless otherwise specified, carbocycles described herein refers to both unsubstituted and substituted carbocycle groups, i.e., optionally substituted carbocycles.

As used herein, the term "heterocycle" or "heterocyclic group" means an optionally substituted mono- or multicyclic system including one or more rings, where at least one ring includes at least one heteroatom. Heteroatoms may be, for example, nitrogen, oxygen, or sulfur atoms. Rings may be three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen membered rings. Heterocycles may include one or more double or triple bonds and may be non-aromatic or aromatic (e.g., heterocycloalkyl or heteroaryl groups). Examples of heterocycles include imidazolyl, imidazolidinyl, oxazolyl, oxazolidinyl, thiazolyl, thiazolidinyl, pyrazolidinyl, pyrazolyl, isoxazolidinyl, isoxazolyl, isothiazolidinyl, isothiazolyl, morpholinyl, pyrrolyl, pyrrolidinyl, furyl, tetrahydrofuryl, thiophenyl, pyridinyl, piperidinyl, quinolyl, and isoquinolyl groups. The term "heterocycloalkyl" as used herein means a non-aromatic heterocycle and may or may not include any double or triple bond. Unless otherwise specified, heterocycles described herein refers to both unsubstituted and substituted heterocycle groups, i.e., optionally substituted heterocycles.

As used herein, the term "heteroalkyl", "heteroalkenyl", or "heteroalkynyl", refers respectively to an alkyl, alkenyl, alkynyl group, as defined herein, which further comprises one or more (e.g., 1, 2, 3, or 4) heteroatoms (e.g., oxygen, sulfur, nitrogen, boron, silicon, phosphorus) wherein the one or more heteroatoms is inserted between adjacent carbon atoms within the parent carbon chain and/or one or more heteroatoms is inserted between a carbon atom and the parent molecule, i.e., between the point of attachment. Unless otherwise specified, heteroalkyls, heteroalkenyls, or heteroalkynyls described herein refers to both unsubstituted and substituted heteroalkyls, heteroalkenyls, or heteroalkynyls, i.e., optionally substituted heteroalkyls, heteroalkenyl s, or heteroalkynyls.

As used herein, a "biodegradable group" is a group that may facilitate faster metabolism of a lipid in a mammalian entity. A biodegradable group may be selected from the group consisting of, but is not limited to, —C(O)O—, —OC(O)—, —C(O)N(R')—, —N(R')C(O)—, —C(O)—, —C(S)—, —C(S)S—, —SC(S)—, —CH(OH)—, —P(O)(OR')O—, —S(O)2-, an aryl group, and a heteroaryl group. As used herein, an "aryl group" is an optionally substituted carbocyclic group including one or more aromatic rings. Examples of aryl groups include phenyl and naphthyl groups. As used herein, a "heteroaryl group" is an optionally substituted heterocyclic group including one or more aromatic rings. Examples of heteroaryl groups include pyrrolyl, furyl, thiophenyl, imidazolyl, oxazolyl, and thiazolyl. Both aryl and heteroaryl groups may be optionally substituted. For example, M and M' can be selected from the non-limiting group consisting of optionally substituted phenyl, oxazole, and thiazole. In the formulas herein, M and M' can be independently selected from the list of biodegradable groups above. Unless otherwise specified, aryl or heteroaryl groups described herein refers to both unsubstituted and substituted groups, i.e., optionally substituted aryl or heteroaryl groups.

Alkyl, alkenyl, and cyclyl (e.g., carbocyclyl and heterocyclyl) groups may be optionally substituted unless otherwise specified. Optional substituents may be selected from the group consisting of, but are not limited to, a halogen atom (e.g., a chloride, bromide, fluoride, or iodide group), a carboxylic acid (e.g., C(O)OH), an alcohol (e.g., a hydroxyl, OH), an ester (e.g., C(O)OR OC(O)R), an aldehyde (e.g., C(O)H), a carbonyl (e.g., C(O)R, alternatively represented by C═O), an acyl halide (e.g., C(O)X, in which X is a halide selected from bromide, fluoride, chloride, and iodide), a carbonate (e.g., OC(O)OR), an alkoxy (e.g., OR), an acetal (e.g., C(OR)2R"", in which each OR are alkoxy groups that can be the same or different and R" " is an alkyl or alkenyl group), a phosphate (e.g., P(O)43-), a thiol (e.g., SH), a sulfoxide (e.g., S(O)R), a sulfinic acid (e.g., S(O)OH), a sulfonic acid (e.g., S(O)2OH), a thial (e.g., C(S)H), a sulfate (e.g., $S(O)_{42}$-), a sulfonyl (e.g., $S(O)_2$), an amide (e.g., $C(O)NR_2$, or N(R)C(O)R), an azido (e.g., N3), a nitro (e.g., NO2), a cyano (e.g., CN), an isocyano (e.g., NC), an acyloxy (e.g., OC(O)R), an amino (e.g., NR2, NRH, or NH2), a carbamoyl (e.g., $OC(O)NR_2$, OC(O)NRH, or OC(O)NH2), a sulfonamide (e.g., S(O)2NR2, S(O)2NRH, S(O)2NH2, N(R)S(O)2R, N(H)S(O)2R, N(R)S(O)2H, or N(H)S(O)2H), an alkyl group, an alkenyl group, and a cyclyl (e.g., carbocyclyl or heterocyclyl) group. In any of the preceding, R is an alkyl or alkenyl group, as defined herein. In some embodiments, the substituent groups themselves may be further substituted with, for example, one, two, three, four, five, or six substituents as defined herein. For example, a C1 6 alkyl group may be further substituted with one, two, three, four, five, or six substituents as described herein.

Compounds of the disclosure that contain nitrogens can be converted to N-oxides by treatment with an oxidizing agent (e.g., 3-chloroperoxybenzoic acid (mCPBA) and/or hydrogen peroxides) to afford other compounds of the disclosure. Thus, all shown and claimed nitrogen-containing

compounds are considered, when allowed by valency and structure, to include both the compound as shown and its N-oxide derivative (which can be designated as N□O or N+—O—). Furthermore, in other instances, the nitrogens in the compounds of the disclosure can be converted to N-hydroxy or N-alkoxy compounds. For example, N-hydroxy compounds can be prepared by oxidation of the parent amine by an oxidizing agent such as m CPBA. All shown and claimed nitrogen-containing compounds are also considered, when allowed by valency and structure, to cover both the compound as shown and its N-hydroxy (i.e., N—OH) and N-alkoxy (i.e., N—OR, wherein R is substituted or unsubstituted C1-C6 alkyl, C1-C6 alkenyl, C1-C6 alkynyl, 3-14-membered carbocycle or 3-14-membered heterocycle) derivatives.

Other Lipid Composition Components

The lipid composition of a pharmaceutical composition disclosed herein can include one or more components in addition to those described above. For example, the lipid composition can include one or more permeability enhancer molecules, carbohydrates, polymers, surface altering agents (e.g., surfactants), or other components. For example, a permeability enhancer molecule can be a molecule described by U.S. Patent Application Publication No. 2005/0222064. Carbohydrates can include simple sugars (e.g., glucose) and polysaccharides (e.g., glycogen and derivatives and analogs thereof).

A polymer can be included in and/or used to encapsulate or partially encapsulate a pharmaceutical composition disclosed herein (e.g., a pharmaceutical composition in lipid nanoparticle form). A polymer can be biodegradable and/or biocompatible. A polymer can be selected from, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates.

The ratio between the lipid composition and the polynucleotide range can be from about 10:1 to about 60:1 (wt/wt).

In some embodiments, the ratio between the lipid composition and the polynucleotide can be about 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 21:1, 22:1, 23:1, 24:1, 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 36:1, 37:1, 38:1, 39:1, 40:1, 41:1, 42:1, 43:1, 44:1, 45:1, 46:1, 47:1, 48:1, 49:1, 50:1, 51:1, 52:1, 53:1, 54:1, 55:1, 56:1, 57:1, 58:1, 59:1 or 60:1 (wt/wt). In some embodiments, the wt/wt ratio of the lipid composition to the polynucleotide encoding a therapeutic agent is about 20:1 or about 15:1.

In some embodiments, the pharmaceutical composition disclosed herein can contain more than one polypeptides. For example, a pharmaceutical composition disclosed herein can contain two or more polynucleotides (e.g., RNA, e.g., mRNA).

In one embodiment, the lipid nanoparticles described herein can comprise polynucleotides (e.g., mRNA) in a lipid:polynucleotide weight ratio of 5:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1 or 70:1, or a range or any of these ratios such as, but not limited to, 5:1 to about 10:1, from about 5:1 to about 15:1, from about 5:1 to about 20:1, from about 5:1 to about 25:1, from about 5:1 to about 30:1, from about 5:1 to about 35:1, from about 5:1 to about 40:1, from about 5:1 to about 45:1, from about 5:1 to about 50:1, from about 5:1 to about 55:1, from about 5:1 to about 60:1, from about 5:1 to about 70:1, from about 10:1 to about 15:1, from about 10:1 to about 20:1, from about 10:1 to about 25:1, from about 10:1 to about 30:1, from about 10:1 to about 35:1, from about 10:1 to about 40:1, from about 10:1 to about 45:1, from about 10:1 to about 50:1, from about 10:1 to about 55:1, from about 10:1 to about 60:1, from about 10:1 to about 70:1, from about 15:1 to about 20:1, from about 15:1 to about 25:1, from about 15:1 to about 30:1, from about 15:1 to about 35:1, from about 15:1 to about 40:1, from about 15:1 to about 45:1, from about 15:1 to about 50:1, from about 15:1 to about 55:1, from about 15:1 to about 60:1 or from about 15:1 to about 70:1.

In one embodiment, the lipid nanoparticles described herein can comprise the polynucleotide in a concentration from approximately 0.1 mg/ml to 2 mg/ml such as, but not limited to, 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml, 0.6 mg/ml, 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1.0 mg/ml, 1.1 mg/ml, 1.2 mg/ml, 1.3 mg/ml, 1.4 mg/ml, 1.5 mg/ml, 1.6 mg/ml, 1.7 mg/ml, 1.8 mg/ml, 1.9 mg/ml, 2.0 mg/ml or greater than 2.0 mg/ml.

Nanoparticle Compositions

In some embodiments, the pharmaceutical compositions disclosed herein are formulated as lipid nanoparticles (LNP). Accordingly, the present disclosure also provides nanoparticle compositions comprising (i) a lipid composition comprising a delivery agent such as compound as described herein, and (ii) a polynucleotide encoding a polypeptide. In such nanoparticle composition, the lipid composition disclosed herein can encapsulate the polynucleotide encoding a polypeptide.

Nanoparticle compositions are typically sized on the order of micrometers or smaller and can include a lipid bilayer. Nanoparticle compositions encompass lipid nanoparticles (LNPs), liposomes (e.g., lipid vesicles), and lipoplexes. For example, a nanoparticle composition can be a liposome having a lipid bilayer with a diameter of 500 nm or less.

Nanoparticle compositions include, for example, lipid nanoparticles (LNPs), liposomes, and lipoplexes. In some embodiments, nanoparticle compositions are vesicles including one or more lipid bilayers. In certain embodiments, a nanoparticle composition includes two or more concentric bilayers separated by aqueous compartments. Lipid bilayers can be functionalized and/or crosslinked to one another. Lipid bilayers can include one or more ligands, proteins, or channels.

In one embodiment, a lipid nanoparticle comprises an ionizable lipid, a structural lipid, a phospholipid, and mRNA. In some embodiments, the LNP comprises an ionizable lipid, a PEG-modified lipid, a sterol and a structural lipid. In some embodiments, the LNP has a molar ratio of about 20-60% ionizable lipid: about 5-25% structural lipid: about 25-55% sterol; and about 0.5-15% PEG-modified lipid.

In some embodiments, the LNP has a polydispersity value of less than 0.4. In some embodiments, the LNP has a net neutral charge at a neutral pH. In some embodiments, the LNP has a mean diameter of 50-150 nm. In some embodiments, the LNP has a mean diameter of 80-100 nm.

As generally defined herein, the term "lipid" refers to a small molecule that has hydrophobic or amphiphilic properties. Lipids may be naturally occurring or synthetic. Examples of classes of lipids include, but are not limited to, fats, waxes, sterol-containing metabolites, vitamins, fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides, and prenol lipids. In some

instances, the amphiphilic properties of some lipids leads them to form liposomes, vesicles, or membranes in aqueous media.

In some embodiments, a lipid nanoparticle (LNP) may comprise an ionizable lipid. As used herein, the term "ionizable lipid" has its ordinary meaning in the art and may refer to a lipid comprising one or more charged moieties. In some embodiments, an ionizable lipid may be positively charged or negatively charged. An ionizable lipid may be positively charged, in which case it can be referred to as "cationic lipid". In certain embodiments, an ionizable lipid molecule may comprise an amine group, and can be referred to as an ionizable amino lipid. As used herein, a "charged moiety" is a chemical moiety that carries a formal electronic charge, e.g., monovalent (+1, or −1), divalent (+2, or −2), trivalent (+3, or −3), etc. The charged moiety may be anionic (i.e., negatively charged) or cationic (i.e., positively charged). Examples of positively-charged moieties include amine groups (e.g., primary, secondary, and/or tertiary amines), ammonium groups, pyridinium group, guanidine groups, and imidizolium groups. In a particular embodiment, the charged moieties comprise amine groups. Examples of negatively-charged groups or precursors thereof, include carboxylate groups, sulfonate groups, sulfate groups, phosphonate groups, phosphate groups, hydroxyl groups, and the like. The charge of the charged moiety may vary, in some cases, with the environmental conditions, for example, changes in pH may alter the charge of the moiety, and/or cause the moiety to become charged or uncharged. In general, the charge density of the molecule may be selected as desired.

It should be understood that the terms "charged" or "charged moiety" does not refer to a "partial negative charge" or "partial positive charge" on a molecule. The terms "partial negative charge" and "partial positive charge" are given its ordinary meaning in the art. A "partial negative charge" may result when a functional group comprises a bond that becomes polarized such that electron density is pulled toward one atom of the bond, creating a partial negative charge on the atom. Those of ordinary skill in the art will, in general, recognize bonds that can become polarized in this way.

In some embodiments, the ionizable lipid is an ionizable amino lipid, sometimes referred to in the art as an "ionizable cationic lipid". In one embodiment, the ionizable amino lipid may have a positively charged hydrophilic head and a hydrophobic tail that are connected via a linker structure.

In addition to these, an ionizable lipid may also be a lipid including a cyclic amine group.

In one embodiment, the ionizable lipid may be selected from, but not limited to, a ionizable lipid described in International Publication Nos. WO2013086354 and WO2013116126; the contents of each of which are herein incorporated by reference in their entirety.

In yet another embodiment, the ionizable lipid may be selected from, but not limited to, formula CLI-CLXXXXII of U.S. Pat. No. 7,404,969; each of which is herein incorporated by reference in their entirety.

In one embodiment, the lipid may be a cleavable lipid such as those described in International Publication No. WO2012170889, herein incorporated by reference in its entirety. In one embodiment, the lipid may be synthesized by methods known in the art and/or as described in International Publication Nos. WO2013086354; the contents of each of which are herein incorporated by reference in their entirety.

Nanoparticle compositions can be characterized by a variety of methods. For example, microscopy (e.g., transmission electron microscopy or scanning electron microscopy) can be used to examine the morphology and size distribution of a nanoparticle composition. Dynamic light scattering or potentiometry (e.g., potentiometric titrations) can be used to measure zeta potentials. Dynamic light scattering can also be utilized to determine particle sizes. Instruments such as the Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) can also be used to measure multiple characteristics of a nanoparticle composition, such as particle size, polydispersity index, and zeta potential.

The size of the nanoparticles can help counter biological reactions such as, but not limited to, inflammation, or can increase the biological effect of the polynucleotide.

As used herein, "size" or "mean size" in the context of nanoparticle compositions refers to the mean diameter of a nanoparticle composition.

In one embodiment, the polynucleotide encoding a polypeptide is formulated in lipid nanoparticles having a diameter from about 10 to about 100 nm such as, but not limited to, about 10 to about 20 nm, about 10 to about 30 nm, about 10 to about 40 nm, about 10 to about 50 nm, about 10 to about 60 nm, about 10 to about 70 nm, about 10 to about 80 nm, about 10 to about 90 nm, about 20 to about 30 nm, about 20 to about 40 nm, about 20 to about 50 nm, about 20 to about 60 nm, about 20 to about 70 nm, about 20 to about 80 nm, about 20 to about 90 nm, about 20 to about 100 nm, about 30 to about 40 nm, about 30 to about 50 nm, about 30 to about 60 nm, about 30 to about 70 nm, about 30 to about 80 nm, about 30 to about 90 nm, about 30 to about 100 nm, about 40 to about 50 nm, about 40 to about 60 nm, about 40 to about 70 nm, about 40 to about 80 nm, about 40 to about 90 nm, about 40 to about 100 nm, about 50 to about 60 nm, about 50 to about 70 nm, about 50 to about 80 nm, about 50 to about 90 nm, about 50 to about 100 nm, about 60 to about 70 nm, about 60 to about 80 nm, about 60 to about 90 nm, about 60 to about 100 nm, about 70 to about 80 nm, about 70 to about 90 nm, about 70 to about 100 nm, about 80 to about 90 nm, about 80 to about 100 nm and/or about 90 to about 100 nm.

In one embodiment, the nanoparticles have a diameter from about 10 to 500 nm. In one embodiment, the nanoparticle has a diameter greater than 100 nm, greater than 150 nm, greater than 200 nm, greater than 250 nm, greater than 300 nm, greater than 350 nm, greater than 400 nm, greater than 450 nm, greater than 500 nm, greater than 550 nm, greater than 600 nm, greater than 650 nm, greater than 700 nm, greater than 750 nm, greater than 800 nm, greater than 850 nm, greater than 900 nm, greater than 950 nm or greater than 1000 nm.

In some embodiments, the largest dimension of a nanoparticle composition is 1 μm or shorter (e.g., 1 μm, 900 nm, 800 nm, 700 nm, 600 nm, 500 nm, 400 nm, 300 nm, 200 nm, 175 nm, 150 nm, 125 nm, 100 nm, 75 nm, 50 nm, or shorter).

A nanoparticle composition can be relatively homogenous. A polydispersity index can be used to indicate the homogeneity of a nanoparticle composition, e.g., the particle size distribution of the nanoparticle composition. A small (e.g., less than 0.3) polydispersity index generally indicates a narrow particle size distribution. A nanoparticle composition can have a polydispersity index from about 0 to about 0.25, such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, or 0.25. In some embodiments,

the polydispersity index of a nanoparticle composition disclosed herein can be from about 0.10 to about 0.20.

The zeta potential of a nanoparticle composition can be used to indicate the electrokinetic potential of the composition. For example, the zeta potential can describe the surface charge of a nanoparticle composition. Nanoparticle compositions with relatively low charges, positive or negative, are generally desirable, as more highly charged species can interact undesirably with cells, tissues, and other elements in the body. In some embodiments, the zeta potential of a nanoparticle composition disclosed herein can be from about −10 mV to about +20 mV, from about −10 mV to about +15 mV, from about 10 mV to about +10 mV, from about −10 mV to about +5 mV, from about −10 mV to about 0 mV, from about −10 mV to about −5 mV, from about −5 mV to about +20 mV, from about −5 mV to about +15 mV, from about −5 mV to about +10 mV, from about −5 mV to about +5 mV, from about −5 mV to about 0 mV, from about 0 mV to about +20 mV, from about 0 mV to about +15 mV, from about 0 mV to about +10 mV, from about 0 mV to about +5 mV, from about +5 mV to about +20 mV, from about +5 mV to about +15 mV, or from about +5 mV to about +10 mV.

In some embodiments, the zeta potential of the lipid nanoparticles can be from about 0 mV to about 100 mV, from about 0 mV to about 90 mV, from about 0 mV to about 80 mV, from about 0 mV to about 70 mV, from about 0 mV to about 60 mV, from about 0 mV to about 50 mV, from about 0 mV to about 40 mV, from about 0 mV to about 30 mV, from about 0 mV to about 20 mV, from about 0 mV to about 10 mV, from about 10 mV to about 100 mV, from about 10 mV to about 90 mV, from about 10 mV to about 80 mV, from about 10 mV to about 70 mV, from about 10 mV to about 60 mV, from about 10 mV to about 50 mV, from about 10 mV to about 40 mV, from about 10 mV to about 30 mV, from about 10 mV to about 20 mV, from about 20 mV to about 100 mV, from about 20 mV to about 90 mV, from about 20 mV to about 80 mV, from about 20 mV to about 70 mV, from about 20 mV to about 60 mV, from about 20 mV to about 50 mV, from about 20 mV to about 40 mV, from about 20 mV to about 30 mV, from about 30 mV to about 100 mV, from about 30 mV to about 90 mV, from about 30 mV to about 80 mV, from about 30 mV to about 70 mV, from about 30 mV to about 60 mV, from about 30 mV to about 50 mV, from about 30 mV to about 40 mV, from about 40 mV to about 100 mV, from about 40 mV to about 90 mV, from about 40 mV to about 80 mV, from about 40 mV to about 70 mV, from about 40 mV to about 60 mV, and from about 40 mV to about 50 mV. In some embodiments, the zeta potential of the lipid nanoparticles can be from about 10 mV to about 50 mV, from about 15 mV to about 45 mV, from about 20 mV to about 40 mV, and from about 25 mV to about 35 mV. In some embodiments, the zeta potential of the lipid nanoparticles can be about 10 mV, about 20 mV, about 30 mV, about 40 mV, about 50 mV, about 60 mV, about 70 mV, about 80 mV, about 90 mV, and about 100 mV.

The term "encapsulation efficiency" of a polynucleotide describes the amount of the polynucleotide that is encapsulated by or otherwise associated with a nanoparticle composition after preparation, relative to the initial amount provided. As used herein, "encapsulation" can refer to complete, substantial, or partial enclosure, confinement, surrounding, or encasement.

Encapsulation efficiency is desirably high (e.g., close to 100%). The encapsulation efficiency can be measured, for example, by comparing the amount of the polynucleotide in a solution containing the nanoparticle composition before and after breaking up the nanoparticle composition with one or more organic solvents or detergents.

Fluorescence can be used to measure the amount of free polynucleotide in a solution. For the nanoparticle compositions described herein, the encapsulation efficiency of a polynucleotide can be at least 50%, for example 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the encapsulation efficiency can be at least 80%. In certain embodiments, the encapsulation efficiency can be at least 90%.

The amount of a polynucleotide present in a pharmaceutical composition disclosed herein can depend on multiple factors such as the size of the polynucleotide, desired target and/or application, or other properties of the nanoparticle composition as well as on the properties of the polynucleotide.

For example, the amount of an mRNA useful in a nanoparticle composition can depend on the size (expressed as length, or molecular mass), sequence, and other characteristics of the mRNA. The relative amounts of a polynucleotide in a nanoparticle composition can also vary.

The relative amounts of the lipid composition and the polynucleotide present in a lipid nanoparticle composition of the present disclosure can be optimized according to considerations of efficacy and tolerability. For compositions including an mRNA as a polynucleotide, the N:P ratio can serve as a useful metric.

As the N:P ratio of a nanoparticle composition controls both expression and tolerability, nanoparticle compositions with low N:P ratios and strong expression are desirable. N:P ratios vary according to the ratio of lipids to RNA in a nanoparticle composition.

In general, a lower N:P ratio is preferred. The one or more RNA, lipids, and amounts thereof can be selected to provide an N:P ratio from about 2:1 to about 30:1, such as 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 12:1, 14:1, 16:1, 18:1, 20:1, 22:1, 24:1, 26:1, 28:1, or 30:1. In certain embodiments, the N:P ratio can be from about 2:1 to about 8:1. In other embodiments, the N:P ratio is from about 5:1 to about 8:1. In certain embodiments, the N:P ratio is between 5:1 and 6:1. In one specific aspect, the N:P ratio is about is about 5.67:1.

In addition to providing nanoparticle compositions, the present disclosure also provides methods of producing lipid nanoparticles comprising encapsulating a polynucleotide. Such method comprises using any of the pharmaceutical compositions disclosed herein and producing lipid nanoparticles in accordance with methods of production of lipid nanoparticles known in the art. See, e.g., Wang et al. (2015) "Delivery of oligonucleotides with lipid nanoparticles" Adv. Drug Deliv. Rev. 87:68-80; Silva et al. (2015) "Delivery Systems for Biopharmaceuticals. Part I: Nanoparticles and Microparticles" Curr. Pharm. Technol. 16: 940-954; Naseri et al. (2015) "Solid Lipid Nanoparticles and Nanostructured Lipid Carriers: Structure, Preparation and Application" Adv. Pharm. Bull. 5:305-13; Silva et al. (2015) "Lipid nanoparticles for the delivery of biopharmaceuticals" Curr. Pharm. Biotechnol. 16:291-302, and references cited therein.

Other Delivery Agents

a. Liposomes, Lipoplexes, and Lipid Nanoparticles

In some embodiments, the compositions or formulations of the present disclosure comprise a delivery agent, e.g., a liposome, a lioplexes, a lipid nanoparticle, or any combination thereof. The polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) can be formulated using one or more lipo-

somes, lipoplexes, or lipid nanoparticles. Liposomes, lipoplexes, or lipid nanoparticles can be used to improve the efficacy of the polynucleotides directed protein production as these formulations can increase cell transfection by the polynucleotide; and/or increase the translation of encoded protein. The liposomes, lipoplexes, or lipid nanoparticles can also be used to increase the stability of the polynucleotides.

Liposomes are artificially-prepared vesicles that can primarily be composed of a lipid bilayer and can be used as a delivery vehicle for the administration of pharmaceutical formulations.

Liposomes can be of different sizes. A multilamellar vesicle (MLV) can be hundreds of nanometers in diameter, and can contain a series of concentric bilayers separated by narrow aqueous compartments. A small unicellular vesicle (SUV) can be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) can be between 50 and 500 nm in diameter. Liposome design can include, but is not limited to, opsonins or ligands to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to, endocytosis. Liposomes can contain a low or a high pH value in order to improve the delivery of the pharmaceutical formulations.

The formation of liposomes can depend on the pharmaceutical formulation entrapped and the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimal size, polydispersity and the shelf-life of the vesicles for the intended application, and the batch-to-batch reproducibility and scale up production of safe and efficient liposomal products, etc.

As a non-limiting example, liposomes such as synthetic membrane vesicles can be prepared by the methods, apparatus and devices described in U.S. Pub. Nos. US20130177638, US20130177637, US20130177636, US20130177635, US20130177634, US20130177633, US20130183375, US20130183373, and US20130183372. In some embodiments, the polynucleotides described herein can be encapsulated by the liposome and/or it can be contained in an aqueous core that can then be encapsulated by the liposome as described in, e.g., Intl. Pub. Nos. WO2012031046, WO2012031043, WO2012030901, WO2012006378, and WO2013086526; and U.S. Pub. Nos. US20130189351, US20130195969 and US20130202684. Each of the references in herein incorporated by reference in its entirety.

In some embodiments, the polynucleotides described herein can be formulated in a cationic oil-in-water emulsion where the emulsion particle comprises an oil core and a cationic lipid that can interact with the polynucleotide anchoring the molecule to the emulsion particle. In some embodiments, the polynucleotides described herein can be formulated in a water-in-oil emulsion comprising a continuous hydrophobic phase in which the hydrophilic phase is dispersed. Exemplary emulsions can be made by the methods described in Intl. Pub. Nos. WO2012006380 and WO201087791, each of which is herein incorporated by reference in its entirety.

In some embodiments, the polynucleotides described herein can be formulated in a lipid-polycation complex. The formation of the lipid-polycation complex can be accomplished by methods as described in, e.g., U.S. Pub. No. US20120178702. As a non-limiting example, the polycation can include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine and the cationic peptides described in Intl. Pub. No. WO2012013326 or U.S. Pub. No. US20130142818. Each of the references is herein incorporated by reference in its entirety.

In some embodiments, the polynucleotides described herein can be formulated in a lipid nanoparticle (LNP) such as those described in Intl. Pub. Nos. WO2013123523, WO2012170930, WO2011127255 and WO2008103276; and U.S. Pub. No. US20130171646, each of which is herein incorporated by reference in its entirety.

Lipid nanoparticle formulations typically comprise one or more lipids. In some embodiments, the lipid is an ionizable lipid (e.g., an ionizable amino lipid), sometimes referred to in the art as an "ionizable cationic lipid". In some embodiments, lipid nanoparticle formulations further comprise other components, including a phospholipid, a structural lipid, and a molecule capable of reducing particle aggregation, for example a PEG or PEG-modified lipid.

Exemplary ionizable lipids include, but not limited to, any one of Compounds 1-342 disclosed herein, DLin-MC3-DMA (MC3), DLin-DMA, DLenDMA, DLin-D-DMA, DLin-K-DMA, DLin-M-C_2-DMA, DLin-K-DMA, DLin-KC2-DMA, DLin-KC3-DMA, DLin-KC4-DMA, DLin-C_2K-DMA, DLin-MP-DMA, DODMA, 98N12-5, $C_{12\text{-}200}$, DLin-C-DAP, DLin-DAC, DLinDAP, DLinAP, DLin-EG-DMA, DLin-2-DMAP, KL10, KL22, KL25, Octyl-CLinDMA, Octyl-CLinDMA (2R), Octyl-CLinDMA (2S), and any combination thereof. Other exemplary ionizable lipids include, (13Z,16Z)—N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine (L608), (20Z,23Z)—N,N-dimethylnonacosa-20,23-dien-10-amine, (17Z,20Z)—N,N-dimemylhexacosa-17,20-dien-9-amine, (16Z,19Z)—N5N-dimethylpentacosa-16,19-dien-8-amine, (13Z,16Z)—N,N-dimethyldocosa-13,16-dien-5-amine, (12Z,15Z)—N,N-dimethylhenicosa-12,15-dien-4-amine, (14Z,17Z)—N,N-dimethyltricosa-14,17-dien-6-amine, (15Z,18Z)—N,N-dimethyltetracosa-15,18-dien-7-amine, (18Z,21Z)—N,N-dimethylheptacosa-18,21-dien-10-amine, (15Z,18Z)—N,N-dimethyltetracosa-15,18-dien-5-amine, (14Z,17Z)—N,N-dimethyltricosa-14,17-dien-4-amine, (19Z,22Z)—N,N-dimethyloctacosa-19,22-dien-9-amine, (18Z,21Z)—N,N-dimethylheptacosa-18,21-dien-8-amine, (17Z,20Z)—N,N-dimethylhexacosa-17,20-dien-7-amine, (16Z,19Z)—N,N-dimethylpentacosa-16,19-dien-6-amine, (22Z,25Z)—N,N-dimethylhentriaconta-22,25-dien-10-amine, (21Z,24Z)—N,N-dimethyltriaconta-21,24-dien-9-amine, (18Z)—N,N-dimetylheptacos-18-en-10-amine, (17Z)—N,N-dimethylhexacos-17-en-9-amine, (19Z,22Z)—N,N-dimethyloctacosa-19,22-dien-7-amine, N,N-dimethylheptacosan-10-amine, (20Z,23Z)—N-ethyl-N-methylnonacosa-20,23-dien-10-amine, 1-[(11Z,14Z)-1-nonylicosa-11,14-dien-1-yl]pyrrolidine, (20Z)—N,N-dimethylheptacos-20-en-10-amine, (15Z)—N,N-dimetyleptacos-15-en-10-amine, (14Z)—N,N-dimethylnonacos-14-en-10-amine, (17Z)—N,N-dimethylnonacos-17-en-10-amine, (24Z)—N,N-dimethyltritriacont-24-en-10-amine, (20Z)—N,N-dimethylnonacos-20-en-10-amine, (22Z)—N,N-dimethylhentriacont-22-en-10-amine, (16Z)—N,N-dimethylpentacos-16-en-8-amine, (12Z,15Z)—N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl] eptadecan-8-amine, 1-[(1S,2R)-2-hexylcyclopropyl]-N,N-dimethylnonadecan-10-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]nonadecan-10-amine, N,N-dimethyl-21-[(1S,2R)-2-octylcyclopropyl]henicosan-10-amine, N,N-

dimethyl-1-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl] methyl}cyclopropyl]nonadecan-10-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]hexadecan-8-amine, N,N-dimethyl-[(1R,2 S)-2-undecylcyclopropyl]tetradecan-5-amine, N,N-dimethyl-3{7-[(1S,2R)-2-octylcyclopropyl] heptyl}dodecan-1-amine, 1-[(1R,2S)-2-heptylcyclopropyl]-N,N-dimethyloctadecan-9-amine, 1-[(1 S,2R)-2-decylcyclopropyl]-N,N-dimethylpentadecan-6-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]pentadecan-8-amine, R—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-(octyloxy)propan-2-amine, S—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-(octyloxy) propan-2-amine, 1-{2-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-1-[(octyloxy)methyl]ethyl} pyrrolidine, (2 S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-[(5Z)-oct-5-en-1-yloxy]propan-2-amine, 1-{2-[(9Z,12Z)-octadeca-9, 12-dien-1-yloxy]-1-[(octyloxy)methyl]ethyl} azetidine, (2S)-1-(hexyloxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, (2 S)-1-(heptyl oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-(nonyl oxy)-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-[(9Z)-octadec-9-en-1-yloxy]-3-(octyloxy) propan-2-amine; (2 S)—N,N-dimethyl-1-[(6Z,9Z,12Z)-octadeca-6,9,12-trien-1-yloxy]-3-(octyloxy)propan-2-amine, (2 S)-1-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethyl-3-(pentyloxy)propan-2-amine, (2 S)-1-(hexyloxy)-3-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethylpropan-2-amine, 1-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, 1-[(13Z,16Z)-docosa-13,16-dien-1-yloxy]-N,N-dimethyl-3-(octyloxy) propan-2-amine, (2S)-1-[(13Z, 16Z)-docosa-13,16-dien-1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, (2 S)-1-[(13Z)-docos-13-en-1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, 1-[(13Z)-docos-13-en-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, 1-[(9Z)-hexadec-9-en-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, (2R)—N,N-dimethyl-H(1-metoyloctyl)oxyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, (2R)-1-[(3,7-dimethyloctyl)oxy]-N,N-dimethyl-3-[(9Z, 12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-(octyloxy)-3-({8-[(1S,2S)-2-{[(1R,2R)-2-pentyl-cyclopropyl]methyl}cyclopropyl]octyl} oxy)propan-2-amine, N,N-dimethyl-1-{[8-(2-oclylcyclopropyl)octyl] oxy}-3-(octyloxy)propan-2-amine, and (11E,20Z,23Z)—N, N-dimethylnonacosa-11,20,2-trien-10-amine, and any combination thereof.

Phospholipids include, but are not limited to, glycerophospholipids such as phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, phosphatidylinositols, phosphatidy glycerols, and phosphatidic acids. Phospholipids also include phosphosphingolipid, such as sphingomyelin. In some embodiments, the phospholipids are DLPC, DMPC, DOPC, DPPC, DSPC, DUPC, 18:0 Diether PC, DLnPC, DAPC, DHAPC, DOPE, 4ME 16:0 PE, DSPE, DLPE, DLnPE, DAPE, DHAPE, DOPG, and any combination thereof. In some embodiments, the phospholipids are MPPC, MSPC, PMPC, PSPC, SMPC, SPPC, DHAPE, DOPG, and any combination thereof. In some embodiments, the amount of phospholipids (e.g., DSPC) in the lipid composition ranges from about 1 mol % to about 20 mol %.

The structural lipids include sterols and lipids containing sterol moieties. In some embodiments, the structural lipids include cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and mixtures thereof. In some embodiments, the structural lipid is cholesterol. In some embodiments, the amount of the structural lipids (e.g., cholesterol) in the lipid composition ranges from about 20 mol % to about 60 mol %.

The PEG-modified lipids include PEG-modified phosphatidylethanolamine and phosphatidic acid, PEG-ceramide conjugates (e.g., PEG-CerC14 or PEG-CerC20), PEG-modified dialkylamines and PEG-modified 1,2-diacyloxypropan-3-amines. Such lipids are also referred to as PEGylated lipids. For example, a PEG lipid can be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG DMPE, PEG-DPPC, or a PEG-DSPE lipid. In some embodiments, the PEG-lipid are 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (PEG-DSPE), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA). In some embodiments, the PEG moiety has a size of about 1000, 2000, 5000, 10,000, 15,000 or 20,000 daltons. In some embodiments, the amount of PEG-lipid in the lipid composition ranges from about 0 mol % to about 5 mol %.

In some embodiments, the LNP formulations described herein can additionally comprise a permeability enhancer molecule. Non-limiting permeability enhancer molecules are described in U.S. Pub. No. US20050222064, herein incorporated by reference in its entirety.

The LNP formulations can further contain a phosphate conjugate. The phosphate conjugate can increase in vivo circulation times and/or increase the targeted delivery of the nanoparticle. Phosphate conjugates can be made by the methods described in, e.g., Intl. Pub. No. WO2013033438 or U.S. Pub. No. US20130196948. The LNP formulation can also contain a polymer conjugate (e.g., a water soluble conjugate) as described in, e.g., U.S. Pub. Nos. US20130059360, US20130196948, and US20130072709. Each of the references is herein incorporated by reference in its entirety.

The LNP formulations can comprise a conjugate to enhance the delivery of nanoparticles of the present invention in a subject. Further, the conjugate can inhibit phagocytic clearance of the nanoparticles in a subject. In some embodiments, the conjugate can be a "self" peptide designed from the human membrane protein CD47 (e.g., the "self" particles described by Rodriguez et al, Science 2013 339, 971-975, herein incorporated by reference in its entirety). As shown by Rodriguez et al. the self peptides delayed macrophage-mediated clearance of nanoparticles which enhanced delivery of the nanoparticles.

The LNP formulations can comprise a carbohydrate carrier. As a non-limiting example, the carbohydrate carrier can include, but is not limited to, an anhydride-modified phytoglycogen or glycogen-type material, phytoglycogen octenyl succinate, phytoglycogen beta-dextrin, anhydride-modified phytoglycogen beta-dextrin (e.g., Intl. Pub. No. WO2012109121, herein incorporated by reference in its entirety).

The LNP formulations can be coated with a surfactant or polymer to improve the delivery of the particle. In some embodiments, the LNP can be coated with a hydrophilic coating such as, but not limited to, PEG coatings and/or coatings that have a neutral surface charge as described in U.S. Pub. No. US20130183244, herein incorporated by reference in its entirety.

The LNP formulations can be engineered to alter the surface properties of particles so that the lipid nanoparticles can penetrate the mucosal barrier as described in U.S. Pat.

No. 8,241,670 or Intl. Pub. No. WO2013110028, each of which is herein incorporated by reference in its entirety.

The LNP engineered to penetrate mucus can comprise a polymeric material (i.e., a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material can include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyeneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates.

LNP engineered to penetrate mucus can also include surface altering agents such as, but not limited to, polynucleotides, anionic proteins (e.g., bovine serum albumin), surfactants (e.g., cationic surfactants such as for example dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (e.g., cyclodextrin), nucleic acids, polymers (e.g., heparin, polyethylene glycol and poloxamer), mucolytic agents (e.g., N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β4 dornase alfa, neltenexine, erdosteine) and various DNases including rhDNase.

In some embodiments, the mucus penetrating LNP can be a hypotonic formulation comprising a mucosal penetration enhancing coating. The formulation can be hypotonic for the epithelium to which it is being delivered. Non-limiting examples of hypotonic formulations can be found in, e.g., Intl. Pub. No. WO2013110028, herein incorporated by reference in its entirety.

In some embodiments, the polynucleotide described herein is formulated as a lipoplex, such as, without limitation, the ATUPLEX™ system, the DACC system, the DBTC system and other siRNA-lipoplex technology from Silence Therapeutics (London, United Kingdom), STEMFECT™ from STEMGENT® (Cambridge, Mass.), and polyethylenimine (PEI) or protamine-based targeted and non-targeted delivery of nucleic acids (Aleku et al. Cancer Res. 2008 68:9788-9798; Strumberg et al. Int J Clin Pharmacol Ther 2012 50:76-78; Santel et al., Gene Ther 2006 13:1222-1234; Santel et al., Gene Ther 2006 13:1360-1370; Gutbier et al., Pulm Pharmacol. Ther. 2010 23:334-344; Kaufmann et al. Microvasc Res 2010 80:286-293Weide et al. J Immunother. 2009 32:498-507; Weide et al. J Immunother. 2008 31:180-188; Pascolo Expert Opin. Biol. Ther. 4:1285-1294; Fotin-Mleczek et al., 2011 J. Immunother. 34:1-15; Song et al., Nature Biotechnol. 2005, 23:709-717; Peer et al., Proc Natl Acad Sci USA. 2007 6; 104:4095-4100; deFougerolles Hum Gene Ther. 2008 19:125-132; all of which are incorporated herein by reference in its entirety).

In some embodiments, the polynucleotides described herein are formulated as a solid lipid nanoparticle (SLN), which can be spherical with an average diameter between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic molecules and can be stabilized with surfactants and/or emulsifiers. Exemplary SLN can be those as described in Intl. Pub. No. WO2013105101, herein incorporated by reference in its entirety.

In some embodiments, the polynucleotides described herein can be formulated for controlled release and/or targeted delivery. As used herein, "controlled release" refers to a pharmaceutical composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome. In one embodiment, the polynucleotides can be encapsulated into a delivery agent described herein and/or known in the art for controlled release and/or targeted delivery. As used herein, the term "encapsulate" means to enclose, surround or encase. As it relates to the formulation of the compounds of the invention, encapsulation can be substantial, complete or partial. The term "substantially encapsulated" means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, or greater than 99% of the pharmaceutical composition or compound of the invention can be enclosed, surrounded or encased within the delivery agent. "Partially encapsulation" means that less than 10, 10, 20, 30, 40 50 or less of the pharmaceutical composition or compound of the invention can be enclosed, surrounded or encased within the delivery agent.

Advantageously, encapsulation can be determined by measuring the escape or the activity of the pharmaceutical composition or compound of the invention using fluorescence and/or electron micrograph. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, or greater than 99% of the pharmaceutical composition or compound of the invention are encapsulated in the delivery agent.

In some embodiments, the polynucleotides described herein can be encapsulated in a therapeutic nanoparticle, referred to herein as "therapeutic nanoparticle polynucleotides." Therapeutic nanoparticles can be formulated by methods described in, e.g., Intl. Pub. Nos. WO2010005740, WO2010030763, WO2010005721, WO2010005723, and WO2012054923; and U.S. Pub. Nos. US20110262491, US20100104645, US20100087337, US20100068285, US20110274759, US20100068286, US20120288541, US20120140790, US20130123351 and US20130230567; and U.S. Pat. Nos. 8,206,747, 8,293,276, 8,318,208 and 8,318,211, each of which is herein incorporated by reference in its entirety.

In some embodiments, the therapeutic nanoparticle polynucleotide can be formulated for sustained release. As used herein, "sustained release" refers to a pharmaceutical composition or compound that conforms to a release rate over a specific period of time. The period of time can include, but is not limited to, hours, days, weeks, months and years. As a non-limiting example, the sustained release nanoparticle of the polynucleotides described herein can be formulated as disclosed in Intl. Pub. No. WO2010075072 and U.S. Pub. Nos. US20100216804, US20110217377, US20120201859 and US20130150295, each of which is herein incorporated by reference in their entirety.

In some embodiments, the therapeutic nanoparticle polynucleotide can be formulated to be target specific, such as those described in Intl. Pub. Nos. WO2008121949, WO2010005726, WO2010005725, WO2011084521 and WO2011084518; and U.S. Pub. Nos. US20100069426, US20120004293 and US20100104655, each of which is herein incorporated by reference in its entirety.

The LNPs can be prepared using microfluidic mixers or micromixers. Exemplary microfluidic mixers can include, but are not limited to, a slit interdigital micromixer including, but not limited to those manufactured by Microinnova (Allerheiligen bei Wildon, Austria) and/or a staggered herringbone micromixer (SHM) (see Zhigaltsevet al., "Bottom-up design and synthesis of limit size lipid nanoparticle systems with aqueous and triglyceride cores using millisecond microfluidic mixing," Langmuir 28:3633-40 (2012); Belliveau et al., "Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA," Molecular Therapy-Nucleic Acids. 1:e37 (2012); Chen et al., "Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation," J. Am. Chem. Soc. 134(16):6948-51 (2012); each of

which is herein incorporated by reference in its entirety). Exemplary micromixers include Slit Interdigital Microstructured Mixer (SIMM-V2) or a Standard Slit Interdigital Micro Mixer (SSIMM) or Caterpillar (CPMM) or Impinging-jet (IJMM,) from the Institut für Mikrotechnik Mainz GmbH, Mainz Germany. In some embodiments, methods of making LNP using SHM further comprise mixing at least two input streams wherein mixing occurs by microstructure-induced chaotic advection (MICA). According to this method, fluid streams flow through channels present in a herringbone pattern causing rotational flow and folding the fluids around each other. This method can also comprise a surface for fluid mixing wherein the surface changes orientations during fluid cycling. Methods of generating LNPs using SHM include those disclosed in U.S. Pub. Nos. US20040262223 and US20120276209, each of which is incorporated herein by reference in their entirety.

In some embodiments, the polynucleotides described herein can be formulated in lipid nanoparticles using microfluidic technology (see Whitesides, George M., "The Origins and the Future of Microfluidics," Nature 442: 368-373 (2006); and Abraham et al., "Chaotic Mixer for Microchannels," Science 295: 647-651 (2002); each of which is herein incorporated by reference in its entirety). In some embodiments, the polynucleotides can be formulated in lipid nanoparticles using a micromixer chip such as, but not limited to, those from Harvard Apparatus (Holliston, Mass.) or Dolomite Microfluidics (Royston, UK). A micromixer chip can be used for rapid mixing of two or more fluid streams with a split and recombine mechanism.

In some embodiments, the polynucleotides described herein can be formulated in lipid nanoparticles having a diameter from about 1 nm to about 100 nm such as, but not limited to, about 1 nm to about 20 nm, from about 1 nm to about 30 nm, from about 1 nm to about 40 nm, from about 1 nm to about 50 nm, from about 1 nm to about 60 nm, from about 1 nm to about 70 nm, from about 1 nm to about 80 nm, from about 1 nm to about 90 nm, from about 5 nm to about from 100 nm, from about 5 nm to about 10 nm, about 5 nm to about 20 nm, from about 5 nm to about 30 nm, from about 5 nm to about 40 nm, from about 5 nm to about 50 nm, from about 5 nm to about 60 nm, from about 5 nm to about 70 nm, from about 5 nm to about 80 nm, from about 5 nm to about 90 nm, about 10 to about 20 nm, about 10 to about 30 nm, about 10 to about 40 nm, about 10 to about 50 nm, about 10 to about 60 nm, about 10 to about 70 nm, about 10 to about 80 nm, about 10 to about 90 nm, about 20 to about 30 nm, about 20 to about 40 nm, about 20 to about 50 nm, about 20 to about 60 nm, about 20 to about 70 nm, about 20 to about 80 nm, about 20 to about 90 nm, about 20 to about 100 nm, about 30 to about 40 nm, about 30 to about 50 nm, about 30 to about 60 nm, about 30 to about 70 nm, about 30 to about 80 nm, about 30 to about 90 nm, about 30 to about 100 nm, about 40 to about 50 nm, about 40 to about 60 nm, about 40 to about 70 nm, about 40 to about 80 nm, about 40 to about 90 nm, about 40 to about 100 nm, about 50 to about 60 nm, about 50 to about 70 nm about 50 to about 80 nm, about 50 to about 90 nm, about 50 to about 100 nm, about 60 to about 70 nm, about 60 to about 80 nm, about 60 to about 90 nm, about 60 to about 100 nm, about 70 to about 80 nm, about 70 to about 90 nm, about 70 to about 100 nm, about 80 to about 90 nm, about 80 to about 100 nm and/or about 90 to about 100 nm.

In some embodiments, the lipid nanoparticles can have a diameter from about 10 to 500 nm. In one embodiment, the lipid nanoparticle can have a diameter greater than 100 nm, greater than 150 nm, greater than 200 nm, greater than 250 nm, greater than 300 nm, greater than 350 nm, greater than 400 nm, greater than 450 nm, greater than 500 nm, greater than 550 nm, greater than 600 nm, greater than 650 nm, greater than 700 nm, greater than 750 nm, greater than 800 nm, greater than 850 nm, greater than 900 nm, greater than 950 nm or greater than 1000 nm.

In some embodiments, the polynucleotides can be delivered using smaller LNPs. Such particles can comprise a diameter from below 0.1 μm up to 100 nm such as, but not limited to, less than 0.1 μm, less than 1.0 μm, less than 5 μm, less than 10 μm, less than 15 um, less than 20 um, less than 25 um, less than 30 um, less than 35 um, less than 40 um, less than 50 um, less than 55 urn, less than 60 um, less than 65 um, less than 70 um, less than 75 um, less than 80 um, less than 85 um, less than 90 um, less than 95 um, less than 100 um, less than 125 um, less than 150 um, less than 175 um, less than 200 um, less than 225 um, less than 250 um, less than 275 um, less than 300 um, less than 325 um, less than 350 um, less than 375 um, less than 400 um, less than 425 um, less than 450 um, less than 475 um, less than 500 um, less than 525 um, less than 550 um, less than 575 um, less than 600 um, less than 625 um, less than 650 um, less than 675 um, less than 700 um, less than 725 um, less than 750 um, less than 775 um, less than 800 um, less than 825 um, less than 850 um, less than 875 um, less than 900 um, less than 925 um, less than 950 um, or less than 975 um.

The nanoparticles and microparticles described herein can be geometrically engineered to modulate macrophage and/or the immune response. The geometrically engineered particles can have varied shapes, sizes and/or surface charges to incorporate the polynucleotides described herein for targeted delivery such as, but not limited to, pulmonary delivery (see, e.g., Intl. Pub. No. WO2013082111, herein incorporated by reference in its entirety). Other physical features the geometrically engineering particles can include, but are not limited to, fenestrations, angled arms, asymmetry and surface roughness, charge that can alter the interactions with cells and tissues.

In some embodiment, the nanoparticles described herein are stealth nanoparticles or target-specific stealth nanoparticles such as, but not limited to, those described in U.S. Pub. No. US20130172406, herein incorporated by reference in its entirety. The stealth or target-specific stealth nanoparticles can comprise a polymeric matrix, which can comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polyesters, polyanhydrides, polyethers, polyurethanes, polymethacrylates, polyacrylates, polycyanoacrylates, or combinations thereof

b. Lipidoids

In some embodiments, the compositions or formulations of the present disclosure comprise a delivery agent, e.g., a lipidoid. The polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) can be formulated with lipidoids. Complexes, micelles, liposomes or particles can be prepared containing these lipidoids and therefore to achieve an effective delivery of the polynucleotide, as judged by the production of an encoded protein, following the injection of a lipidoid formulation via localized and/or systemic routes of administration. Lipidoid complexes of polynucleotides can be

administered by various means including, but not limited to, intravenous, intramuscular, or subcutaneous routes.

The synthesis of lipidoids is described in literature (see Mahon et al., Bioconjug. Chem. 2010 21:1448-1454; Schroeder et al., J Intern Med. 2010 267:9-21; Akinc et al., Nat Biotechnol. 2008 26:561-569; Love et al., Proc Natl Acad Sci USA. 2010 107:1864-1869; Siegwart et al., Proc Natl Acad Sci USA. 2011 108:12996-3001; all of which are incorporated herein in their entireties).

Formulations with the different lipidoids, including, but not limited to penta[3-(1-laurylaminopropionyl)]-triethylenetetramine hydrochloride (TETA-5LAP; also known as 98N12-5, see Murugaiah et al., Analytical Biochemistry, 401:61 (2010)), C_{12-200} (including derivatives and variants), and MD1, can be tested for in vivo activity. The lipidoid "98N12-5" is disclosed by Akinc et al., Mol Ther. 2009 17:872-879. The lipidoid "C_{12-200}" is disclosed by Love et al., Proc Natl Acad Sci USA. 2010 107:1864-1869 and Liu and Huang, Molecular Therapy. 2010 669-670. Each of the references is herein incorporated by reference in its entirety.

In one embodiment, the polynucleotides described herein can be formulated in an aminoalcohol lipidoid. Aminoalcohol lipidoids can be prepared by the methods described in U.S. Pat. No. 8,450,298 (herein incorporated by reference in its entirety).

The lipidoid formulations can include particles comprising either 3 or 4 or more components in addition to polynucleotides. Lipidoids and polynucleotide formulations comprising lipidoids are described in Intl. Pub. No. WO 2015051214 (herein incorporated by reference in its entirety.

c. Hyaluronidase

In some embodiments, the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) and hyaluronidase for injection (e.g., intramuscular or subcutaneous injection). Hyaluronidase catalyzes the hydrolysis of hyaluronan, which is a constituent of the interstitial barrier. Hyaluronidase lowers the viscosity of hyaluronan, thereby increases tissue permeability (Frost, Expert Opin. Drug Deliv. (2007) 4:427-440).

Alternatively, the hyaluronidase can be used to increase the number of cells exposed to the polynucleotides administered intramuscularly, or subcutaneously.

d. Nanoparticle Mimics

In some embodiments, the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) is encapsulated within and/or absorbed to a nanoparticle mimic. A nanoparticle mimic can mimic the delivery function organisms or particles such as, but not limited to, pathogens, viruses, bacteria, fungus, parasites, prions and cells. As a non-limiting example, the polynucleotides described herein can be encapsulated in a non-viron particle that can mimic the delivery function of a virus (see e.g., Intl. Pub. No. WO2012006376 and U.S. Pub. Nos. US20130171241 and US20130195968, each of which is herein incorporated by reference in its entirety).

e. Self-Assembled Nanoparticles, or Self-Assembled Macromolecules

In some embodiments, the compositions or formulations of the present disclosure comprise the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) in self-assembled nanoparticles, or amphiphilic macromolecules (AMs) for delivery. AMs comprise biocompatible amphiphilic polymers that have an alkylated sugar backbone covalently linked to poly(ethylene glycol). In aqueous solution, the AMs self-assemble to form micelles. Nucleic acid self-assembled nanoparticles are described in Intl. Appl. No. PCT/US2014/027077, and AMs and methods of forming AMs are described in U.S. Pub. No. US20130217753, each of which is herein incorporated by reference in its entirety.

f. Cations and Anions

In some embodiments, the compositions or formulations of the present disclosure comprise the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) and a cation or anion, such as Zn2+, Ca2+, Cu2+, Mg2+ and combinations thereof. Exemplary formulations can include polymers and a polynucleotide complexed with a metal cation as described in, e.g., U.S. Pat. Nos. 6,265,389 and 6,555,525, each of which is herein incorporated by reference in its entirety. In some embodiments, cationic nanoparticles can contain a combination of divalent and monovalent cations. The delivery of polynucleotides in cationic nanoparticles or in one or more depot comprising cationic nanoparticles can improve polynucleotide bioavailability by acting as a long-acting depot and/or reducing the rate of degradation by nucleases.

g. Amino Acid Lipids

In some embodiments, the compositions or formulations of the present disclosure comprise the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) that is formulation with an amino acid lipid. Amino acid lipids are lipophilic compounds comprising an amino acid residue and one or more lipophilic tails. Non-limiting examples of amino acid lipids and methods of making amino acid lipids are described in U.S. Pat. No. 8,501,824. The amino acid lipid formulations can deliver a polynucleotide in releasable form that comprises an amino acid lipid that binds and releases the polynucleotides. As a non-limiting example, the release of the polynucleotides described herein can be provided by an acid-labile linker as described in, e.g., U.S. Pat. Nos. 7,098,032, 6,897,196, 6,426,086, 7,138,382, 5,563,250, and 5,505,931, each of which is herein incorporated by reference in its entirety.

h. Interpolyelectrolyte Complexes

In some embodiments, the compositions or formulations of the present disclosure comprise the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) in an interpolyelectrolyte complex. Interpolyelectrolyte complexes are formed when charge-dynamic polymers are complexed with one or more anionic molecules. Non-limiting examples of charge-dynamic polymers and interpolyelectrolyte complexes and methods of making interpolyelectrolyte complexes are described in U.S. Pat. No. 8,524,368, herein incorporated by reference in its entirety.

i. Crystalline Polymeric Systems

In some embodiments, the compositions or formulations of the present disclosure comprise the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) in crystalline polymeric systems. Crystalline polymeric systems are polymers with crystalline moieties and/or terminal units comprising crystalline moieties. Exemplary polymers are described in U.S. Pat. No. 8,524,259 (herein incorporated by reference in its entirety).

j. Polymers, Biodegradable Nanoparticles, and Core-Shell Nanoparticles

In some embodiments, the compositions or formulations of the present disclosure comprise the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) and a natural

and/or synthetic polymer. The polymers include, but not limited to, polyethenes, polyethylene glycol (PEG), poly(l-lysine)(PLL), PEG grafted to PLL, cationic lipopolymer, biodegradable cationic lipopolymer, polyethyleneimine (PEI), cross-linked branched poly(alkylene imines), a polyamine derivative, a modified poloxamer, elastic biodegradable polymer, biodegradable copolymer, biodegradable polyester copolymer, biodegradable polyester copolymer, multiblock copolymers, poly[α-(4-aminobutyl)-L-glycolic acid) (PAGA), biodegradable cross-linked cationic multi-block copolymers, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly (orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), amine-containing polymers, dextran polymers, dextran polymer derivatives or combinations thereof.

Exemplary polymers include, DYNAMIC POLYCONJUGATE® (Arrowhead Research Corp., Pasadena, Calif.) formulations from MIRUS® Bio (Madison, Wis.) and Roche Madison (Madison, Wis.), PHASERX™ polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGY™ (PHASERX®, Seattle, Wash.), DMRI/DOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, Calif.), chitosan, cyclodextrin from Calando Pharmaceuticals (Pasadena, Calif.), dendrimers and poly (lactic-co-glycolic acid) (PLGA) polymers. RONDEL™ (RNAi/Oligonucleotide Nanoparticle Delivery) polymers (Arrowhead Research Corporation, Pasadena, Calif.) and pH responsive co-block polymers such as PHASERX® (Seattle, Wash.).

The polymer formulations allow a sustained or delayed release of the polynucleotide (e.g., following intramuscular or subcutaneous injection). The altered release profile for the polynucleotide can result in, for example, translation of an encoded protein over an extended period of time. The polymer formulation can also be used to increase the stability of the polynucleotide. Sustained release formulations can include, but are not limited to, PLGA microspheres, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, Fla.), HYLENEX® (Halozyme Therapeutics, San Diego Calif.), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, Ga.), TISSELL® (Baxter International, Inc. Deerfield, Ill.), PEG-based sealants, and COSEAL® (Baxter International, Inc. Deerfield, Ill.).

As a non-limiting example modified mRNA can be formulated in PLGA microspheres by preparing the PLGA microspheres with tunable release rates (e.g., days and weeks) and encapsulating the modified mRNA in the PLGA microspheres while maintaining the integrity of the modified mRNA during the encapsulation process. EVAc are non-biodegradable, biocompatible polymers that are used extensively in pre-clinical sustained release implant applications (e.g., extended release products Ocusert a pilocarpine ophthalmic insert for glaucoma or progestasert a sustained release progesterone intrauterine device; transdermal delivery systems Testoderm, Duragesic and Selegiline; catheters). Poloxamer F-407 NF is a hydrophilic, non-ionic surfactant triblock copolymer of polyoxyethylene-polyoxypropylene-polyoxyethylene having a low viscosity at temperatures less than 5° C. and forms a solid gel at temperatures greater than 15° C.

As a non-limiting example, the polynucleotides described herein can be formulated with the polymeric compound of PEG grafted with PLL as described in U.S. Pat. No. 6,177,274. As another non-limiting example, the polynucleotides described herein can be formulated with a block copolymer such as a PLGA-PEG block copolymer (see e.g., U.S. Pub. No. US20120004293 and U.S. Pat. Nos. 8,236,330 and 8,246,968), or a PLGA-PEG-PLGA block copolymer (see e.g., U.S. Pat. No. 6,004,573). Each of the references is herein incorporated by reference in its entirety.

In some embodiments, the polynucleotides described herein can be formulated with at least one amine-containing polymer such as, but not limited to polylysine, polyethylene imine, poly(amidoamine) dendrimers, poly(amine-co-esters) or combinations thereof. Exemplary polyamine polymers and their use as delivery agents are described in, e.g., U.S. Pat. Nos. 8,460,696, 8,236,280, each of which is herein incorporated by reference in its entirety.

In some embodiments, the polynucleotides described herein can be formulated in a biodegradable cationic lipopolymer, a biodegradable polymer, or a biodegradable copolymer, a biodegradable polyester copolymer, a biodegradable polyester polymer, a linear biodegradable copolymer, PAGA, a biodegradable cross-linked cationic multi-block copolymer or combinations thereof as described in, e.g., U.S. Pat. Nos. 6,696,038, 6,517,869, 6,267,987, 6,217,912, 6,652,886, 8,057,821, and 8,444,992; U.S. Pub. Nos. US20030073619, US20040142474, US20100004315, US2012009145 and US20130195920; and Intl Pub. Nos. WO2006063249 and WO2013086322, each of which is herein incorporated by reference in its entirety.

In some embodiments, the polynucleotides described herein can be formulated in or with at least one cyclodextrin polymer as described in U.S. Pub. No. US20130184453. In some embodiments, the polynucleotides described herein can be formulated in or with at least one crosslinked cation-binding polymers as described in Intl. Pub. Nos. WO2013106072, WO2013106073 and WO2013106086. In some embodiments, the polynucleotides described herein can be formulated in or with at least PEGylated albumin polymer as described in U.S. Pub. No. US20130231287. Each of the references is herein incorporated by reference in its entirety.

In some embodiments, the polynucleotides disclosed herein can be formulated as a nanoparticle using a combination of polymers, lipids, and/or other biodegradable agents, such as, but not limited to, calcium phosphate. Components can be combined in a core-shell, hybrid, and/or layer-by-layer architecture, to allow for fine-tuning of the nanoparticle for delivery (Wang et al., Nat Mater. 2006 5:791-796; Fuller et al., Biomaterials. 2008 29:1526-1532; DeKoker et al., Adv Drug Deliv Rev. 2011 63:748-761; Endres et al., Biomaterials. 2011 32:7721-7731; Su et al., Mol Pharm. 2011 Jun. 6; 8(3):774-87; herein incorporated by reference in their entireties). As a non-limiting example, the nanoparticle can comprise a plurality of polymers such as, but not limited to hydrophilic-hydrophobic polymers (e.g., PEG-PLGA), hydrophobic polymers (e.g., PEG) and/or hydrophilic polymers (Intl. Pub. No. WO20120225129, herein incorporated by reference in its entirety).

The use of core-shell nanoparticles has additionally focused on a high-throughput approach to synthesize cationic cross-linked nanogel cores and various shells (Siegwart et al., Proc Natl Acad Sci USA. 2011 108:12996-13001; herein incorporated by reference in its entirety). The complexation, delivery, and internalization of the polymeric nanoparticles can be precisely controlled by altering the chemical composition in both the core and shell components of the nanoparticle. For example, the core-shell nanopar-

ticles can efficiently deliver siRNA to mouse hepatocytes after they covalently attach cholesterol to the nanoparticle.

In some embodiments, a hollow lipid core comprising a middle PLGA layer and an outer neutral lipid layer containing PEG can be used to delivery of the polynucleotides as described herein. In some embodiments, the lipid nanoparticles can comprise a core of the polynucleotides disclosed herein and a polymer shell, which is used to protect the polynucleotides in the core. The polymer shell can be any of the polymers described herein and are known in the art. The polymer shell can be used to protect the polynucleotides in the core.

Core—shell nanoparticles for use with the polynucleotides described herein are described in U.S. Pat. No. 8,313,777 or Intl. Pub. No. WO2013124867, each of which is herein incorporated by reference in their entirety.

k. Peptides and Proteins

In some embodiments, the compositions or formulations of the present disclosure comprise the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) that is formulated with peptides and/or proteins to increase transfection of cells by the polynucleotide, and/or to alter the biodistribution of the polynucleotide (e.g., by targeting specific tissues or cell types), and/or increase the translation of encoded protein (e.g., Intl. Pub. Nos. WO2012110636 and WO2013123298. In some embodiments, the peptides can be those described in U.S. Pub. Nos. US20130129726, US20130137644 and US20130164219. Each of the references is herein incorporated by reference in its entirety.

l. Conjugates

In some embodiments, the compositions or formulations of the present disclosure comprise the polynucleotides described herein (e.g., a polynucleotide comprising a nucleotide sequence encoding a polypeptide) that is covalently linked to a carrier or targeting group, or including two encoding regions that together produce a fusion protein (e.g., bearing a targeting group and therapeutic protein or peptide) as a conjugate. The conjugate can be a peptide that selectively directs the nanoparticle to neurons in a tissue or organism, or assists in crossing the blood-brain barrier.

The conjugates include a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or globulin); an carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid, an oligonucleotide (e.g., an aptamer). Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly (L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

In some embodiments, the conjugate can function as a carrier for the polynucleotide disclosed herein. The conjugate can comprise a cationic polymer such as, but not limited to, polyamine, polylysine, polyalkylenimine, and polyethylenimine that can be grafted to with poly(ethylene glycol). Exemplary conjugates and their preparations are described in U.S. Pat. No. 6,586,524 and U.S. Pub. No. US20130211249, each of which herein is incorporated by reference in its entirety.

The conjugates can also include targeting groups, e.g., a cell or tissue targeting agent, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, an RGD peptide, an RGD peptide mimetic or an aptamer.

Targeting groups can be proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies e.g., an antibody, that binds to a specified cell type such as an endothelial cell or bone cell. Targeting groups can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent frucose, or aptamers. The ligand can be, for example, a lipopolysaccharide, or an activator of p38 MAP kinase.

The targeting group can be any ligand that is capable of targeting a specific receptor. Examples include, without limitation, folate, GalNAc, galactose, mannose, mannose-6P, apatamers, integrin receptor ligands, chemokine receptor ligands, transferrin, biotin, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL, and HDL ligands. In particular embodiments, the targeting group is an aptamer. The aptamer can be unmodified or have any combination of modifications disclosed herein. As a non-limiting example, the targeting group can be a glutathione receptor (GR)-binding conjugate for targeted delivery across the blood-central nervous system barrier as described in, e.g., U.S. Pub. No. US2013021661012 (herein incorporated by reference in its entirety).

In some embodiments, the conjugate can be a synergistic biomolecule-polymer conjugate, which comprises a long-acting continuous-release system to provide a greater therapeutic efficacy. The synergistic biomolecule-polymer conjugate can be those described in U.S. Pub. No. US20130195799. In some embodiments, the conjugate can be an aptamer conjugate as described in Intl. Pat. Pub. No. WO2012040524. In some embodiments, the conjugate can be an amine containing polymer conjugate as described in U.S. Pat. No. 8,507,653. Each of the references is herein incorporated by reference in its entirety. In some embodiments, the polynucleotides can be conjugated to SMARTT POLYMER TECHNOLOGY® (PHASERX®, Inc. Seattle, Wash.).

In some embodiments, the polynucleotides described herein are covalently conjugated to a cell penetrating polypeptide, which can also include a signal sequence or a targeting sequence. The conjugates can be designed to have increased stability, and/or increased cell transfection; and/or altered the biodistribution (e.g., targeted to specific tissues or cell types).

In some embodiments, the polynucleotides described herein can be conjugated to an agent to enhance delivery. In some embodiments, the agent can be a monomer or polymer

such as a targeting monomer or a polymer having targeting blocks as described in Intl. Pub. No. WO2011062965. In some embodiments, the agent can be a transport agent covalently coupled to a polynucleotide as described in, e.g., U.S. Pat. Nos. 6,835.393 and 7,374,778. In some embodiments, the agent can be a membrane barrier transport enhancing agent such as those described in U.S. Pat. Nos. 7,737,108 and 8,003,129. Each of the references is herein incorporated by reference in its entirety.

Pharmaceutical Compositions

The present disclosure includes pharmaceutical compositions comprising an mRNA or a nanoparticle (e.g., a lipid nanoparticle) described herein, in combination with one or more pharmaceutically acceptable excipient, carrier or diluent. In particular embodiments, the mRNA is present in a nanoparticle, e.g., a lipid nanoparticle. In particular embodiments, the mRNA or nanoparticle is present in a pharmaceutical composition. In various embodiments, the one or more mRNA present in the pharmaceutical composition is encapsulated in a nanoparticle, e.g., a lipid nanoparticle. In particular embodiments, the molar ratio of the first mRNA to the second mRNA is about 1:50, about 1:25, about 1:10, about 1:5, about 1:4, about 1:3, about 1:2, about 1:1, about 2:1, about 3:1, about 4:1, or about 5:1, about 10:1, about 25:1 or about 50:1. In particular embodiments, the molar ratio of the first mRNA to the second mRNA is greater than 1:1.

In some embodiments, a composition described herein comprises an mRNA encoding a polypeptide. In some embodiments, the polypeptide is a therapeutic polypeptide. In some embodiments, the polypeptide is an enzyme. In some embodiments, the polypeptide is an antibody. In some embodiments, the polypeptide comprises an antigen.

Pharmaceutical compositions may optionally include one or more additional active substances, for example, therapeutically and/or prophylactically active substances. Pharmaceutical compositions of the present disclosure may be sterile and/or pyrogen-free. General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference in its entirety). In particular embodiments, a pharmaceutical composition comprises an mRNA and a lipid nanoparticle, or complexes thereof.

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

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may include between 0.1% and 100%, e.g., between 0.5% and 70%, between 1% and 30%, between 5% and 80%, or at least 80% (w/w) active ingredient.

The mRNAs of the disclosure can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (e.g., from a depot formulation of the mRNA); (4) alter the biodistribution (e.g., target the mRNA to specific tissues or cell types); (5) increase the translation of a polypeptide encoded by the mRNA in vivo; and/or (6) alter the release profile of a polypeptide encoded by the mRNA in vivo. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients of the present disclosure can include, without limitation, lipidoids, liposomes, lipid nanoparticles (e.g., liposomes and micelles), polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, carbohydrates, cells transfected with mRNAs (e.g., for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof. Accordingly, the formulations of the disclosure can include one or more excipients, each in an amount that together increases the stability of the mRNA, increases cell transfection by the mRNA, increases the expression of a polypeptide encoded by the mRNA, and/or alters the release profile of a mRNA-encoded polypeptide. Further, the mRNAs of the present disclosure may be formulated using self-assembled nucleic acid nanoparticles.

Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: *The Science and Practice of Pharmacy,* 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, Md., 2006; incorporated herein by reference in its entirety). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

In some embodiments, the formulations described herein may include at least one pharmaceutically acceptable salt. Examples of pharmaceutically acceptable salts that may be included in a formulation of the disclosure include, but are not limited to, acid addition salts, alkali or alkaline earth metal salts, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, acetic acid, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzene sulfonic acid, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecyl sulfate, ethanesulfonate, fumarate, glucoheptonate,

glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methyl amine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

In some embodiments, the formulations described herein may contain at least one type of polynucleotide. As a non-limiting example, the formulations may contain 1, 2, 3, 4, 5 or more than 5 mRNAs described herein. In some embodiments, the formulations described herein may contain at least one mRNA encoding a polypeptide and at least one nucleic acid sequence such as, but not limited to, an siRNA, an shRNA, a snoRNA, and an miRNA.

Liquid dosage forms for e.g., parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, nanoemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and/or suspending agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as CREMAPHOR®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables. Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

In some embodiments, pharmaceutical compositions including at least one mRNA described herein are administered to mammals (e.g., humans). Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, e.g., to a non-human mammal. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys. In particular embodiments, a subject is provided with two or more mRNAs described herein. In particular embodiments, the first and second mRNAs are provided to the subject at the same time or at different times, e.g., sequentially. In particular embodiments, the first and second mRNAs are provided to the subject in the same pharmaceutical composition or formulation, e.g., to facilitate uptake of both mRNAs by the same cells.

The present disclosure also includes kits comprising a container comprising a mRNA encoding a polypeptide that enhances an immune response. In another embodiment, the kit comprises a container comprising a mRNA encoding a polypeptide that enhances an immune response, as well as one or more additional mRNAs encoding one or more antigens or interest. In other embodiments, the kit comprises a first container comprising the mRNA encoding a polypeptide that enhances an immune response and a second container comprising one or more mRNAs encoding one or more antigens of interest. In particular embodiments, the mRNAs for enhancing an immune response and the mRNA(s) encoding an antigen(s) are present in the same or different nanoparticles and/or pharmaceutical compositions. In particular embodiments, the mRNAs are lyophilized, dried, or freeze-dried.

Methods And Use

The disclosure provides methods using the mRNAs, compositions, lipid nanoparticles, or pharmaceutical compositions disclosed herein. In some aspects, the mRNAs described herein are used to increase the amount and/or quality of a polypeptide (e.g., a therapeutic polypeptide) encoded by and translated from the mRNA. In some embodiments, the mRNAs described herein are used to reduce the translation of partial, aberrant, or otherwise undesirable open reading frames within the mRNA. In some embodiments, the mRNA described herein are used to initiate translation of a polypeptide (e.g., a therapeutic polypeptide) at a desired initiator codon.

In some embodiments, the methods described herein are useful for increasing the potency of an mRNA encoding a polypeptide. In one embodiment, the disclosure provides a method of inhibiting or reducing leaky scanning of an mRNA by a PIC or ribosome, the method comprising contacting a cell with an mRNA, a composition, a lipid nanoparticle, or a pharmaceutical composition according to the disclosure.

In some embodiments, the disclosure provides a method of increasing an amount of a polypeptide translated from a full open reading frame comprising an mRNA, the method

comprising contacting a cell with an mRNA, a composition, a lipid nanoparticle, or a pharmaceutical composition according to the disclosure.

In some embodiments, the disclosure provides a method of increasing potency of a polypeptide translated from an mRNA, the method comprising contacting a cell with an mRNA, a composition, a lipid nanoparticle, or a pharmaceutical composition according to the disclosure.

In some embodiments, the disclosure provides a method of increasing initiation of polypeptide synthesis at or from an initiation codon comprising an mRNA, the method comprising contacting a cell with an mRNA, a composition, a lipid nanoparticle, or a pharmaceutical composition according to the disclosure.

In some embodiments, the disclosure provides a method of inhibiting or reducing initiation of polypeptide synthesis at any codon within an mRNA other than an initiation codon, the method comprising contacting a cell with an mRNA, a composition, a lipid nanoparticle, or a pharmaceutical composition according to the disclosure.

In some embodiments, the disclosure provides a method of inhibiting or reducing an amount of polypeptide translated from any open reading frame within an mRNA other than a full open reading frame, the method comprising contacting a cell with an mRNA, a composition, a lipid nanoparticle, or a pharmaceutical composition according to the disclosure.

In some embodiments, the disclosure provides method of inhibiting or reducing translation of truncated or aberrant translation products from an mRNA, the method comprising contacting a cell with an mRNA, a composition, a lipid nanoparticle, or a pharmaceutical composition according to the disclosure.

In one embodiment, the method comprises administering to the subject a composition of the disclosure (or lipid nanoparticle thereof, or pharmaceutical composition thereof) comprising at least one mRNA construct encoding a polypeptide (e.g., a therapeutic polypeptide)

Compositions of the disclosure are administered to the subject at an effective amount or effective dose. In general, an effective amount of the composition will allow for efficient production of the encoded polypeptide in the cell. Metrics for efficiency may include polypeptide translation (indicated by polypeptide expression), level of mRNA degradation, and immune response indicators.

Therapeutic Methods

The mRNA provided by the disclosure can be used in a variety of clinical or therapeutic applications. In some embodiments, the disclosure provides method of treating a disease, the method comprising administering an mRNA, a composition, a lipid nanoparticle, or a pharmaceutical composition according to the disclosure.

In some embodiments, a subject having a disease is provided with or administered a nanoparticle (e.g., a lipid nanoparticle) comprising the mRNA(s). In further related embodiments, the subject is provided with or administered a pharmaceutical composition of the disclosure to the subject. In particular embodiments, the pharmaceutical composition comprises an mRNA(s) encoding a polypeptide as described herein, or it comprises a nanoparticle comprising the mRNA(s). In particular embodiments, the mRNA(s) is present in a nanoparticle, e.g., a lipid nanoparticle. In particular embodiments, the mRNA(s) or nanoparticle is present in a pharmaceutical composition.

In certain embodiments, the subject in need thereof has been diagnosed with a disease (e.g., cancer) or is considered to be at risk of developing a disease In some embodiments, the disease is, for example, an infectious disease, a cardiovascular disease, a rare genetic disease, or cancer. In some embodiments, the cancer is liver cancer, colorectal cancer, a melanoma cancer, a pancreatic cancer, a NSCLC, a cervical cancer or a head or neck cancer. In some embodiments, the cancer is a hematopoietic cancer. In some embodiments, the cancer is an acute myeloid leukemia, a chronic myeloid leukemia, a chronic myelomonocytic leukemia, a myelodystrophic syndrome (including refractory anemias and refractory cytopenias) or a myeloproliferative neoplasm or disease (including polycythemia vera, essential thrombocytosis and primary myelofibrosis). In other embodiments, the cancer is a blood-based cancer or a hematopoetic cancer. Selectivity for a particular cancer type can be achieved through the combination of use of an appropriate LNP formulation (e.g., targeting specific cell types) in combination with appropriate regulatory site(s) (e.g., microRNAs) engineered into the mRNA constructs.

In some embodiments, the mRNA(s), nanoparticle, or pharmaceutical composition is administered to the patient parenterally. In particular embodiments, the subject is a mammal, e.g., a human. In various embodiments, the subject is provided with an effective amount of the mRNA(s).

The methods of treating cancer can further include treatment of the subject with additional agents that enhance an anti-tumor response in the subject and/or that are cytotoxic to the tumor (e.g., chemotherapeutic agents). Suitable therapeutic agents for use in combination therapy include small molecule chemotherapeutic agents, including protein tyrosine kinase inhibitors, as well as biological anti-cancer agents, such as anti-cancer antibodies, including but not limited to those discussed further below. Combination therapy can include administering to the subject an immune checkpoint inhibitor to enhance anti-tumor immunity, such as PD-1 inhibitors, PD-L1 inhibitors and CTLA-4 inhibitors. Other modulators of immune checkpoints may target OX-40, OX-40L or ICOS. In one embodiment, an agent that modulates an immune checkpoint is an antibody. In another embodiment, an agent that modulates an immune checkpoint is a protein or small molecule modulator. In another embodiment, the agent (such as an mRNA) encodes an antibody modulator of an immune checkpoint. Non-limiting examples of immune checkpoint inhibitors that can be used in combination therapy include pembrolizumab, alemtuzumab, nivolumab, pidilizumab, ofatumumab, rituximab, MEDI0680 and PDR001, AMP-224, PF-06801591, BGB-A317, REGN2810, SHR-1210, TSR-042, affimer, avelumab (MSB0010718C), atezolizumab (MPDL3280A), durvalumab (MEDI4736), BMS936559, ipilimumab, tremelimumab, AGEN1884, MEDI6469 and MOXR0916.

A pharmaceutical composition including one or more mRNAs of the disclosure may be administered to a subject by any suitable route. In some embodiments, compositions of the disclosure are administered by one or more of a variety of routes, including parenteral (e.g., subcutaneous, intracutaneous, intravenous, intraperitoneal, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, or intracranial injection, as well as any suitable infusion technique), oral, trans- or intra-dermal, interdermal, rectal, intravaginal, topical (e.g., by powders, ointments, creams, gels, lotions, and/or drops), mucosal, nasal, buccal, enteral, vitreal, intratumoral, sublingual, intranasal; by intratracheal instillation, bronchial instillation, and/or inhalation; as an oral spray and/or powder, nasal spray, and/or aerosol, and/or through a portal vein catheter. In some embodiments, a composition may be administered intravenously, intramuscularly, intradermally, intra-arteri-

ally, intratumorally, subcutaneously, or by inhalation. In some embodiments, a composition is administered intramuscularly. However, the present disclosure encompasses the delivery of compositions of the disclosure by any appropriate route taking into consideration likely advances in the sciences of drug delivery. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the pharmaceutical composition including one or more mRNAs (e.g., its stability in various bodily environments such as the bloodstream and gastrointestinal tract), and the condition of the patient (e.g., whether the patient is able to tolerate particular routes of administration).

In certain embodiments, compositions of the disclosure may be administered at dosage levels sufficient to deliver from about 0.0001 mg/kg to about 10 mg/kg, from about 0.001 mg/kg to about 10 mg/kg, from about 0.005 mg/kg to about 10 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, from about 1 mg/kg to about 10 mg/kg, from about 2 mg/kg to about 10 mg/kg, from about 5 mg/kg to about 10 mg/kg, from about 0.0001 mg/kg to about 5 mg/kg, from about 0.001 mg/kg to about 5 mg/kg, from about 0.005 mg/kg to about 5 mg/kg, from about 0.01 mg/kg to about 5 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, from about 1 mg/kg to about 5 mg/kg, from about 2 mg/kg to about 5 mg/kg, from about 0.0001 mg/kg to about 1 mg/kg, from about 0.001 mg/kg to about 1 mg/kg, from about 0.005 mg/kg to about 1 mg/kg, from about 0.01 mg/kg to about 1 mg/kg, or from about 0.1 mg/kg to about 1 mg/kg in a given dose, where a dose of 1 mg/kg provides 1 mg of mRNA or nanoparticle per 1 kg of subject body weight. In particular embodiments, a dose of about 0.005 mg/kg to about 5 mg/kg of mRNA or nanoparticle of the disclosure may be administrated.

A dose may be administered one or more times per day, in the same or a different amount, to obtain a desired level of mRNA expression and/or effect (e.g., a therapeutic effect) . The desired dosage may be delivered, for example, three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage may be delivered using multiple administrations (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). In some embodiments, a single dose may be administered, for example, prior to or after a surgical procedure or in the instance of an acute disease, disorder, or condition. The specific therapeutically effective, prophylactically effective, or otherwise appropriate dose level for any particular patient will depend upon a variety of factors including the severity and identify of a disorder being treated, if any; the one or more mRNAs employed; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific pharmaceutical composition employed; the duration of the treatment; drugs used in combination or coincidental with the specific pharmaceutical composition employed; and like factors well known in the medical arts.

An mRNA or composition (e.g., a pharmaceutical composition) of the disclosure may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited, to intradermal, intramuscular, intranasal, and/or subcutaneous administration. The present disclosure provides methods comprising administering RNA compositions and lipid nanoparticles of the disclosure to a subject in need thereof. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. RNA compositions and lipid nanoparticles of the disclosure are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of RNA (e.g., mRNA) compositions may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

The effective amount of an RNA composition or lipid nanoparticle of the disclosure, as provided herein, may be as low as 10 μg, administered for example as a single dose or as two 5 μg doses. In some embodiments, the effective amount is a total dose of 10 μg-300 μg. For example, the effective amount may be a total dose of 10 μg, 20 μg, 25 μg, 30 μg, 35 μg, 40 μg, 45 μg, 50 μg, 55 μg, 60 μg, 65 μg, 70 μg, 75 μg, 80 μg, 85 μg, 90 μg, 95 μg, 100 μg, 110 μg, 120 μg, 130 μg, 140 μg, 150 μg, 160 μg, 170 μg, 180 μg, 190 pig or 200 μg, 210 μg, 220 μg, 230 μg, 240 μg, 250 μg, 260 μg, 270 μg, 280 μg, 290 μg or 300 μg. In some embodiments, the effective amount is a total dose of 10 μg-300 μg. In some embodiments, the effective amount is a total dose of 30 μg-100 μg or 50 μg-200 μg.

In some embodiments, RNA (e.g., mRNA) compositions and lipid nanoparticles may be administered at dosage levels sufficient to deliver 0.0001 mg/kg to 100 mg/kg, 0.001 mg/kg to 0.05 mg/kg, 0.005 mg/kg to 0.05 mg/kg, 0.001 mg/kg to 0.005 mg/kg, 0.05 mg/kg to 0.5 mg/kg, 0.01 mg/kg to 50 mg/kg, 0.1 mg/kg to 40 mg/kg, 0.5 mg/kg to 30 mg/kg, 0.01 mg/kg to 10 mg/kg, 0.1 mg/kg to 10 mg/kg, or 1 mg/kg to 25 mg/kg, of subject body weight per day, one or more times a day, per week, per month, etc. to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect (see e.g., the range of unit doses described in International Publication No. WO2013078199, herein incorporated by reference in its entirety). The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, every four weeks, every 2 months, every three months, every 6 months, etc. In certain embodiments, the desired dosage may be delivered using multiple administrations (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. In exemplary embodiments, RNA (e.g., mRNA) compositions may be administered at dosage levels sufficient to deliver 0.0005 mg/kg to 0.01 mg/kg, e.g., about 0.0005 mg/kg to about 0.0075 mg/kg, e.g., about 0.0005 mg/kg, about 0.001 mg/kg, about 0.002 mg/kg, about 0.003 mg/kg, about 0.004 mg/kg or about 0.005 mg/kg.

In some embodiments, RNA (e.g., mRNA) compositions may be administered once or twice (or more) at dosage levels sufficient to deliver 0.025 mg/kg to 0.250 mg/kg,

0.025 mg/kg to 0.500 mg/kg, 0.025 mg/kg to 0.750 mg/kg, or 0.025 mg/kg to 1.0 mg/kg.

In some embodiments, RNA (e.g., mRNA) compositions may be administered twice (e.g., Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5 years later, or Day 0 and 10 years later) at a total dose of or at dosage levels sufficient to deliver a total dose of 0.0100 mg, 0.025 mg, 0.050 mg, 0.075 mg, 0.100 mg, 0.125 mg, 0.150 mg, 0.175 mg, 0.200 mg, 0.225 mg, 0.250 mg, 0.275 mg, 0.300 mg, 0.325 mg, 0.350 mg, 0.375 mg, 0.400 mg, 0.425 mg, 0.450 mg, 0.475 mg, 0.500 mg, 0.525 mg, 0.550 mg, 0.575 mg, 0.600 mg, 0.625 mg, 0.650 mg, 0.675 mg, 0.700 mg, 0.725 mg, 0.750 mg, 0.775 mg, 0.800 mg, 0.825 mg, 0.850 mg, 0.875 mg, 0.900 mg, 0.925 mg, 0.950 mg, 0.975 mg, or 1.0 mg. Higher and lower dosages and frequency of administration are encompassed by the present disclosure. For example, a RNA (e.g., mRNA) composition may be administered three or four times.

In some embodiments, RNA (e.g., mRNA) compositions or lipid nanoparticles comprising the same may be administered twice (e.g., Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5 years later, or Day 0 and 10 years later) at a total dose of or at dosage levels sufficient to deliver a total dose of 0.010 mg, 0.025 mg, 0.100 mg or 0.400 mg.

In some embodiments, the RNA (e.g., mRNA)composition or lipid nanoparticles comprising the same for use in a method of vaccinating a subject is administered the subject a single dosage of between 10 μg/kg and 400 μg/kg of the nucleic acid vaccine in an effective amount to vaccinate the subject. In some embodiments, the RNA composition or lipid nanoparticles comprising the same for use in a method of vaccinating a subject is administered the subject a single dosage of between 10 μg and 400 μg of the nucleic acid vaccine in an effective amount to vaccinate the subject. In some embodiments, a RNA (e.g., mRNA) composition or lipid nanoparticles comprising the same for use in a method of vaccinating a subject is administered to the subject as a single dosage of 25-1000 μg (e.g., a single dosage of mRNA encoding an antigen). In some embodiments, a RNA composition is administered to the subject as a single dosage of 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 μg. For example, a RNA composition may be administered to a subject as a single dose of 25-100, 25-500, 50-100, 50-500, 50-1000, 100-500, 100-1000, 250-500, 250-1000, or 500-1000 μs. In some embodiments, a RNA (e.g., mRNA) composition or lipid nanoparticles comprising the same for use in a method of vaccinating a subject is administered to the subject as two dosages, the combination of which equals 25-1000 ng of the RNA (e.g., mRNA) composition.

An RNA (e.g., mRNA) composition or lipid nanoparticles comprising the same described herein can be formulated into a dosage form described herein, such as an intranasal, intratracheal, or injectable (e.g., intravenous, intraocular, intravitreal, intramuscular, intradermal, intracardiac, intraperitoneal, and subcutaneous).

In some embodiments, a pharmaceutical composition of the disclosure may be administered in combination with another agent, for example, another therapeutic agent, a prophylactic agent, and/or a diagnostic agent. By "in combination with," it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. For example, one or more compositions including one or more different mRNAs may be administered in combination. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of compositions of the disclosure, or imaging, diagnostic, or prophylactic compositions thereof in combination with agents that improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body.

Exemplary therapeutic agents that may be administered in combination with the compositions of the disclosure include, but are not limited to, cytotoxic, chemotherapeutic, and other therapeutic agents. Cytotoxic agents may include, for example, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxyanthracinedione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, rachelmycin, and analogs thereof. Radioactive ions may also be used as therapeutic agents and may include, for example, radioactive iodine, strontium, phosphorous, palladium, cesium, iridium, cobalt, yttrium, samarium, and praseodymium. Other therapeutic agents may include, for example, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, and 5-fluorouracil, and decarbazine), alkylating agents (e.g., mechlorethamine, thiotepa, chlorambucil, rachelmycin, melphalan, carmustine, lomustine, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP), and cisplatin), anthracyclines (e.g., daunorubicin and doxorubicin), antibiotics (e.g., dactinomycin, bleomycin, mithramycin, and anthramycin), and antimitotic agents (e.g., vincristine, vinblastine, taxol, and maytansinoids).

The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, a composition useful for treating cancer may be administered concurrently with a chemotherapeutic agent), or they may achieve different effects (e.g., control of any adverse effects).

Immune checkpoint inhibitors such as pembrolizumab or nivolumab, which target the interaction between programmed death receptor 1/programmed death ligand 1 (PD-1/PD-L1) and PD-L2, have been recently approved for the treatment of various malignancies and are currently being investigated in clinical trials for various cancers including melanoma, head and neck squamous cell carcinoma (HNSCC).

Accordingly, one aspect of the disclosure relates to combination therapy in which a subject is previously treated with a PD-1 antagonist prior to administration of a lipid nanoparticle or composition of the present disclosure. In another aspect, the subject has been treated with a monoclonal

antibody that binds to PD-1 prior to administration of a lipid nanoparticle or composition of the present disclosure. In another aspect, the subject has been administered a lipid nanoparticle or composition of the disclosure prior to treatment with an anti-PD-1 monoclonal antibody therapy. In some aspects, the anti-PD-1 monoclonal antibody therapy comprises nivolumab, pembrolizumab, pidilizumab, or any combination thereof. In some aspects, the anti-PD-1 monoclonal antibody comprises pembrolizumab.

In another aspect, the subject has been treated with a monoclonal antibody that binds to PD-L1 prior to administration of a lipid nanoparticle or composition of the present disclosure. In another aspect, the subject is administered a lipid nanoparticle or composition prior to treatment with an anti-PD-L1 monoclonal antibody therapy. In some aspects, the anti-PD-L1 monoclonal antibody therapy comprises durvalumab, avelumab, MEDI473, BMS-936559, aezolizumab, or any combination thereof.

In some aspects, the subject has been treated with a CTLA-4 antagonist prior to treatment with the compositions of present disclosure. In another aspect, the subject has been previously treated with a monoclonal antibody that binds to CTLA-4 prior to administration of a lipid nanoparticle or composition of the present disclosure. In some aspects, the subject has been administered a lipid nanoparticle or composition prior to treatment with an anti-CTLA-4 monoclonal antibody. In some aspects, the anti-CTLA-4 antibody therapy comprises ipilimumab or tremelimumab.

In any of the foregoing or related aspects, the disclosure provides a lipid nanoparticle, and an optional pharmaceutically acceptable carrier, or a pharmaceutical composition for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of the composition in combination with a second composition, wherein the second composition comprises a checkpoint inhibitor polypeptide and an optional pharmaceutically acceptable carrier.

In any of the foregoing or related aspects, the disclosure provides use of a lipid nanoparticle, and an optional pharmaceutically acceptable carrier, in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the lipid nanoparticle and an optional pharmaceutically acceptable carrier and wherein the treatment comprises administration of the medicament in combination with a composition comprising a checkpoint inhibitor polypeptide and an optional pharmaceutically acceptable carrier.

In any of the foregoing or related aspects, the disclosure provides a kit comprising a container comprising a lipid nanoparticle, and an optional pharmaceutically acceptable carrier, or a pharmaceutical composition, and a package insert comprising instructions for administration of the lipid nanoparticle or pharmaceutical composition for treating or delaying progression of cancer in an individual. In some aspects, the package insert further comprises instructions for administration of the lipid nanoparticle or pharmaceutical composition in combination with a composition comprising a checkpoint inhibitor polypeptide and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

In any of the foregoing or related aspects, the disclosure provides a kit comprising a medicament comprising a lipid nanoparticle, and an optional pharmaceutically acceptable carrier, or a pharmaceutical composition, and a package insert comprising instructions for administration of the medicament alone or in combination with a composition comprising a checkpoint inhibitor polypeptide and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual. In some aspects, the kit further comprises a package insert comprising instructions for administration of the first medicament prior to, current with, or subsequent to administration of the second medicament for treating or delaying progression of cancer in an individual.

In any of the foregoing or related aspects, the disclosure provides a lipid nanoparticle, a composition, or the use thereof, or a kit comprising a lipid nanoparticle or a composition as described herein, wherein the checkpoint inhibitor polypeptide inhibits PD1, PD-L1, CTLA4, or a combination thereof. In some aspects, the checkpoint inhibitor polypeptide is an antibody. In some aspects, the checkpoint inhibitor polypeptide is an antibody selected from an anti-CTLA4 antibody or antigen-binding fragment thereof that specifically binds CTLA4, an anti-PD1 antibody or antigen-binding fragment thereof that specifically binds PD1, an anti-PD-L1 antibody or antigen-binding fragment thereof that specifically binds PD-L1, and a combination thereof. In some aspects, the checkpoint inhibitor polypeptide is an anti-PD-L1 antibody selected from atezolizumab, avelumab, or durvalumab. In some aspects, the checkpoint inhibitor polypeptide is an anti-CTLA-4 antibody selected from tremelimumab or ipilimumab. In some aspects, the checkpoint inhibitor polypeptide is an anti-PD1 antibody selected from nivolumab or pembrolizumab. In some aspects, the checkpoint inhibitor polypeptide is an anti-PD1 antibody, wherein the anti-PD1 antibody is pembrolizumab.

In related aspects, the disclosure provides a method of reducing or decreasing a size of a tumor or inhibiting a tumor growth in a subject in need thereof comprising administering to the subject any of the foregoing or related lipid nanoparticles of the disclosure, or any of the foregoing or related compositions of the disclosure.

In related aspects, the disclosure provides a method inducing an anti-tumor response in a subject with cancer comprising administering to the subject any of the foregoing or related lipid nanoparticles of the disclosure, or any of the foregoing or related compositions of the disclosure. In some aspects, the anti-tumor response comprises a T-cell response. In some aspects, the T-cell response comprises CD8+ T cells.

In some aspects of the foregoing methods, the method further comprises administering a second composition comprising a checkpoint inhibitor polypeptide, and an optional pharmaceutically acceptable carrier. In some aspects, the checkpoint inhibitor polypeptide inhibits PD1, PD-L1, CTLA4, or a combination thereof. In some aspects, the checkpoint inhibitor polypeptide is an antibody. In some aspects, the checkpoint inhibitor polypeptide is an antibody selected from an anti-CTLA4 antibody or antigen-binding fragment thereof that specifically binds CTLA4, an anti-PD1 antibody or antigen-binding fragment thereof that specifically binds PD1, an anti-PD-L1 antibody or antigen-binding fragment thereof that specifically binds PD-L1, and a combination thereof. In some aspects, the checkpoint inhibitor polypeptide is an anti-PD-L1 antibody selected from atezolizumab, avelumab, or durvalumab. In some aspects, the checkpoint inhibitor polypeptide is an anti-CTLA-4 antibody selected from tremelimumab or ipilimumab. In some aspects, the checkpoint inhibitor polypeptide is an anti-PD1 antibody selected from nivolumab or pembrolizumab. In some aspects, the checkpoint inhibitor polypeptide is an anti-PD1 antibody, wherein the anti-PD1 antibody is pembrolizumab.

In some aspects of any of the foregoing or related methods, the composition comprising the checkpoint inhibitor polypeptide is administered by intravenous injection. In some aspects, the composition comprising the checkpoint inhibitor polypeptide is administered once every 2 to 3 weeks. In some aspects, the composition comprising the checkpoint inhibitor polypeptide is administered once every 2 weeks or once every 3 weeks. In some aspects, the composition comprising the checkpoint inhibitor polypeptide is administered prior to, concurrent with, or subsequent to administration of the lipid nanoparticle or pharmaceutical composition thereof.

In any of the foregoing or related aspects, the disclosure provides pharmaceutical composition comprising the lipid nanoparticle, and a pharmaceutically acceptable carrier. In some aspects, the pharmaceutical composition is formulated for intramuscular delivery.

DETAILED DESCRIPTION

Definitions

As used herein, the terms "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).

Base Composition: As used herein, the term "base composition" refers to the proportion of the total bases of a nucleic acid consisting of guanine+cytosine or thymine (or uracil)+adenine nucleotides.

Base Pair: As used herein, the term "base pair" refers to two nucleobases on opposite complementary nucleic acid strands that interact via the formation of specific hydrogen bonds. As used herein, the term "Watson-Crick base pairing", used interchangeably with "complementary base pairing", refers to a set of base pairing rules, wherein a purine always binds with a pyrimidine such that the nucleobase adenine (A) forms a complementary base pair with thymine (T) and guanine (G) forms a complementary base pair with cytosine (C) in DNA molecules. In RNA molecules, thymine is replaced by uracil (U), which, similar to thymine (T), forms a complementary base pair with adenine (A). The complementary base pairs are bound together by hydrogen bonds and the number of hydrogen bonds differs between base pairs. As in known in the art, guanine (G)-cytosine (C) base pairs are bound by three (3) hydrogen bonds and adenine (A)-thymine (T) or uracil (U) base pairs are bound by two (2) hydrogen bonds. Base pairing interactions that do not follow these rules can occur in natural, non-natural, and synthetic nucleic acids and are referred to herein as "non-Watson-Crick base pairing" or alternatively "non-complementary base pairing".

Codon: As used herein, the term "codon" refers to a sequence of three nucleotides that together form a unit of genetic code in a DNA or RNA molecule. A codon is operationally defined by the initial nucleotide from which translation starts and sets the frame for a run of successive nucleotide triplets, which is known as an "open reading frame" (ORF). For example, the string GGGAAACCC, if read from the first position, contains the codons GGG, AAA, and CCC; if read from the second position, it contains the codons GGA and AAC; and if read from the third position, GAA and ACC. Thus, every nucleic sequence read in its 5'→3' direction comprises three reading frames, each producing a possibly distinct amino acid sequence (in the given example, Gly-Lys-Pro, Gly-Asn, or Glu-Thr, respectively). DNA is double-stranded defining six possible reading frames, three in the forward orientation on one strand and three reverse on the opposite strand. Open reading frames encoding polypeptides are typically defined by a start codon, usually the first AUG codon in the sequence.

Conjugated: As used herein, the term "conjugated," when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, e.g., physiological conditions. In some embodiments, two or more moieties may be conjugated by direct covalent chemical bonding. In other embodiments, two or more moieties may be conjugated by ionic bonding or hydrogen bonding.

Contacting: As used herein, the term "contacting" means establishing a physical connection between two or more entities. For example, contacting a cell with an mRNA or a lipid nanoparticle composition means that the cell and mRNA or lipid nanoparticle are made to share a physical connection. Methods of contacting cells with external entities both in vivo, in vitro, and ex vivo are well known in the biological arts. In exemplary embodiments of the disclosure, the step of contacting a mammalian cell with a composition (e.g., an isolated mRNA, nanoparticle, or pharmaceutical composition of the disclosure) is performed in vivo. For example, contacting a lipid nanoparticle composition and a cell (for example, a mammalian cell) which may be disposed within an organism (e.g., a mammal) may be performed by any suitable administration route (e.g., parenteral administration to the organism, including intravenous, intramuscular, intradermal, and subcutaneous administration). For a cell present in vitro, a composition (e.g., a lipid nanoparticle or an isolated mRNA) and a cell may be contacted, for example, by adding the composition to the culture medium of the cell and may involve or result in transfection. Moreover, more than one cell may be contacted by a nanoparticle composition.

Denaturation: As used herein, the term "denaturation" refers to the process by which the hydrogen bonding between base paired nucleotides in a nucleic acid is disrupted, resulting in the loss of secondary and/or tertiary nucleic acid structure (e.g. the separation of previously annealed strands). Denaturation can occur by the application of an external substance, energy, or biochemical process to a nucleic acid. For example, local denaturation of nucleic acid structure by enzymatic activity occurs when biologically important transactions such as DNA replication, transcription, translation, or DNA repair need to occur. Folded structures (e.g. secondary and tertiary nucleic acid structures) of an mRNA can constitute a barrier to the scanning function of the PIC or the elongation function of the ribosome, resulting in a lower translation rate. During translation initiation, helicase activity provided by eIFs (e.g. eIF4A) can denature or unwind duplexed, double-stranded RNA structure to facilitate PIC scanning.

Epitope Tag: As used herein, the term "epitope tag" refers to an artificial epitope, also known as an antigenic determinant, which is fused to a polypeptide sequence by placing the sequence encoding the epitope in-frame with the coding

sequence or open reading frame of a polypeptide. An epitope-tagged polypeptides is considered a fusion protein. Epitope tags are relatively short peptide sequences ranging from about 10-30 amino acids in length. Epitope tags are usually fused to either the N- or C-terminus in order to minimize tertiary structure disruptions that may alter protein function. Epitope tags are reactive to high-affinity antibodies that can be reliably produced in many different species. Exemplary epitope tags include the V5-tag, Myc-tag, HA-tag and 3×FLAG-tag. These tags are useful for detection or purification of fusion proteins by Western blotting, immunofluorescence, or immunoprecipitation techniques.

Expression: As used herein, "expression" of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

Identity: As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, e.g., between polynucleotide molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using methods such as those described in *Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988*; Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993*; Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987*; Computer Analysis of Sequence Data*, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; each of which is incorporated herein by reference. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4:11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., *SIAM J Applied Math.,* 48:1073 (1988); incorporated herein by reference. Techniques for determining identity are codified in publicly available computer programs. Exemplary computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux et al., *Nucleic Acids Research,* 12(1): 387,1984, BLASTP, BLASTN, and FASTA, Altschul, S. F. et al., *J. Molec. Biol.,* 215, 403, 1990.

Fragment: A "fragment," as used herein, refers to a portion. For example, fragments of proteins may include polypeptides obtained by digesting full-length protein isolated from cultured cells or obtained through recombinant DNA techniques.

Fusion Protein: The term "fusion protein" means a polypeptide sequence that is comprised of two or more polypeptide sequences linked by a peptide bond(s). "Fusion proteins" that do not occur in nature can be generated using recombinant DNA techniques.

GC-rich: As used herein, the term "GC-rich" refers to the nucleobase composition of a polynucleotide (e.g., mRNA), or any portion thereof (e.g., an RNA element), comprising guanine (G) and/or cytosine (C) nucleobases, or derivatives or analogs thereof, wherein the GC-content is greater than 50%. The term "GC-rich" refers to all, or to a portion, of a polynucleotide, including, but not limited to, a gene, a non-coding region, a 5' UTR, a 3' UTR, an open reading frame, an RNA element, a sequence motif, or any discrete sequence, fragment, or segment thereof which comprises greater than 50% GC-content. In some embodiments of the disclosure, GC-rich polynucleotides, or any portions thereof, are exclusively comprised of guanine (G) and/or cytosine (C) nucleobases.

GC-content: As used herein, the term "GC-content" refers to the percentage of nucleobases in a polynucleotide (e.g., mRNA), or a portion thereof (e.g., an RNA element), that are either guanine (G) and cytosine (C) nucleobases, or derivatives or analogs thereof, (from a total number of possible nucleobases, including adenine (A) and thymine (T) or uracil (U), and derivatives or analogs thereof, in DNA and in RNA). The term "GC-content" refers to all, or to a portion, of a polynucleotide, including, but not limited to, a gene, a non-coding region, a 5' or 3' UTR, an open reading frame, an RNA element, a sequence motif, or any discrete sequence, fragment, or segment thereof.

Genetic code: As used herein, the term "genetic code" refers to the set of rules by which genetic information encoded within genetic material (DNA or RNA sequences) is translated by the ribosome into polypeptides. The code defines how sequences of nucleotide triplets, referred to as "codons", specify which amino acid will be added next during protein synthesis. A three-nucleotide codon in a nucleic acid sequence specifies a single amino acid. The vast majority of genes are encoded with a single scheme of rules referred to as the canonical or standard genetic code, or simply the genetic code, though variant codes (such as in human mitochondria) exist.

Heterologous: As used herein, "heterologous" indicates that a sequence (e.g., an amino acid sequence or the polynucleotide that encodes an amino acid sequence) is not normally present in a given natural polypeptide or polynucleotide. For example, an amino acid sequence that corresponds to a domain or motif of one protein may be heterologous to a second protein.

Hybridization: As used herein, the term "hybridization" refers to the process of a first single-stranded nucleic acid,

or a portion, fragment, or region thereof, annealing to a second single-stranded nucleic acid, or a portion, fragment, or region thereof, either from the same or separate nucleic acid molecules, mediated by Watson-Crick base pairing to form a secondary and/or tertiary structure. Complementary strands of linked nucleobases able to undergo hybridization can be from either the same or separate nucleic acids. Due to the thermodynamically favorable hydrogen bonding interaction between complementary base pairs, hybridization is a fundamental property of complementary nucleic acid sequences. Such hybridization of nucleic acids, or a portion or fragment thereof, may occur with "near" or "substantial" complementarity, as well as with exact complementarity.

Initiation Codon: As used herein, the term "initiation codon", used interchangeably with the term "start codon", refers to the first codon of an open reading frame that is translated by the ribosome and is comprised of a triplet of linked adenine-uracil-guanine nucleobases. The initiation codon is depicted by the first letter codes of adenine (A), uracil (U), and guanine (G) and is often written simply as "AUG". Although natural mRNAs may use codons other than AUG as the initiation codon, which are referred to herein as "alternative initiation codons", the initiation codons of polynucleotides described herein use the AUG codon. During the process of translation initiation, the sequence comprising the initiation codon is recognized via complementary base-pairing to the anticodon of an initiator tRNA (Met-tRNA$_i^{Met}$) bound by the ribosome. Open reading frames may contain more than one AUG initiation codon, which are referred to herein as "alternate initiation codons".

The initiation codon plays a critical role in translation initiation. The initiation codon is the first codon of an open reading frame that is translated by the ribosome. Typically, the initiation codon comprises the nucleotide triplet AUG, however, in some instances translation initiation can occur at other codons comprised of distinct nucleotides. The initiation of translation in eukaryotes is a multistep biochemical process that involves numerous protein-protein, protein-RNA, and RNA-RNA interactions between messenger RNA molecules (mRNAs), the 40S ribosomal subunit, other components of the translation machinery (e.g., eukaryotic initiation factors; elFs). The current model of mRNA translation initiation postulates that the pre-initiation complex (alternatively "43S pre-initiation complex"; abbreviated as "PIC") translocates from the site of recruitment on the mRNA (typically the 5' cap) to the initiation codon by scanning nucleotides in a 5' to 3' direction until the first AUG codon that resides within a specific translation-promotive nucleotide context (the Kozak sequence) is encountered (Kozak (1989) J Cell Biol 108:229-241).

Scanning by the PIC ends upon complementary base-pairing between nucleotides comprising the anticodon of the initiator Met-tRNAi^{Met} transfer RNA and nucleotides comprising the initiation codon of the mRNA. Productive base-pairing between the AUG codon and the Met-tRNA$_i^{Met}$ anticodon elicits a series of structural and biochemical events that culminate in the joining of the large 60S ribosomal subunit to the PIC to form an active ribosome that is competent for translation elongation.

Insertion: As used herein, an "insertion" or an "addition" refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to a molecule as compared to a reference sequence, for example, the sequence found in a naturally-occurring molecule.

Insertion Site: As used herein, an "insertion site" is a position or region of a scaffold polypeptide that is amenable to insertion of an amino acid sequence of a heterologous polypeptide. It is to be understood that an insertion site also may refer to the position or region of the polynucleotide that encodes the polypeptide (e.g., a codon of a polynucleotide that codes for a given amino acid in the scaffold polypeptide). In some embodiments, insertion of an amino acid sequence of a heterologous polypeptide into a scaffold polypeptide has little to no effect on the stability (e.g., conformational stability), expression level, or overall secondary structure of the scaffold polypeptide.

Isolated: As used herein, the term "isolated" refers to a substance or entity that has been separated from at least some of the components with which it was associated (whether in nature or in an experimental setting). Isolated substances may have varying levels of purity in reference to the substances from which they have been associated. Isolated substances and/or entities may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated agents are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components.

Kozak Sequence: The term "Kozak sequence" (also referred to as "Kozak consensus sequence") refers to a translation initiation enhancer element to enhance expression of a gene or open reading frame, and which in eukaryotes, is located in the 5' UTR. The Kozak consensus sequence was originally defined as the sequence GCCRCC, where R=a purine, following an analysis of the effects of single mutations surrounding the initiation codon (AUG) on translation of the preproinsulin gene (Kozak (1986) Cell 44:283-292). Polynucleotides disclosed herein comprise a Kozak consensus sequence, or a derivative or modification thereof. (Examples of translational enhancer compositions and methods of use thereof, see U.S. Pat. No. 5,807,707 to Andrews et al., incorporated herein by reference in its entirety; U.S. Pat. No. 5,723,332 to Chernajovsky, incorporated herein by reference in its entirety; U.S. Pat. No. 5,891,665 to Wilson, incorporated herein by reference in its entirety.)

Leaky scanning: As used herein, the term "leaky scanning" refers to a biological phenomenon whereby the PIC bypasses the initiation codon of an mRNA and instead continues scanning downstream until an alternate or alternative initiation codon is recognized. Depending on the frequency of occurrence, the bypass of the initiation codon by the PIC can result in a decrease in translation efficiency. Furthermore, translation from this downstream AUG codon can occur, which will result in the production of an undesired, aberrant translation product that may not be capable of eliciting the desired therapeutic response. In some cases, the aberrant translation product may in fact cause a deleterious response (Kracht et al., (2017) Nat Med 23(4):501-507).

mRNA: As used herein, an "mRNA" refers to a messenger ribonucleic acid. An mRNA may be naturally or non-naturally occurring or synthetic. For example, an mRNA may include modified and/or non-naturally occurring components such as one or more nucleobases, nucleosides, nucleotides, or linkers. An mRNA may include a cap structure, a 5' transcript leader, a 5' untranslated region, an initiator codon, an open reading frame, a stop codon, a chain

terminating nucleoside, a stem-loop, a hairpin, a polyA sequence, a polyadenylation signal, and/or one or more cis-regulatory elements. An mRNA may have a nucleotide sequence encoding a polypeptide. Translation of an mRNA, for example, in vivo translation of an mRNA inside a mammalian cell, may produce a polypeptide. Traditionally, the basic components of a natural mRNA molecule include at least a coding region, a 5'-untranslated region (5'-UTR), a 3'UTR, a 5' cap and a polyA sequence.

Modified: As used herein "modified" or "modification" refers to a changed state or a change in composition or structure of a polynucleotide (e.g., mRNA). Polynucleotides may be modified in various ways including chemically, structurally, and/or functionally. For example, polynucleotides may be structurally modified by the incorporation of one or more RNA elements, wherein the RNA element comprises a sequence and/or an RNA secondary structure(s) that provides one or more functions (e.g., translational regulatory activity). Accordingly, polynucleotides of the disclosure may be comprised of one or more modifications (e.g., may include one or more chemical, structural, or functional modifications, including any combination thereof).

Nucleobase: As used herein, the term "nucleobase" (alternatively "nucleotide base" or "nitrogenous base") refers to a purine or pyrimidine heterocyclic compound found in nucleic acids, including any derivatives or analogs of the naturally occurring purines and pyrimidines that confer improved properties (e.g., binding affinity, nuclease resistance, chemical stability) to a nucleic acid or a portion or segment thereof. Adenine, cytosine, guanine, thymine, and uracil are the nucleobases predominately found in natural nucleic acids. Other natural, non-natural, and/or synthetic nucleobases, as known in the art and/or described herein, can be incorporated into nucleic acids.

Nucleoside/Nucleotide: As used herein, the term "nucleoside" refers to a compound containing a sugar molecule (e.g., a ribose in RNA or a deoxyribose in DNA), or derivative or analog thereof, covalently linked to a nucleobase (e.g., a purine or pyrimidine), or a derivative or analog thereof (also referred to herein as "nucleobase"), but lacking an internucleoside linking group (e.g., a phosphate group). As used herein, the term "nucleotide" refers to a nucleoside covalently bonded to an internucleoside linking group (e.g., a phosphate group), or any derivative, analog, or modification thereof that confers improved chemical and/or functional properties (e.g., binding affinity, nuclease resistance, chemical stability) to a nucleic acid or a portion or segment thereof.

Nucleic acid: As used herein, the term "nucleic acid" is used in its broadest sense and encompasses any compound and/or substance that includes a polymer of nucleotides, or derivatives or analogs thereof. These polymers are often referred to as "polynucleotides". Accordingly, as used herein the terms "nucleic acid" and "polynucleotide" are equivalent and are used interchangeably. Exemplary nucleic acids or polynucleotides of the disclosure include, but are not limited to, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), DNA-RNA hybrids, RNAi-inducing agents, RNAi agents, siRNAs, shRNAs, mRNAs, modified mRNAs, miRNAs, antisense RNAs, ribozymes, catalytic DNA, RNAs that induce triple helix formation, threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β-D-ribo configuration, α-LNA having an α-L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino-α-LNA having a T-amino functionalization) or hybrids thereof.

Nucleic Acid Structure: As used herein, the term "nucleic acid structure" (used interchangeably with "polynucleotide structure") refers to the arrangement or organization of atoms, chemical constituents, elements, motifs, and/or sequence of linked nucleotides, or derivatives or analogs thereof, that comprise a nucleic acid (e.g., an mRNA). The term also refers to the two-dimensional or three-dimensional state of a nucleic acid. Accordingly, the term "RNA structure" refers to the arrangement or organization of atoms, chemical constituents, elements, motifs, and/or sequence of linked nucleotides, or derivatives or analogs thereof, comprising an RNA molecule (e.g., an mRNA) and/or refers to a two-dimensional and/or three dimensional state of an RNA molecule. Nucleic acid structure can be further demarcated into four organizational categories referred to herein as "molecular structure", "primary structure", "secondary structure", and "tertiary structure" based on increasing organizational complexity.

Open Reading Frame: As used herein, the term "open reading frame", abbreviated as "ORF", refers to a segment or region of an mRNA molecule that encodes a polypeptide. The ORF comprises a continuous stretch of non-overlapping, in-frame codons, beginning with the initiation codon and ending with a stop codon, and is translated by the ribosome.

Pre-Initiation Complex: As used herein, the term "pre-initiation complex" (alternatively "43S pre-initiation complex"; abbreviated as "PIC") refers to a ribonucleoprotein complex comprising a 40S ribosomal subunit, eukaryotic initiation factors (eIF1, eIF1A, eIF3, eIF5), and the eIF2-GTP-Met-tRNA$_i^{Met}$ ternary complex, that is intrinsically capable of attachment to the 5' cap of an mRNA molecule and, after attachment, of performing ribosome scanning of the 5' UTR.

Polypeptide: As used herein, the term "polypeptide" or "polypeptide of interest" refers to a polymer of amino acid residues typically joined by peptide bonds that can be produced naturally (e.g., isolated or purified) or synthetically.

Potency: As used herein, the term "potency" refers to an amount, level or concentration of a substance (e.g., an mRNA) that is required to produce a given response or effect. The potency of a substance may be defined by its EC_{50} value if the substance produces an agonistic response or effect or its IC_{50} value if the substance produces an antagonistic response or effect. As used herein, the term "EC_{50}" refers to the concentration of a substance (e.g., an mRNA) which induces a response or effect, either in an in vitro or an in vivo assay, which is 50% of the maximal response or effect, i.e., halfway between the maximal response or effect and the baseline. As used herein, the term "IC_{50}" refers to the concentration of a substance (e.g., an mRNA) which inhibits a response or effect, either in an in vitro or an in vivo assay, which is 50% of the maximal response or effect, i.e., halfway between the maximal response or effect and the baseline.

Increase in Potency: As used herein, the term "increase in potency" (e.g., of a substance, for example, an mRNA) refers to a potency which is improved (increased, or enhanced) relative to the potency of a similar or comparable substance for which the potency has not been improved. Increased potency is typically observed as a decrease in the amount, level or concentration of a substance (e.g., an mRNA) required to produce a given response or effect. In some embodiments, an increase in potency can be observed

as an improved (increased or enhanced) response or effect, resulting from a given amount, level or concentration of a substance (e.g., an mRNA).

In some embodiments, an increase in potency of an mRNA results from an RNA element (e.g., a G C-rich RNA element located in the 5' UTR of the mRNA) that provides a desired translational regulatory activity. In some embodiments, an increase in potency results from an RNA element (e.g., a G C-rich RNA element located in the 5' UTR of the mRNA) which increases an amount of polypeptide translated from an mRNA. In some embodiments, an increase in the potency of an mRNA results from an RNA element (e.g., a G C-rich RNA element located in the 5' UTR of the mRNA) which increases the number of polypeptide molecules translated per mRNA molecule. In some embodiments, an increase in the potency of an mRNA results from an RNA element (e.g., a G C-rich RNA element located in the 5' UTR of the mRNA) which increases the number of polypeptide molecules translated per mRNA molecule per unit time. In some embodiments, an increase in potency of an mRNA results from an RNA element (e.g., a G C-rich RNA element located in the 5' UTR of the mRNA) which increases an amount of functional polypeptide translated from an mRNA relative to the total amount of polypeptide translated from an mRNA. In some embodiments, an increase in potency of an mRNA results from an RNA element (e.g., a G C-rich RNA element located in the 5' UTR of the mRNA) due to an increase in mRNA translation fidelity by (i) an inhibition or reduction in leaky scanning (ii) an increase in codon decoding fidelity, or (iii) minimizing or inhibiting stop codon read through, or any combination of (i), (ii) and (iii). In some embodiments, an increase in potency of an mRNA results from an RNA element (e.g., a G C-rich RNA element located in the 5' UTR of the mRNA) due to an increase in an amount of functional polypeptide at a particular site or location (e.g., by targeting the polypeptide to a specific site or location in a cell or in the extracellular environment). In some embodiments, an increase in potency of an mRNA results from an RNA element (e.g., a G C-rich RNA element located in the 5' UTR of the mRNA) which increases an amount of polypeptide translated from an mRNA by increasing the half-life of the mRNA.

In some embodiments, the disclosure provides an mRNA comprising a 5' UTR comprising an RNA element that increases the potency of the mRNA. In some embodiments, the RNA element is any one of the GC-rich RNA elements described herein. In some embodiments, the RNA element is any one of the stable RNA secondary structures described herein. In some embodiments, the disclosure provides an mRNA comprising a modification that increases potency of the mRNA. In some embodiments, potency of the mRNA is increased 1-fold, 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 10-fold relative to an mRNA without the modification (e.g., without the RNA element). In some embodiments, the potency of the mRNA molecule is increased by 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%. In some embodiments, the potency of the mRNA molecule is increased by about 5%-10%, by about 10%-20%, by about 20%-40%, by about 40%-60%, by about 60%-80%, by about 90% relative to an mRNA without the modification (e.g., without the RNA element).

RNA element: As used herein, the term "RNA element" refers to a portion, fragment, or segment of an RNA molecule that provides a biological function and/or has biological activity (e.g., translational regulatory activity). Modification of a polynucleotide by the incorporation of one or more RNA elements, such as those described herein, provides one or more desirable functional properties to the modified polynucleotide. RNA elements, as described herein, can be naturally-occurring, non-naturally occurring, synthetic, engineered, or any combination thereof. For example, naturally-occurring RNA elements that provide a regulatory activity include elements found throughout the transcriptomes of viruses, prokaryotic and eukaryotic organisms (e.g., humans). RNA elements in particular eukaryotic mRNAs and translated viral RNAs have been shown to be involved in mediating many functions in cells. Exemplary natural RNA elements include, but are not limited to, translation initiation elements (e.g., internal ribosome entry site (IRES), see Kieft et al., (2001) RNA 7(2):194-206), translation enhancer elements (e.g., the APP mRNA translation enhancer element, see Rogers et al., (1999) J Biol Chem 274(10):6421-6431), mRNA stability elements (e.g., AU-rich elements (AREs), see Garneau et al., (2007) Nat Rev Mol Cell Biol 8(2):113-126), translational repression element (see e.g., Blumer et al., (2002) Mech Dev 110(1-2):97-112), protein-binding RNA elements (e.g., iron-responsive element, see Selezneva et al., (2013) J Mol Biol 425(18):3301-3310), cytoplasmic polyadenylation elements (Villalba et al., (2011) Curr Opin Genet Dev 21(4):452-457), and catalytic RNA elements (e.g., ribozymes, see Scott et al., (2009) Biochim Biophys Acta 1789(9-10):634-641).

Residence time: As used herein, the term "residence time" refers to the time of occupancy of a pre-initiation complex (PIC) or a ribosome at a discrete position or location along an mRNA molecule.

Stable RNA Secondary Structure: As used herein, the term "stable RNA secondary structure" refers to a structure, fold, or conformation adopted by an RNA molecule, or local segment or portion thereof, that is persistently maintained under physiological conditions and characterized by a low free energy state. Typical examples of stable RNA secondary structures include duplexes, hairpins, and stem-loops. Stable RNA secondary structures are known in the art to exhibit various biological activities.

Subject: As used herein, the term "subject" refers to any organism to which a composition in accordance with the disclosure may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants. In some embodiments, a subject may be a patient.

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.

Suffering from: An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of a disease, disorder, and/or condition.

Transcription start site: As used herein, the term "transcription start site" refers to a specific nucleotide in the sense strand of a DNA molecule where transcription by an RNA polymerase initiates and that corresponds to the first nucleotide in the transcript. The transcription start site is typically located downstream of a promoter, which is a region of DNA that initiations transcription. For example, the T7 RNA polymerase initiates transcription at the underlined G in the

promoter sequence 5' TAATACGACTCACTATAG 3'. The polymerase then transcribes using the opposite DNA strand as a template. In some embodiments, the transcription start site for a T7 RNA polymerase is referred to as a "T7 start site". The first base in the transcript will be a G. The DNA contacts made by T7 RNA polymerase have been mapped during binding and during the subsequent initiation of transcription. The RNA polymerase alone protects 19 bases in a region from −21 to −3. Synthesis of the trinucleotide r(GGG) expands the length of the sequence protected by the RNA polymerase and stabilizes the complex. The formation of a hexanucleotide mRNA, r(GGGAGA) further extends the protected region, stabilizes the complex, and results in increased transcriptional efficiency (Ikeda and Richardson (1986) Proc Natl Acad Sci 83:3614-3618). The sequence GGGAGA is referred to as a "T7 leader sequence". Accordingly, in some embodiments, the mRNAs provided by the disclosure comprise a 5' UTR comprising a T7 leader sequence at the 5' end of the 5' UTR. In some embodiments, the mRNA of the disclosure comprises a 5' UTR comprising a T7 leader sequence comprising the sequence GGGAGA at the 5' end of the 5' UTR. In some embodiments, the mRNA of the disclosure comprises a 5' UTR comprising a T7 leader sequence comprising the sequence GGGAAA at the 5' end of the 5' UTR. In some embodiments, the mRNA comprises a 5' UTR which does not comprise a T7 leader sequence.

Targeting moiety: As used herein, a "targeting moiety" is a compound or agent that may target a nanoparticle to a particular cell, tissue, and/or organ type.

Therapeutic Agent: The term "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

Translational Regulatory Activity: As used herein, the term "translational regulatory activity" (used interchangeably with "translational regulatory function") refers to a biological function, mechanism, or process that modulates (e.g., regulates, influences, controls, varies) the activity of the translational apparatus, including the activity of the PIC and/or ribosome. In some aspects, the desired translation regulatory activity promotes and/or enhances the translational fidelity of mRNA translation. In some aspects, the desired translational regulatory activity reduces and/or inhibits leaky scanning.

Translation of a polynucleotide comprising an open reading frame encoding a polypeptide can be controlled and regulated by a variety of mechanisms that are provided by various cis-acting nucleic acid structures. For example, naturally-occurring, cis-acting RNA elements that form hairpins or other higher-order (e.g., pseudoknot) intramolecular mRNA secondary structures can provide a translational regulatory activity to a polynucleotide, wherein the RNA element influences or modulates the initiation of polynucleotide translation, particularly when the RNA element is positioned in the 5' UTR close to the 5'-cap structure (Pelletier and Sonenberg (1985) Cell 40(3):515-526; Kozak (1986) Proc Natl Acad Sci 83:2850-2854). Cis-acting RNA elements can also affect translation elongation, being involved in numerous frameshifting events (Namy et al., (2004) Mol Cell 13(2):157-168). Internal ribosome entry sequences (IRES) represent another type of cis-acting RNA element that are typically located in 5' UTRs, but have also been reported to be found within the coding region of naturally-occurring mRNAs (Holcik et al. (2000) Trends Genet 16(10):469-473). In cellular mRNAs, IBES often coexist with the 5'-cap structure and provide mRNAs with the functional capacity to be translated under conditions in which cap-dependent translation is compromised (Gebauer et al., (2012) Cold Spring Harb Perspect Biol 4(7):a012245). Another type of naturally-occurring cis-acting RNA element comprises upstream open reading frames (uORFs). Naturally-occurring uORFs occur singularly or multiply within the 5' UTRs of numerous mRNAs and influence the translation of the downstream major ORF, usually negatively (with the notable exception of GCN4 mRNA in yeast and ATF4 mRNA in mammals, where uORFs serve to promote the translation of the downstream major ORF under conditions of increased eIF2 phosphorylation (Hinnebusch (2005) Annu Rev Microbiol 59:407-450)). Additional exemplary translational regulatory activities provided by components, structures, elements, motifs, and/or specific sequences comprising polynucleotides (e.g., mRNA) include, but are not limited to, mRNA stabilization or destabilization (Baker & Parker (2004) Curr Opin Cell Biol 16(3):293-299), translational activation (Villalba et al., (2011) Curr Opin Genet Dev 21(4):452-457), and translational repression (Blumer et al., (2002) Mech Dev 110(1-2):97-112). Studies have shown that naturally-occurring, cis-acting RNA elements can confer their respective functions when used to modify, by incorporation into, heterologous polynucleotides (Goldberg-Cohen et al., (2002) J Biol Chem 277(16):13635-13640).

Transfect: As used herein, the terms "transfect", "transfection" or "transfecting" refer to the act or method of introducing a molecule, usually a nucleic acid, into a cell.

Unmodified: As used herein, "unmodified" refers to any substance, compound or molecule prior to being changed in any way. Unmodified may, but does not always, refer to the wild type or native form of a biomolecule. Molecules may undergo a series of modifications whereby each modified molecule may serve as the "unmodified" starting molecule for a subsequent modification.

Uridine Content: The terms "uridine content" or "uracil content" are interchangeable and refer to the amount of uracil or uridine present in a certain nucleic acid sequence. Uridine content or uracil content can be expressed as an absolute value (total number of uridine or uracil in the sequence) or relative (uridine or uracil percentage respect to the total number of nucleobases in the nucleic acid sequence).

Uridine-Modified Sequence: The terms "uridine-modified sequence" refers to a sequence optimized nucleic acid (e.g., a synthetic mRNA sequence) with a different overall or local uridine content (higher or lower uridine content) or with different uridine patterns (e.g., gradient distribution or clustering) with respect to the uridine content and/or uridine patterns of a candidate nucleic acid sequence. In the content of the present disclosure, the terms "uridine-modified sequence" and "uracil-modified sequence" are considered equivalent and interchangeable.

A "high uridine codon" is defined as a codon comprising two or three uridines, a "low uridine codon" is defined as a codon comprising one uridine, and a "no uridine codon" is a codon without any uridines. In some embodiments, a uridine-modified sequence comprises substitutions of high uridine codons with low uridine codons, substitutions of high uridine codons with no uridine codons, substitutions of low uridine codons with high uridine codons, substitutions of low uridine codons with no uridine codons, substitution of no uridine codons with low uridine codons, substitutions of no uridine codons with high uridine codons, and combinations thereof. In some embodiments, a high uridine codon can be replaced with another high uridine codon. In some embodiments, a low uridine codon can be replaced with another low uridine codon. In some embodiments, a no

uridine codon can be replaced with another no uridine codon. A uridine-modified sequence can be uridine enriched or uridine rarefied.

Uridine Enriched: As used herein, the terms "uridine enriched" and grammatical variants refer to the increase in uridine content (expressed in absolute value or as a percentage value) in a sequence optimized nucleic acid (e.g., a synthetic mRNA sequence) with respect to the uridine content of the corresponding candidate nucleic acid sequence. Uridine enrichment can be implemented by substituting codons in the candidate nucleic acid sequence with synonymous codons containing less uridine nucleobases. Uridine enrichment can be global (i.e., relative to the entire length of a candidate nucleic acid sequence) or local (i.e., relative to a subsequence or region of a candidate nucleic acid sequence).

Uridine Rarefied: As used herein, the terms "uridine rarefied" and grammatical variants refer to a decrease in uridine content (expressed in absolute value or as a percentage value) in an sequence optimized nucleic acid (e.g., a synthetic mRNA sequence) with respect to the uridine content of the corresponding candidate nucleic acid sequence. Uridine rarefication can be implemented by substituting codons in the candidate nucleic acid sequence with synonymous codons containing less uridine nucleobases. Uridine rarefication can be global (i.e., relative to the entire length of a candidate nucleic acid sequence) or local (i.e., relative to a subsequence or region of a candidate nucleic acid sequence).

Other Embodiments

E1. A modified messenger RNA (mmRNA), wherein the mmRNA comprises: a 5'untranslated region (UTR), an initiation codon, a full open reading frame encoding a polypeptide, a 3' UTR, and at least one modification, wherein the modification provides a translational regulatory activity selected from:

(a) increasing residence time of a 43S pre-initiation complex (PIC) or ribosome at, or proximal to, the initiation codon;

(b) increasing initiation of polypeptide synthesis at or from the initiation codon;

(c) increasing an amount of polypeptide translated from the full open reading frame;

(d) increasing fidelity of initiation codon decoding by the PIC or ribosome;

(e) inhibiting or reducing leaky scanning by the PIC or ribosome;

decreasing a rate of decoding the initiation codon by the PIC or ribosome;

(g) inhibiting or reducing initiation of polypeptide synthesis at any codon within the mmRNA other than the initiation codon;

(h) inhibiting or reducing the amount of polypeptide translated from any open reading frame within the mmRNA other than the full open reading frame;

(i) inhibiting or reducing the production of aberrant translation products; and a combination of any of (a)-(i).

E2. The mmRNA of embodiment 1, wherein the at least one modification is a structural modification selected from: a RNA element, a GC-rich RNA element, a viral RNA element, a protein-binding RNA element, a translation initiation element, a translation enhancer element, a translation fidelity enhancing element, an mRNA nuclear export element, a codon optimized open reading frame, or a modification of base composition.

E3. The mmRNA of embodiment 1, wherein the at least one modification is a chemical modification selected from: one or more chemically modified nucleotides, one or more deoxyribonucleotides, or one or more chemical modifications to the mmRNA backbone.

E4. The mmRNA of any of embodiments 1-3, wherein the 5' UTR comprises the at least one modification.

E5. The mmRNA of any of embodiments 1-4, wherein the initiation codon comprises the at least one modification.

E6. The mmRNA of any of embodiments 1-5, wherein the full open reading frame encoding a polypeptide comprises the at least one modification.

E7. The mmRNA of any of embodiments 1-6, wherein the 3' UTR comprises the at least one modification.

E8. The mmRNA of any of embodiments 1-7, wherein the at least one modification is a GC-rich RNA element comprising a sequence of linked nucleotides, or derivatives or analogs thereof, located upstream of a Kozak consensus sequence in the 5' UTR.

E9. The mmRNA of embodiment 8, wherein the GC-rich RNA element is located about 30, about 25, about 20, about 15, about 10, or about 5 nucleotides upstream of a Kozak consensus sequence in the 5' UTR.

E10. The mmRNA of embodiment 8, wherein the GC-rich RNA element is located about 20, about 15, about 10 or about 5 nucleotides upstream of a Kozak consensus sequence in the 5' UTR.

E11. The mmRNA of embodiment 8, wherein the GC-rich RNA element is located about 5, about 4, about 3, about 2, or about 1 nucleotide upstream of a Kozak consensus sequence in the 5' UTR.

E12. The mmRNA of embodiment 8, wherein the GC-rich RNA element is located about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides upstream of a Kozak consensus sequence in the 5' UTR.

E13. The mmRNA of embodiment 8, wherein the GC-rich RNA element is upstream of and immediately adjacent to a Kozak consensus sequence in the 5' UTR.

E14. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of about 30, about 20-30, about 20, about 10-20, about 15, about 10-15, about 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, or 3 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is about 70% cytosine, about 60%-70% cytosine, about 60% cytosine, about 50%-60% cytosine, about 50% cytosine, about 40%-50% cytosine, about 40% cytosine, about 30%-40% cytosine, about 30% cytosine.

E15. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 3 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E16. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 4 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E17. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 5 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E18. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 6 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E19. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of

7 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E20. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 8 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E21. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 9 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E22. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 10 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E23. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 11 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E24. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 12 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E25. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 13 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E26. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 14 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E27. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 15 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E28. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 16 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E29. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 17 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E30. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 18 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E31. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 19 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E32. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of 20 nucleotides, or derivatives or analogs thereof, linked in any order, wherein the sequence is >50% cytosine.

E33. The mmRNA of any one of embodiments 8-13, wherein the GC-rich RNA element comprises a sequence of about 3-30 guanine and cytosine nucleotides, or derivatives or analogues thereof, wherein the sequence comprises a repeating GC-motif.

E34. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[CCG]_n$, wherein n=1 to 10.

E35. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[CCG]_n$, where n=1 to 5.

E36. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[CCG]_n$, where n=3.

E37. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[CCG]_n$, where n=2.

E38. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[CCG]_n$, where n=1.

E39. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[GCC]_n$, where n=1 to 10.

E40. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[GCC]_n$, where n=1 to 5.

E41. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[GCC]_n$, where n=3.

E42. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[GCC]_n$, where n=2.

E43. The mmRNA of embodiment 33, wherein the repeating GC-motif is $[GCC]_n$, where n=1.

E44. The mmRNA of any one of embodiments 8-13, wherein the sequence of the GC-rich RNA element comprises the sequence of EK1 [CCCGCC] (SEQ ID NO: 9) as set forth in Table 1.

E45. The mmRNA of any one of embodiments 8-13, wherein the sequence of the GC-rich RNA element comprises the sequence of EK2 [GCCGCC] (SEQ ID NO: 10) as set forth in Table 1.

E46. The mmRNA of any one of embodiments 8-13, wherein the sequence of the GC-rich RNA element comprises the sequence of EK3 [CCGCCG] (SEQ ID NO: 11) as set forth in Table 1.

E47. The mmRNA of any one of embodiments 8-13, wherein the sequence of the GC-rich RNA element comprises the sequence of V1 [CCCCGGCGCC] (SEQ ID NO: 2) as set forth in Table 1.

E48. The mmRNA of any one of embodiments 8-13, wherein the sequence of the GC-rich RNA element comprises the sequence of V2 [CCCCGGC] (SEQ ID NO: 3) as set forth in Table 1.

E49. The mmRNA of any one of embodiments 8-13, wherein the sequence of the GC-rich RNA element comprises the sequence of CG1 [GCGCCCCGCGGCG CCCCGCG] (SEQ ID NO: 4) as set forth in Table 1.

E50. The mmRNA of any one of embodiments 8-13, wherein the sequence of the GC-rich RNA element comprises the sequence of CG2 [CCCGCCCGCCCC GCCCCGCC] (SEQ ID NO: 5) as set forth in Table 1.

E51. The mmRNA of any one of embodiments 1-7, wherein the at least one modification is a GC-rich RNA element comprising a stable RNA secondary structure located upstream of a Kozak consensus sequence in the 5' UTR.

E52. The mmRNA of embodiment 51, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 30, about 25, about 20, about 15, about 10, or about 5 nucleotides upstream of a Kozak consensus sequence in the 5' UTR.

E53. The mmRNA of embodiment 51, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 20, about 15, about 10 or about 5 nucleotides upstream of a Kozak consensus sequence in the 5' UTR.

E54. The mmRNA of embodiment 51, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 5, about 4, about 3, about 2, or about 1 nucleotide upstream of a Kozak consensus sequence in the 5' UTR.

E55. The mmRNA of embodiment 51, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides upstream of a Kozak consensus sequence in the 5' UTR.

E56. The mmRNA of embodiment 51, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located upstream of and immediately adjacent to a Kozak consensus sequence in the 5' UTR.

E57. The mmRNA of any one of embodiments 1-7, wherein the at least one modification is a GC-rich RNA element comprising a stable RNA secondary structure located downstream of the initiation codon.

E58. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 30, about 25, about 20, about 15, about 10, or about 5 nucleotides downstream of the initiation codon.

E59. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 20, about 15, about 10 or about 5 nucleotides downstream of the initiation codon.

E60. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 5, about 4, about 3, about 2, about 1 nucleotide downstream of the initiation codon.

E61. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides downstream of the initiation codon.

E62. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 nucleotides downstream of the initiation codon.

E63. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located 15 nucleotides downstream of the initiation codon.

E64. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located 14 nucleotides downstream of the initiation codon.

E64. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located 13 nucleotides downstream of the initiation codon.

E66. The mmRNA of embodiment 57, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located 12 nucleotides downstream of the initiation codon.

E67. The mmRNA of any one of embodiments 1-7, wherein the at least one modification is a GC-rich RNA element comprising a stable RNA secondary structure located upstream of the initiation codon in the 5' UTR.

E68. The mmRNA of embodiments 67, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 40, about 35, about 30, about 25, about 20, about 15, about 10, or about 5 nucleotides upstream of the initiation codon.

E69. The mmRNA of embodiment 67, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 20, about 15, about 10 or about 5 nucleotides upstream of the initiation codon.

E70. The mmRNA of embodiment 67, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 5, about 4, about 3, about 2, about 1 nucleotide upstream of the initiation codon.

E71. The mmRNA of embodiment 67, wherein the GC-rich RNA element comprising a stable RNA secondary structure is located about 15-40, about 15-30, about 15-20, about 15-25, about 10-15, or about 5-10 nucleotides upstream of the initiation codon.

E72. The mmRNA of any one of embodiments 1-7, wherein the at least one modification is a GC-rich RNA element comprising a stable RNA secondary structure, wherein the stable RNA secondary structure comprises the initiation codon and one or more additional nucleotides upstream, downstream, or upstream and downstream of the initiation codon.

E73. The mmRNA of any one of embodiments 51-72, wherein the sequence of the GC-rich RNA element comprising a stable RNA secondary structure comprises the sequence of SL1 [CCGCGGCGCCCCGCGG] (SEQ ID NO: 28) as set forth in Table 1.

E74. The mmRNA of any one of embodiments 51-72, wherein the sequence of the GC-rich RNA element comprising a stable RNA secondary structure comprises the sequence of SL2 [GCGCGCAUAUAGCGCGC] (SEQ ID NO: 29) as set forth in Table 1.

E75. The mmRNA of any one of embodiments 51-72, wherein the sequence of the GC-rich RNA element comprising a stable RNA secondary structure comprises the sequence of SL3 [CATGGTGGCGGCCCGCCGCCACCATG] (SEQ ID NO: 30) as set forth in Table 1.

E76. The mmRNA of any one of embodiments 51-72, wherein the sequence of the GC-rich RNA element comprising a stable RNA secondary structure comprises the sequence of SL4 [CATGGTGGCCCGCCGCCACCATG] (SEQ ID NO: 31) as set forth in Table 1.

E77. The mmRNA of any one of embodiments 51-72, wherein the sequence of the GC-rich RNA element comprising a stable RNA secondary structure comprises the sequence of SL5 [CATGGTGCCCGCCGCCACCATG] (SEQ ID NO: 32) as set forth in Table 1.

E78. The mmRNA of any one of the preceding embodiments, wherein the stable RNA secondary structure is a hairpin or a stem-loop.

E79. The mmRNA of any of the preceding embodiments, wherein the stable RNA secondary structure has a deltaG of about −30 kcal/mol, about −20 to −30 kcal/mol, about −20 kcal/mol, about −10 to −20 kcal/mol, about −10 kcal/mol, about −5 to ~10 kcal/mol.

E80. The mmRNA of any one of embodiments 1-7, wherein the at least one modification is one or more chemically modified nucleotides, wherein the sequence comprising the initiation codon comprises one or more modified nucleotides that increases binding affinity with the initiator Met-tRNA$_i^{Met}$.

E81. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises 2-thiouridine.

E82. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises 2'-O-methyl-2-thiouridine.

E83. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises 2-selenouridine.

E84. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises 2'-O-methyl ribose.

E85. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises a modified nucleotide in which the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon.

E86. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises inosine.

E87. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises 2-methyl-guanosine.

E88. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises 6-methyl-adenosine.

E89. The mmRNA of embodiment 80, wherein the one or more chemically modified nucleotides comprises a deoxyribonucleotide.

E90. The mmRNA of any of the preceding embodiments, wherein the mmRNA comprises:

(i) a first polynucleotide, wherein the first polynucleotide is chemically synthesized, and wherein the first polynucleotide comprises a 5' UTR, an initiation codon, and at least one modification, and;

(ii) a second polynucleotide, wherein the second polynucleotide is synthesized by in vitro translation, and, wherein the second polynucleotide comprises a full open reading frame encoding a polypeptide, and a 3' UTR.

E91. The mmRNA of embodiment 90, wherein the first polynucleotide and the second polynucleotide are chemically cross-linked.

E92. The mmRNA of embodiment 90, wherein the first polynucleotide and the second polynucleotide are enzymatically ligated.

E93. The mmRNA of embodiment 90-92, wherein the first polynucleotide and the second polynucleotide are operably linked.

E94. A modified mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the 5' UTR comprises the sequence of the V1-UTR [GGGAAATAAGAGAGAAAAG AAGAGTAAGAAGAAATATAAGACC CCGGCGCC GCCA CC] (SEQ ID NO: 34) as set forth in Table 1.

E95. A modified mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the 5' UTR comprises the sequence of the V2-UTR [GGGAAATAAGAGAGAAAA GAAGAGTAAGAAGAAATATAAGACCCCGGCGC-CACC] (SEQ ID NO: 54) as set forth in Table 1.

E96. A modified mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the 5' UTR comprises the sequence of the CG1-UTR [GGGAAATAAGAGAGAAAAG AAGAGTAAGAAGAAATATAAGAGCGCCCCGCGGCG CCCCGCGGCCACC] (SEQ ID NO: 73) as set forth in Table 1.

E97. A modified mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the 5' UTR comprises the sequence of the CG2-UTR [GGGAAATAAGAGAGAAAAG AAGAGTAAGAAGAAATATAAGACCCGCCCGCCCC GC CCCGCCGCCACC] (SEQ ID NO: 92) as set forth in Table 1.

E98. A modified mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the 5' UTR comprises the sequence of the KT1-UTR [GGGCCCGCCGCCAAC] (SEQ ID NO: 472) as set forth in Table 1.

E99. A modified mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the 5' UTR comprises the sequence of the KT2-UTR [GGGCCCGCCGCCACC] (SEQ ID NO: 473) as set forth in Table 1.

E100. A modified mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the 5' UTR comprises the sequence of the KT3-UTR [GGGCCCGCCGCCGAC] (SEQ ID NO: 474) as set forth in Table 1.

E101. A modified mRNA comprising a 5' UTR, an initiation codon, a full open reading frame encoding a polypeptide, and a 3' UTR, wherein the 5' UTR comprises the sequence of the KT4-UTR [GGGCCCGCCGCCGCC] (SEQ ID NO: 475) as set forth in Table 1.

E102. A method of isolating/identifying a modification having translational regulatory activity, the method comprising:

(i) synthesizing a 1^{st} control mRNA comprising

(a) a polynucleotide sequence comprising an open reading frame encoding eGFP, and; an 1' AUG codon upstream of, in-frame, and operably linked to, the open reading frame encoding eGFP, and; a coding sequence for a 3×FLAG epitope tag upstream of, in-frame, and operably linked to the 1^{st} AUG codon, and; a 2^{nd} AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the 3×FLAG epitope tag, and; a coding sequence for a V5 epitope tag upstream of, in-frame, and operably linked to the 2" AUG codon, and; a 3^{rd} AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the V5 epitope tag, and; a 5' UTR and a 3' UTR; and

(ii) synthesizing a 2' test mmRNA comprising

(b) a polynucleotide sequence comprising an open reading frame encoding eGFP, and; an 1^{st} AUG codon upstream of, in-frame, and operably linked to, the open reading frame encoding eGFP, and; a coding sequence for a 3×FLAG epitope tag upstream of, in-frame, and operably linked to the 1^{st} AUG codon, and; a 2^{nd} AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the 3×FLAG epitope tag, and; a coding sequence for a V5 epitope tag upstream of, in-frame, and operably linked to the 2^{nd} AUG codon, and; a 3^{rd} AUG codon upstream of, in-frame, and operably linked to, the coding sequence for the V5 epitope tag, and; a 5' UTR, a 3' UTR, and a candidate modification.

(iii) introducing the 1^{st} control mRNA and 2^{nd} test mmRNA to conditions suitable for translation of the polynucleotide sequence encoding the reporter polypeptide;

(iv) measuring the effect of the candidate modification on the initiation of translation of the polynucleotide sequence encoding the reporter polypeptide from each of the three AUG codons.

EXAMPLES

Materials & Methods

Synthesis of mRNA. mRNAs were synthesized in vitro from linearized DNA templates which include the 5' UTR, 3'UTR and polyA tail, followed by addition of a 5' CAP. All 5' UTRs depicted in the Figures are shown as DNA sequences for purposes of in vitro transcription. 5' UTR sequences tested in the Examples are summarized in Table 8 and are depicted as RNA.

TABLE 8

5'UTR	Sequence	GC-Rich RNA Element
Standard	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUA AGAGCCACC (SEQ ID NO: 33)	none
6nt	GGGAAA (SEQ ID NO: 529)	none
6nt (TISU)	GGCAAG (SEQ ID NO: 530)	none

TABLE 8-continued

5'UTR	Sequence	GC-Rich RNA Element
Tubulin-like	GUACACCGGCAUCGACUAAUCAGGGCCAGGCUCGAGGC UUUGUCUCCCUACCGCGCGCCGAUUCUCCCGCCUCCCA GCCCCGGCGCACGCGCGCCCCGCCCAGCCUGCUUUCCC UCCGCGCCCUCCCCCUCUCCUUUCUCCCUCUCAGAACCU UCCUGCCGUCGCGUUUGCACCUCGCUGCUCCAGCCUCU CGCAUUCCAACCUUCCAGCCUGCGACCUGCGGAGACUU AGCCCCAUACAUACCUUGAGGCGAGCUUUUAACC (SEQ ID NO: 531)	none
V1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUA AGACCCCGGCGCCGCCACC (SEQ ID NO: 35)	(V1) CCCCGGCGCC
V2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUA AGACCCCGGCGCCACC (SEQ ID NO: 54)	(V2) CCCCGGC
V3-UTR	GGGAAAUAAGAGAGAAAAGAAGACCCCGGCGCCGUAAG AAGAAAUAUAAGAGCCACC (SEQ ID NO: 52)	(V1) CCCCGGCGCC
V4-UTR	GGGCCCCGGCGCCAAAUAAGAGAGAAAAGAAGAGUAAG AAGAAAUAUAAGAGCCAC (SEQ ID NO: 53)	(V1) CCCCGGCGCC
GC Scramble #1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUA AGAGGGGCGCCCGGCCACC (SEQ ID NO: 532)	(GC Scramble #1) GGGGCGCCCG
GC Scramble #2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUA AGAGCCCGCCCGCGCCACC (SEQ ID NO: 533)	(GC Scramble #2) GCCCGCCCGC
GC Scramble #3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUA AGAGCGCCCCGCGGCCACC (SEQ ID NO: 534)	(GC Scramble #3-UTR) GCGCCCCGCG
GC1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUA AGAGCGCCCCGCGGCGCCCCGCGGCCACC (SEQ ID NO: 535)	(GC1) GCGCCCCGCGG CGCCCCGCG

Cell culture and transfection. HeLa (ATCC), primary human hepatocytes (BioReclamation IVT), AML12 (ATCC #CRL-2254) and MEF cells (Oriental Bioservice Inc., Minami-yayamashiro Laboratory) were cultured under standard conditions. Cells were transfected with reporter mRNA using Lipofectamine 2000 or MC3 following standard protocols.

Luciferase assay in mice. Animal studies were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Moderna Therapeutics. Female BALB/c mice, 8 weeks old, weighing 18-23 g and female Sprague Dawley rats, 8 weeks old, weighing 275-300 g (Charles River Laboratories, Wilmington, Mass.), were pre-warmed using a heating lamp before injected in the lateral tail vein using a 1-mL syringe with a 27G ½" needle (Becton Dickson, San Diego, Mass.) with MC3-encapsulated 0.05 mg/kg mRNA encoding Luc. Luciferin, the substrate of luciferase, was injected intraperitoneally into mice or rat at a dose of 150 mg/kg body weight. 20 minutes after Luciferin injection, animals were euthanized. Whole body imaging imaging was carried out on the IVIS spectrum by using Living Image Software (Perkin Elmer, Waltham, Mass.).

Analysis of leaky scanning using an eGFP reporter construct. Cells were harvested and lysed using 5×RIPA Buffer (Boston BIOproducts; Cat: BP-115-5X) in the presence of protease and phosphatase inhibitors (ThermoScientific; Halt Protease & Phosphatase Inhibitor Cat: 78446). Protein concentration was assessed by BioRad DC Protein Assay (Cat: 5000113) following the manufacturer's instructions. Total protein lysates were analyzed by SDS-PAGE/Western blot analysis using primary antibodies against eGFP (Abcam; ab290 rabbit, ab6673 goat), V5-tag (Abcam; ab27671 mouse) and FLAG-tag (Abcam; ab18230 mouse) in combination with secondary antibodies (LICOR; Green, goat, anti-mouse 926-32210; Red, goat, anti-rabbit 926-68071; Red, donkey, anti-mouse 926-68072). An antibody against vinculin (Abcam; ab18058 mouse) was used as loading control. A LI-COR Odyssey CLx system was used for imaging of Western blots and densitometric analysis of translation products. The amounts of eGFP synthesized starting at the first (M1), second (M2) or third AUG (M3) codon, respectively, were quantitated. The percent of truncated protein was determined as (M2+M3)/(M1+M2+M3), setting (M1+M2+M3) to 100%. Total eGFP expression was determined as (M1+M2+M3)/(vinculin).

40S footprinting. Cells were lysed, then immediately crosslinked with formaldehyde at a final concentration of 1.5%. Following buffer exchange, the lysate was treated with a cocktail of RNases T1, A, and I. The digested lysate was centrifuged through a sucrose gradient and the small subunit peak selected for reverse crosslinking and RNA extraction. rRNA was depleted using the NEBNext rRNA Depletion kit, and the resulting RNA was converted into a cDNA library using the NEBNext Small RNA Library Prep Set. Following deep sequencing, reads were mapped to the human transcriptome HeLa cells of human hepatocytes as indicated.

Example 1: The Length and Base Composition of 5' UTRs Comprising Reporter mRNAs Affects Leaky Scanning and the Fidelity of Translation Initiation

DNA plasmid constructs were generated and used to produce reporter mRNAs, via in vitro transcription, as described in the Materials & Methods. The reporter mRNAs contain a 5' UTR with a Kozak consensus sequence preceding, or upstream of, a sequence encoding a V5 epitope tag and a 3× FLAG epitope tag fused in-frame with a sequence encoding eGFP, followed by a 3' UTR. The sequences encoding the V5 epitope tag and the 3× FLAG epitope tag are each preceded by an in-frame AUG codon upstream of the eGFP AUG codon, as is shown in FIG. **1**A. The reporter mRNAs are designed such that translation initiation from the 1^{st} AUG codon downstream of the 5' UTR would produce an eGFP polypeptide fused to a V5 epitope tag and to a 3×FLAG epitope tag at the N-terminus. Translation initiation from the 2^{nd} AUG codon downstream of the 5' UTR would produce an eGFP polypeptide fused only to a 3×FLAG epitope tag at the N-terminus. Translation initiation at the 3^{rd} AUG codon downstream of the 5' UTR would produce only an eGFP polypeptide containing no epitope tags. This design provides the ability to assess the effect of various 5' UTRs (FIG. **1**B) on translation initiation at each AUG codon as a function of the production of polypeptides of discrete lengths (each detectable using an anti-GFP antibody) and with differential reactivity to anti-V5 and/or anti-FLAG antibodies, depending on the presence or absence of the corresponding epitope tag. The production of a full-length translation product (reactive to a V5-specific antibody) and products from leaky scanning arising from translation initiation at the 2nd and 3rd AUG (not reactive to a V5-specific antibody, but reactive to FLAG- and eGFP-specific antibodies, respectively) is monitored by standard SDS-PAGE/Western blot techniques, as described in the Materials & Methods.

In cell-based experiments, a full-length translation product (V5-Flag-eGFP (M1)) and truncated translation products (Flag-eGFP (M2); eGFP(M3)) were detected by Western blotting (FIG. **2**A) after electrophoretic separation of proteins from HeLa cells or murine embryonic fibroblasts (MEFs) that were independently transfected with reporter mRNAs containing 5' UTRs varying in length and/or base composition, as described in the Materials & Methods (Table 8). Strikingly, a relatively long 5' UTR derived from the mammalian tubulin gene (labeled "262nt tub-like"; FIGS. **2**A and **2**B) drastically reduced the formation of the truncated translation products FLAG-eGFP (M2) and eGFP (M3), demonstrating that the length of the 5' UTR of the reporter mRNAs affects translation initiation and leaky scanning in these cell types. In addition, the amount of truncated protein products translated from reporter mRNAs containing two short 5' UTRs (labeled "6nt" and "6nt (TISU)"; FIG. **2**A) varying only in base composition was evaluated. Cells transfected with reporter mRNA containing the 6nt (TISU) 5' UTR produced less truncated translation products relative to cells transfected with reporter mRNA containing the 6nt 5' UTR, demonstrating that the base composition of the 5' UTRs also affects translation initiation and leaky scanning. The amount of truncated products translated from the reporter mRNAs was quantified by densitometry and is shown as a percentage of the total amount of all translation products detectable by Western blot (FIGS. **2**C and **2**D). Similar results were obtained from in vivo experiments using cells derived from liver after intravenous administration of 0.5 mg/kg of reporter mRNAs as shown in FIGS. **2**B and **2**D.

Example 2: Increasing the Length of Reporter mRNA 5' UTRs Decreases Both Leaky Scanning and Translation Efficiency

To better reveal the contribution of 5' UTR length on leaky scanning, reporter mRNAs were generated containing 5' UTRs of increasing length (Table 8) upstream of a sequence encoding a 3× FLAG epitope tag fused in-frame with a sequence encoding eGFP, followed by a 3' UTR. The sequence encoding the 3× FLAG epitope tag is preceded by an in-frame AUG codon and is upstream of the eGFP AUG codon, as shown in FIG. **3**A. The reporter mRNAs are designed such that translation initiation from the 1" AUG codon downstream of the 5' UTR would produce an eGFP polypeptide fused to a 3×FLAG epitope tag at the N-terminus. Translation initiation from the 2^{nd} AUG codon downstream of the 5' UTR would produce only an eGFP polypeptide containing no epitope tags. This design provides the ability to assess the effect of 5' UTR length on translation initiation at each AUG codon as a function of the production of polypeptides of discrete lengths (each detectable using an anti-GFP antibody) and with differential reactivity to an anti-FLAG antibody, depending on the presence or absence of the epitope tag. The production of a full-length translation product (reactive to both FLAG- and eGFP-specific antibody) and products from leaky scanning arising from translation initiation at the 2^{nd} AUG (only reactive to eGFP-specific antibodies) is monitored by standard SDS-PAGE/Western blot techniques, as described in the Materials & Methods.

In cell-based experiments, a full-length translation product (FLAG-eGFP (M1)) and a truncated translation product (eGFP (M2)) were detected by Western blotting after electrophoretic separation of proteins from HeLa cells that were independently transfected with reporter mRNAs containing 5' UTRs varying in length, as shown (FIG. **3**B). As was suggested by the results of experiments described in Example 1, reporter mRNAs containing 5' UTRs of increasing length correlated with less translation of the truncated translation product eGFP (M2) (FIG. **3**C), again demonstrating that the length of the 5' UTR of the reporter mRNAs can affect translation initiation and leaky scanning. In addition, the amount of total translation product translated from reporter mRNAs decreased with increasing length of the 5' UTR (FIG. **3**D), as measured by the densitometric analysis of all anti-eGFP reactive bands from the Western blot in FIG. **3**B.

These findings are also supported by analysis of small ribosomal subunit footprinting on cellular mRNAs. FIG. **4**A illustrates the relative density of small subunits, where deep sequencing reads were mapped to the transcriptome of HeLa cells and the number of reads overlapping with each AUG in each mRNA was counted. The number of reads overlapping with each AUG was then normalized to the 1^{st} AUG, showing a significant density of small ribosomal subunits at the 2^{nd}, 3^{rd} etc. AUG codon. In a separate experiment performed in the absence of cross-linking, a similar pattern is observed in both HeLa cells (FIG. **12**A) and mouse spleens (FIG. **12**B), where the density of small ribosomal subunits at the 1^{st} AUG decreases with each subsequent AUG in the mRNA.

The frequency of leaky scanning dependent on 5' UTR length (FIG. **4**B) for each mRNA in primary human hepa-

tocytes was estimated by dividing the mean small subunit read density in the first 500 nt of the coding sequence by the mean small subunit read density in the 5'UTR. In FIG. **4**B, leaky scanning was plotted against the length of 5'UTR; each point represents an individual mRNA with at least 100 mapped reads. The black line represents a moving average.

Example 3: GC-rich RNA Elements Located Proximal to The Kozak Consensus Sequence of Reporter mRNAs Decrease Leaky Scanning and Increase the Fidelity of Translation Initiation

The Kozak consensus sequence [GCCACC] located immediately upstream of the 1st AUG codon from the 5' end is not enough to guarantee a high fidelity of translation initiation for the reporter mRNAs described in the preceding Examples, as shown by a significant level of leaky scanning observed by two independent assay systems.

To better reveal the contribution of 5' UTR base composition on leaky scanning, reporter mRNAs were generated with 5' UTRs containing GC-rich RNA elements (Table 8). The approximate location of these GC-rich RNA elements is depicted in FIG. **5**A. These 5' UTRs are followed by a sequence encoding a 3× FLAG epitope tag fused in-frame with a sequence encoding eGFP, followed by a 3' UTR. The sequence encoding the 3× FLAG epitope tag is preceded by an in-frame AUG codon and is upstream of the eGFP AUG codon, as shown in FIG. **5**A. As in the previous Examples, these reporter mRNAs are designed such that translation initiation from the 1' AUG codon downstream of the 5' UTR would produce an eGFP polypeptide fused to a 3×FLAG epitope tag at the N-terminus. Translation initiation from the 2nd AUG codon downstream of the 5' UTR would produce only an eGFP polypeptide containing no epitope tags. This design provides the ability to assess the effect of the presence and position of GC-rich RNA elements on translation initiation at each AUG codon as a function of the production of polypeptides of discrete lengths (each detectable using an anti-GFP antibody) and with differential reactivity to an anti-FLAG antibody, depending on the presence or absence of the epitope tag. The production of a full-length translation product (reactive to both FLAG- and eGFP-specific antibody) and products from leaky scanning arising from translation initiation at the 2nd AUG (only reactive to eGFP-specific antibodies) is monitored by standard SDS-PAGE/Western blot techniques, as described in the Materials & Methods.

In cell-based experiments, a full-length translation product (Flag-eGFP (M1)) and a truncated translation product (eGFP(M2)) were detected by Western blotting after electrophoretic separation of proteins from HeLa cells or MEFs that were independently transfected with reporter mRNAs containing 5' UTRs encoding GC-rich RNA elements located proximal to or distal from the Kozak consensus sequence preceding the 1st AUG codon from the 5' end, as shown (FIG. **5**A). The insertion of a 10 nt RNA element composed of C and G residues [CCCCGGCGCC; V1] (SEQ ID NO: 2) upstream of the Kozak consensus sequence significantly reduced leaky scanning (FIG. **5**B), without affecting the overall translational efficiency as illustrated for two different reporter constructs, human Erythropoietin (Epo, FIGS. **6**A and B) and luciferase (Luc, FIGS. **6**C and D). A related 7 nt RNA element inserted upstream of the Kozak consensus sequence also composed of C and G residues [CCCCGGC; V2] (SEQ ID NO: 3) also decreased the amount of the truncated translation product eGFP (M2) in both HeLa cells and MEFs. As was suggested by the results of experiments described in Example 1, modifying the base composition of 5' UTRs by insertion of GC-rich RNA elements correlated with less translation of the truncated translation product eGFP (M2) (FIG. **5**B), again demonstrating that the base composition of the 5' UTR of the reporter mRNAs can affect translation initiation and leaky scanning in these cell types. Furthermore, the position of the V1 GC-rich RNA element was also shown to have an effect on leaky scanning. As shown in FIGS. **5**A and **5**B, leaky scanning is reduced when these GC-rich RNA elements are proximal to the Kozak consensus sequence or initiation codon (M1). The V3-UTR (V3) and V4-UTRs (V4), which comprise the V1 GC-rich RNA element but located farther upstream from the initiation codon AUG (M1) (Table 8), are not as effective at decreasing leaky scanning, as shown in FIG. **5**B.

Example 4: GC Content of The 20 nts Preceding the Kozak Consensus Sequence in Reporter mRNAs Correlates with Leaky Scanning

To assess the impact of GC content on leaky scanning, 254 different 5' UTRs from natural and synthetic sources, varying base composition and length, were tested with the eGFP reporter described in Example 3, where translation initiation from the 1st AUG codon downstream of the 5' UTR would produce an eGFP polypeptide fused to a 3×FLAG epitope tag at the N-terminus. Translation initiation from the 2nd AUG codon downstream of the 5' UTR would produce only an eGFP polypeptide containing no epitope tags. The top 24 sequences that performed well in terms of overall translation efficiency were analyzed further for leaky scanning. FIG. **7**A shows leaky scanning observed for each of the 5' UTR constructs, all shorter than 100 nucleotides in length, normalized to leaky scanning observed for the standard 5' UTR (FIG. **1**, Table 8). Clearly, increased GC content in the final nucleotides of the 5' UTR, i.e. those nucleotides preceding the initiation codon, decreases leaky scanning. As shown above, the insertion of a 10 nt RNA element composed of C and G residues [CCCCGGCGCC; V1] (SEQ ID NO: 2) into the standard 5' UTR resulted in a significant decrease of leaky scanning.

A similar correlation is found globally across human mRNAs. In FIG. **7**B, the frequency of leaky scanning for each mRNA in primary hepatocytes was estimated by dividing the mean small subunit read density in the first 500 nt of the coding sequence by the mean small subunit read density in the 5' UTR and plotted against the number of G and C bases in the final 20 nt of the 5' UTR; each point represents an individual mRNA with at least 100 mapped reads. The black line represents a moving average.

Example 5: mRNAs with 5' UTRs Comprising GC-rich RNA Elements with Greater than 40% Cytosine Located Upstream of the Kozak Consensus Sequence Decrease Leaky Scanning

To further characterize the ability of GC-rich RNA elements to decrease leaky scanning, 5' UTRs with GC-rich RNA elements comprising greater than 40% cytosine nucleobases were tested with the eGFP reporter described in Example 1. The 5' UTRs (tested are shown in the table in FIG. **8**A. A schematic of the reporter construct with the relative location of the GC-rich RNA elements is shown in FIG. **8**B.

Similar to the results shown in FIG. **5**B, the presence of the GC-rich RNA element V1, as well as GC scramble #2,

GC scramble #3 and GC1, which comprise 60%-70% cytosine nucleobases, decreased leaky scanning of the reporter mRNA, as shown as a reduction in the amount of FLAG-eGFP (M2) and eGFP (M3) polypeptide (FIGS. **9**A and **9**B), determined by standard SDS-PAGE/Western blot techniques, as described in the Materials & Methods. The 5' UTR containing the GC-rich RNA element GC scramble #1, which comprises 40% cytosine nucleobases, did not appreciably decrease leaky scanning compared to the standard 5' UTR, which does not comprise a GC-rich RNA element. Taken together, these data demonstrate that the cytosine content of the GC-rich RNA element impacts the ability of the 5' UTR to decrease leaky scanning.

Example 6: mRNAs with 5' UTR Comprising GC-rich RNA Elements Increase Potency of Translated Polypeptides

To determine the effect of GC-rich RNA elements on the potency of polypeptides translated from an mRNA, reporter mRNAs encoding luciferase or eGFP were generated containing 5' UTRs comprising the GC-rich RNA elements VI or V2, as described in Table 8, and evaluated both in vivo and in vitro.

To evaluate the potency of polypeptides translated from mRNA comprising 5' UTRs with GC-rich RNA elements in vivo, BALB/c mice were injected intravenously with 0.05 mg/kg mRNA encoding luciferase downstream of an 5'UTR comprising a GC-rich RNA element (V1 or V2) formulated in an lipid nanoparticle. At various time points post-injection, as indicated, whole body imaging using IVIS was performed to quantify the luciferase signal (total flux). At 6 hours, mRNA encoding luciferase and comprising the VI-UTR or V2-UTR produced higher luminescence than with the comparator control mRNA that does not comprise a GC-rich RNA element (FIG. **10**) or with mRNA comprising V3-UTR or V4-UTR. The V3-UTR (V3) and V4-UTRs (V4) (Table 8) comprise the V1 GC-rich RNA element but located farther upstream from the initiation codon AUG of the luciferase gene. Notably, the V1-UTR produced the highest luciferase signal. These data suggest that GC-rich RNA elements tested increase the potency of the polypeptide translated from the mRNA.

To evaluate the potency of polypeptides translated from mRNA comprising 5' UTRs with GC-rich RNA elements in vitro, HeLa cells (FIG. **11**A), AML12 (mouse hepatocyte cell line) cells (FIG. **11**B), and primary human hepatocytes (FIG. **11**C) were transfected with mRNA encoding deg-eGFP (eGFP fused to a PEST domain on the C-terminal end to mediate rapid degradation of the protein) and comprising an 5' UTR with the V1 GC-rich RNA element (v1) or with a control mRNA encoding eGFP and comprising a 5' UTR that does not contain a GC-rich RNA element (Ctrl). An image of the fluorescent cells was taken every hour for 48 hours using a live-cell analysis system (IncuCyte). The total fluorescent intensity of the cells (AUC) for each cell type transfected with each mRNA is shown in FIGS. **11**A, **11**B, and **11**C. Total fluorescence is higher in all cell types transfected with the mRNA comprising V1 compared to control mRNA, suggesting the V1 GC-rich RNA element increased the potency of the eGFP polypeptide in vitro.

Equivalents and Scope

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the disclosure described herein. The scope of the present disclosure is not intended to be limited to the Description below, but rather is as set forth in the appended claims.

In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" is thus also encompassed and disclosed.

Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control.

TABLE 9

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
1	Kozak Consensus Sequence	GCCRCC, where R = A or G
2	V1	CCCCGGCGCC
3	V2	CCCCGGC
4	CG1	GCGCCCCGCGGCGCCCCGCG
5	CG2	CCCGCCCGCCCCGCCCCGCC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
6	GC Scramble #1	GGGGCGCCCG
7	GC Scramble #2	GCCCGCCCGC
8	GC Scramble #3	GCGCCCCGCG
9	EK1	CCCGCC
10	EK2	GCCGCC
11	EK3	CCGCCG
12	(CCG)3	CCGCCGCCG
13	(CCG)4	CCGCCGCCGCCG
14	(CCG)5	CCGCCGCCGCCGCCG
15	(CCG)6	CCGCCGCCGCCGCCGCCG
16	(CCG)7	CCGCCGCCGCCGCCGCCGCCG
17	(CCG)8	CCGCCGCCGCCGCCGCCGCCGCCG
18	(CCG)9	CCGCCGCCGCCGCCGCCGCCGCCGCCG
19	(CCG)10	CCGCCGCCGCCGCCGCCGCCGCCGCCGCCG
20	(GCC)3	GCCGCCGCC
21	(GCC)4	GCCGCCGCCGCC
22	(GCC)5	GCCGCCGCCGCCGCC
23	(GCC)6	GCCGCCGCCGCCGCCGCC
24	(GCC)7	GCCGCCGCCGCCGCCGCCGCC
25	(GCC)8	GCCGCCGCCGCCGCCGCCGCCGCC
26	(GCC)9	GCCGCCGCCGCCGCCGCCGCCGCCGCC
27	(GCC)10	GCCGCCGCCGCCGCCGCCGCCGCCGCCGCC
28	SL1	CCGCGGCGCCCCGCGG
29	SL2	GCGCGCAUAUAGCGCGC
30	SL3	CAUGGUGGCGGCCCGCCGCCACCAUG
31	SL4	CAUGGUGGCCCGCCGCCACCAUG
32	SL5	CAUGGUGCCCGCCGCCACCAUG
33	Standard UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCACC
34	V1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CCCGGCGCCGCCACC
35	V1-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCCC CGGCGCCAGCCACC
36	V1-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCCC GGCGCCGAGCCACC
37	V1-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCCCG GCGCCAGAGCCACC
38	V1-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCCCGG CGCCAAGAGCCACC
39	V1-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCCCGGC GCCUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
40	V1-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCCCGGCG CCAUAAGAGCCACC
41	V1-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCCCGGCGC CUAUAAGAGCCACC
42	V1-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCCCGGCGCC AUAUAAGAGCCACC
43	V1-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCCCGGCGCCA AUAUAAGAGCCACC
44	V1-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCCCGGCGCCAA AUAUAAGAGCCACC
45	V1-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCCCGGCGCCGAA AUAUAAGAGCCACC
46	V1-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCCCGGCGCCAGAA AUAUAAGAGCCACC
47	V1-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCCCGGCGCCAAGAA AUAUAAGAGCCACC
48	V1-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCCCGGCGCCGAAGAA AUAUAAGAGCCACC
49	V1-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCCCGGCGCCAGAAGAA AUAUAAGAGCCACC
50	V1-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCCCGGCGCCAAGAAGAA AUAUAAGAGCCACC
51	V1-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCCCGGCGCCUAAGAAGAA AUAUAAGAGCCACC
52	V3-UTR	GGGAAAUAAGAGAGAAAAGAAGACCCCGGCGCCGUAAGAAGAA AUAUAAGAGCCACC
53	V4-UTR	GGGCCCCGGCGCCAAAUAAGAGAGAAAAGAAGAGUAAGAAGAA AUAUAAGAGCCACC
54	V2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CCCGGCGCCACC
55	V2-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCCC CGGCAGCCACC
56	V2-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCCC GGCGAGCCACC
57	V2-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCCCG GCAGAGCCACC
58	V2-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCCCGG CAAGAGCCACC
59	V2-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCCCGGC UAAGAGCCACC
60	V2-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCCCGGCA UAAGAGCCACC
61	V2-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCCCGGCUA UAAGAGCCACC
62	V2-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCCCGGCAUA UAAGAGCCACC
63	V2-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCCCGGCAAUA UAAGAGCCACC
64	V2-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCCCGGCAAAUA UAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
65	V2-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCCCGGCGAAAUA UAAGAGCCACC
66	V2-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCCCGGCAGAAAUA UAAGAGCCACC
67	V2-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCCCGGCAAGAAAUA UAAGAGCCACC
68	V2-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCCCGGCGAAGAAAUA UAAGAGCCACC
69	V2-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCCCGGCAGAAGAAAUA UAAGAGCCACC
70	V2-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCCCGGCAAGAAGAAAUA UAAGAGCCACC
71	V2-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCCCGGCUAAGAAGAAAUA UAAGAGCCACC
72	V2-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCCCGGCGUAAGAAGAAAUA UAAGAGCCACC
73	CG1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CGCCCCGCGGCGCCCCGCGGCCACC
74	CG1-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC GCCCCGCGGCGCCCCGCGAGCCACC
75	CG1-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCG CCCCGCGGCGCCCCGCGGAGCCACC
76	CG1-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCGC CCCGCGGCGCCCCGCGAGAGCCACC
77	CG1-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCGCCC CGCGGCGCCCCGCGAAGAGCCACC
78	CG1-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCGCCCC GCGGCGCCCCGCGUAAGAGCCACC
79	CG1-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCGCCCCG CGGCGCCCCGCGAUAAGAGCCACC
80	CG1-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCGCCCCGC GGCGCCCCGCGUAUAAGAGCCACC
81	CG1-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCGCCCCGCG GCGCCCCGCGAUAUAAGAGCCACC
82	CG1-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCGCCCCGCGG CGCCCCGCGAAUAUAAGAGCCACC
83	CG1-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCGCCCCGCGGC GCCCCGCGAAAUAUAAGAGCCACC
84	CG1-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCGCCCCGCGGCG CCCCGCGGAAAUAUAAGAGCCACC
85	CG1-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCGCCCCGCGGCGC CCCGCGAGAAAUAUAAGAGCCACC
86	CG1-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGGCGCCCCGCGGCGCC CCGCGAAGAAAUAUAAGAGCCACC
87	CG1-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCGCCCCGCGGCGCCC CGCGGAAGAAAUAUAAGAGCCACC
88	CG1-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCGCCCCGCGGCGCCCC GCGAGAAGAAAUAUAAGAGCCACC
89	CG1-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCGCCCCGCGGCGCCCCG CGAAGAAGAAAUAUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
90	CG1-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCGCCCCGCGGCGCCCCGC GUAAGAAGAAAUAUAAGAGCCACC
91	CG1-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCGCCCCGCGGCGCCCCGCG GUAAGAAGAAAUAUAAGAGCCACC
92	CG2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CCGCCCGCCCCGCCCCGCCGCCACC
93	CG2-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCCC GCCCGCCCCGCCCCGCCAGCCACC
94	CG2-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCCG CCCGCCCCGCCCCGCCGAGCCACC
95	CG2-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCCGC CCGCCCCGCCCCGCCAGAGCCACC
96	CG2-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCCGCC CGCCCCGCCCCGCCAAGAGCCACC
97	CG2-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCCGCCC GCCCCGCCCCGCCUAAGAGCCACC
98	CG2-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCCGCCCG CCCCGCCCCGCCAUAAGAGCCACC
99	CG2-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCCGCCCGC CCCGCCCCGCCUAUAAGAGCCACC
100	CG2-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCCGCCCGCC CCGCCCCGCCAUAUAAGAGCCACC
101	CG2-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCCGCCCGCCC CGCCCCGCCAAUAUAAGAGCCACC
102	CG2-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCCGCCCGCCCC GCCCCGCCAAAUAUAAGAGCCACC
103	CG2-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCCGCCCGCCCCG CCCCGCCGAAAUAUAAGAGCCACC
104	CG2-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCCGCCCGCCCCGC CCCGCCAGAAAUAUAAGAGCCACC
105	CG2-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCCGCCCGCCCCGCC CCGCCAAGAAAUAUAAGAGCCACC
106	CG2-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCCGCCCGCCCCGCCCC GCCGAAGAAAUAUAAGAGCCACC
107	CG2-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCCGCCCGCCCCGCCCCG CCAGAAGAAAUAUAAGAGCCACC
108	CG2-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCCGCCCGCCCCGCCCCGC CAAGAAGAAAUAUAAGAGCCACC
109	CG2-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCCGCCCGCCCCGCCCCGCC UAAGAAGAAAUAUAAGAGCCACC
110	CG2-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCCGCCCGCCCCGCCCCGCCG UAAGAAGAAAUAUAAGAGCCACC
111	EK1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CCGCCGCCACC
112	EK1-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCCC GCCAGCCACC
113	EK1-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCCG CCGAGCCACC
114	EK1-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCCGC CAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
115	EK1-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCCGCC AAGAGCCACC
116	EK1-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCCGCCU AAGAGCCACC
117	EK1-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCCGCCAU AAGAGCCACC
118	EK1-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCCGCCUAU AAGAGCCACC
119	EK1-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCCGCCAUAU AAGAGCCACC
120	EK1-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCCGCCAAUAU AAGAGCCACC
121	EK1-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCCGCCAAAUAU AAGAGCCACC
122	EK1-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCCGCCGAAAUAU AAGAGCCACC
123	EK1-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCCGCCAGAAAUAU AAGAGCCACC
124	EK1-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCCGCCAAGAAAUAU AAGAGCCACC
125	EK1-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCCGCCGAAGAAAUAU AAGAGCCACC
126	EK1-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCCGCCAGAAGAAAUAU AAGAGCCACC
127	EK1-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCCGCCAAGAAGAAAUAU AAGAGCCACC
128	EK1-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCCGCCUAAGAAGAAAUAU AAGAGCCACC
129	EK1-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCCGCCGUAAGAAGAAAUAU AAGAGCCACC
130	EK2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCGCCGCCACC
131	EK2-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCAGCCACC
132	EK2-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGAGCCACC
133	EK2-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCAGAGCCACC
134	EK2-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC AAGAGCCACC
135	EK2-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCCGCCU AAGAGCCACC
136	EK2-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCCGCCAU AAGAGCCACC
137	EK2-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCUAU AAGAGCCACC
138	EK2-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCCGCCAUAU AAGAGCCACC
139	EK2-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCCGCCAAUAU AAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
140	EK2-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCCGCCAAAUAU AAGAGCCACC
141	EK2-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGAAAUAU AAGAGCCACC
142	EK2-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCCGCCAGAAAUAU AAGAGCCACC
143	EK2-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGGCCGCCAAGAAAUAU AAGAGCCACC
144	EK2-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGAAGAAAUAU AAGAGCCACC
145	EK2-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCAGAAGAAAUAU AAGAGCCACC
146	EK2-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCAAGAAGAAAUAU AAGAGCCACC
147	EK2-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCUAAGAAGAAAUAU AAGAGCCACC
148	EK2-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGUAAGAAGAAAUAU AAGAGCCACC
149	EK3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CGCCGGCCACC
150	EK3-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGAGCCACC
151	EK3-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCGC CGGAGCCACC
152	EK3-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCGCC GAGAGCCACC
153	EK3-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCGCCG AAGAGCCACC
154	EK3-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCGCCGU AAGAGCCACC
155	EK3-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCGCCGAU AAGAGCCACC
156	EK3-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGUAU AAGAGCCACC
157	EK3-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGAUAU AAGAGCCACC
158	EK3-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGAAUAU AAGAGCCACC
159	EK3-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGAAAUAU AAGAGCCACC
160	EK3-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGGAAAUAU AAGAGCCACC
161	EK3-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGAGAAAUAU AAGAGCCACC
162	EK3-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGAAGAAAUAU AAGAGCCACC
163	EK3-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGGAAGAAAUAU AAGAGCCACC
164	EK3-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGAGAAGAAAUAU AAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
165	EK3-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGAAGAAGAAAUAU AAGAGCCACC
166	EK3-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGUAAGAAGAAAUAU AAGAGCCACC
167	EK3-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGGUAAGAAGAAAUAU AAGAGCCACC
168	(CCG)3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CGCCGCCGGCCACC
169	(CCG)3-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGAGCCACC
170	(CCG)3-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCGC CGCCGGAGCCACC
171	(CCG)3-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCGCC GCCGAGAGCCACC
172	(CCG)3-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCGCCG CCGAAGAGCCACC
173	(CCG)3-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCGCCGC CGUAAGAGCCACC
174	(CCG)3-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCGCCGCC GAUAAGAGCCACC
175	(CCG)3-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGCCG UAUAAGAGCCACC
176	(CCG)3-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGCCGA UAUAAGAGCCACC
177	(CCG)3-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGCCGAA UAUAAGAGCCACC
178	(CCG)3-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGAAA UAUAAGAGCCACC
179	(CCG)3-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGCCGGAAA UAUAAGAGCCACC
180	(CCG)3-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGCCGAGAAA UAUAAGAGCCACC
181	(CCG)3-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGAAGAAA UAUAAGAGCCACC
182	(CCG)3-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGCCGGAAGAAA UAUAAGAGCCACC
183	(CCG)3-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGCCGAGAAGAAA UAUAAGAGCCACC
184	(CCG)3-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGCCGAAGAAGAAA UAUAAGAGCCACC
185	(CCG)3-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGUAAGAAGAAA UAUAAGAGCCACC
186	(CCG)3-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGCCGGUAAGAAGAAA UAUAAGAGCCACC
187	(CCG)4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CGCCGCCGCCGGCCACC
188	(CCG)4-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGAGCCACC
189	(CCG)4-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCGC CGCCGCCGGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
190	(CCG)4-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCGCC GCCGCCGAGAGCCACC
191	(CCG)4-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCGCCG CCGCCGAAGAGCCACC
192	(CCG)4-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCGCCGC CGCCGUAAGAGCCACC
193	(CCG)4-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCGCCGCC GCCGAUAAGAGCCACC
194	(CCG)4-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGCCG CCGUAUAAGAGCCACC
195	(CCG)4-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGCCGC CGAUAUAAGAGCCACC
196	(CCG)4-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGCCGCC GAAUAUAAGAGCCACC
197	(CCG)4-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG AAAUAUAAGAGCCACC
198	(CCG)4-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGCCGCCGG AAAUAUAAGAGCCACC
199	(CCG)4-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGCCGCCGAG AAAUAUAAGAGCCACC
200	(CCG)4-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGAAG AAAUAUAAGAGCCACC
201	(CCG)4-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGCCGCCGGAAG AAAUAUAAGAGCCACC
202	(CCG)4-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGCCGCCGAGAAG AAAUAUAAGAGCCACC
203	(CCG)4-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGCCGCCGAAGAAG AAAUAUAAGAGCCACC
204	(CCG)4-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGUAAGAAG AAAUAUAAGAGCCACC
205	(CCG)4-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGCCGCCGGUAAGAAG AAAUAUAAGAGCCACC
206	(CCG)5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CGCCGCCGCCGCCGGCCACC
207	(CCG)5-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGAGCCACC
208	(CCG)5-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCGC CGCCGCCGCCGGAGCCACC
209	(CCG)5-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCGCC GCCGCCGCCGAGAGCCACC
210	(CCG)5-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCGCCG CCGCCGCCGAAGAGCCACC
211	(CCG)5-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCGCCGC CGCCGCCGUAAGAGCCACC
212	(CCG)5-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCGCCGCC GCCGCCGAUAAGAGCCACC
213	(CCG)5-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGCCG CCGCCGUAUAAGAGCCACC
214	(CCG)5-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGCCGC CGCCGAUAUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
215	(CCG) 5-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGCCGCC GCCGAAUAUAAGAGCCACC
216	(CCG) 5-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGAAAUAUAAGAGCCACC
217	(CCG) 5-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGCCGCCGC CGGAAAUAUAAGAGCCACC
218	(CCG) 5-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGCCGCCGCC GAGAAAUAUAAGAGCCACC
219	(CCG) 5-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG AAGAAAUAUAAGAGCCACC
220	(CCG) 5-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGCCGCCGCCGG AAGAAAUAUAAGAGCCACC
221	(CCG) 5-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGCCGCCGCCGAG AAGAAAUAUAAGAGCCACC
222	(CCG) 5-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGCCGCCGCCGAAG AAGAAAUAUAAGAGCCACC
223	(CCG) 5-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGUAAG AAGAAAUAUAAGAGCCACC
224	(CCG) 5-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGCCGCCGCCGGUAAG AAGAAAUAUAAGAGCCACC
225	(CCG) 6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAAUAUAAGAC CGCCGCCGCCGCCGCCGGCCACC
226	(CCG) 6-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAAUAUAAGCC GCCGCCGCCGCCGCCGAGCCACC
227	(CCG) 6-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAAUAUAACCGC CGCCGCCGCCGCCGGAGCCACC
228	(CCG) 6-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAAUAUACCGCC GCCGCCGCCGCCGAGAGCCACC
229	(CCG) 6-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAAUAUCCGCCG CCGCCGCCGCCGAAGAGCCACC
230	(CCG) 6-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAAUACCGCCGC CGCCGCCGCCGUAAGAGCCACC
231	(CCG) 6-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAAUCCGCCGCC GCCGCCGCCGAUAAGAGCCACC
232	(CCG) 6-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGCCG CCGCCGCCGUAUAAGAGCCACC
233	(CCG) 6-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGCCGC CGCCGCCGAUAUAAGAGCCACC
234	(CCG) 6-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGCCGCC GCCGCCGAAUAUAAGAGCCACC
235	(CCG) 6-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGAAAUAUAAGAGCCACC
236	(CCG) 6-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGCCGCCGC CGCCGGAAAUAUAAGAGCCACC
237	(CCG) 6-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGCCGCCGCC GCCGAGAAAUAUAAGAGCCACC
238	(CCG) 6-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGAAGAAAUAUAAGAGCCACC
239	(CCG) 6-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGCCGCCGCCGC CGGAAGAAAUAUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
240	(CCG) 6-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGCCGCCGCCGCC GAGAAGAAAUAUAAGAGCCACC
241	(CCG) 6-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGCCGCCGCCGCCG AAGAAGAAAUAUAAGAGCCACC
242	(CCG) 6-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGU AAGAAGAAAUAUAAGAGCCACC
243	(CCG) 6-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGCCGCCGCCGCCGGU AAGAAGAAAUAUAAGAGCCACC
244	(CCG) 7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CGCCGCCGCCGCCGCCGCCGGCCACC
245	(CCG) 7-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGCCGAGCCACC
246	(CCG) 7-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCGC CGCCGCCGCCGCCGCCGGAGCCACC
247	(CCG) 7-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCGCC GCCGCCGCCGCCGCCGAGAGCCACC
248	(CCG) 7-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCGCCG CCGCCGCCGCCGCCGAAGAGCCACC
249	(CCG) 7-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCGCCGC CGCCGCCGCCGCCGUAAGAGCCACC
250	(CCG) 7-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCGCCGCC GCCGCCGCCGCCGAUAAGAGCCACC
251	(CCG) 7-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGCCG CCGCCGCCGCCGUAUAAGAGCCACC
252	(CCG) 7-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGCCGC CGCCGCCGCCGAUAUAAGAGCCACC
253	(CCG) 7-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGCCGCC GCCGCCGCCGAAUAUAAGAGCCACC
254	(CCG) 7-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGCCGAAAUAUAAGAGCCACC
255	(CCG) 7-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGCCGCCGC CGCCGCCGGAAAUAUAAGAGCCACC
256	(CCG) 7-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGCCGCCGCC GCCGCCGAGAAAUAUAAGAGCCACC
257	(CCG) 7-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGCCGAAGAAAUAUAAGAGCCACC
258	(CCG) 7-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGCCGCCGCCGC CGCCGGAAGAAAUAUAAGAGCCACC
259	(CCG) 7-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGCCGCCGCCGCC GCCGAGAAGAAAUAUAAGAGCCACC
260	(CCG) 7-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGCCGCCGCCGCCG CCGAAGAAGAAAUAUAAGAGCCACC
261	(CCG) 7-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGC CGUAAGAAGAAAUAUAAGAGCCACC
262	(CCG) 7-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGCCGCCGCCGCCGCC GGUAAGAAGAAAUAUAAGAGCCACC
263	(CCG) 8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CGCCGCCGCCGCCGCCGCCGGCCACC
264	(CCG) 8-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGCCGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
265	(CCG)8-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCGC CGCCGCCGCCGCCGCCGCCGGAGCCACC
266	(CCG)8-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCGCC GCCGCCGCCGCCGCCGCCGAGAGCCACC
267	(CCG)8-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCGCCG CCGCCGCCGCCGCCGCCGAAGAGCCACC
268	(CCG)8-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCGCCGC CGCCGCCGCCGCCGCCGUAAGAGCCACC
269	(CCG)8-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCGCCGCC GCCGCCGCCGCCGCCGAUAAGAGCCACC
270	(CCG)8-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGCCG CCGCCGCCGCCGCCGUAUAAGAGCCACC
271	(CCG)8-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGCCGC CGCCGCCGCCGCCGAUAUAAGAGCCACC
272	(CCG)8-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGCCGCC GCCGCCGCCGCCGAAUAUAAGAGCCACC
273	(CCG)8-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGCCGCCGAAAUAUAAGAGCCACC
274	(CCG)8-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGCCGCCGC CGCCGCCGCCGGAAAUAUAAGAGCCACC
275	(CCG)8-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGCCGCCGCC GCCGCCGCCGAGAAAUAUAAGAGCCACC
276	(CCG)8-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGCCGCCGAAGAAAUAUAAGAGCCACC
277	(CCG)8-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGCCGCCGCCGC CGCCGCCGGAAGAAAUAUAAGAGCCACC
278	(CCG)8-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGCCGCCGCCGCC GCCGCCGAGAAGAAAUAUAAGAGCCACC
279	(CCG)8-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGCCGCCGCCGCCG CCGCCGAAGAAGAAAUAUAAGAGCCACC
280	(CCG)8-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGC CGCCGUAAGAAGAAAUAUAAGAGCCACC
281	(CCG)8-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGCCGCCGCCGCCGCC GCCGGUAAGAAGAAAUAUAAGAGCCACC
282	(CCG)9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CGCCGCCGCCGCCGCCGCCGCCGGCCACC
283	(CCG)9-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGCCGCCGAGCCACC
284	(CCG)9-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCGC CGCCGCCGCCGCCGCCGCCGGAGCCACC
285	(CCG)9-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCGCC GCCGCCGCCGCCGCCGCCGAGAGCCACC
286	(CCG)9-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCGCCG CCGCCGCCGCCGCCGCCGAAGAGCCACC
287	(CCG)9-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCGCCGC CGCCGCCGCCGCCGCCGUAAGAGCCACC
288	(CCG)9-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCGCCGCC GCCGCCGCCGCCGCCGAUAAGAGCCACC
289	(CCG)9-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGCCG CCGCCGCCGCCGCCGCCGUAUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
290	(CCG) 9-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGCCGC CGCCGCCGCCGCCGCCGAUAUAAGAGCCACC
291	(CCG) 9-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGCCGCC GCCGCCGCCGCCGCCGAAUAUAAGAGCCACC
292	(CCG) 9-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGCCGCCGCCGAAAUAUAAGAGCCACC
293	(CCG) 9-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGCCGCCGC CGCCGCCGCCGCCGGAAAUAUAAGAGCCACC
294	(CCG) 9-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGCCGCCGCC GCCGCCGCCGCCGAGAAAUAUAAGAGCCACC
295	(CCG) 9-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGCCGCCGCCGAAGAAAUAUAAGAGCCACC
296	(CCG) 9-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGCCGCCGCCGC CGCCGCCGCCGGAAGAAAUAUAAGAGCCACC
297	(CCG) 9-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGCCGCCGCCGCC GCCGCCGCCGAGAAGAAAUAUAAGAGCCACC
298	(CCG) 9-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGCCGCCGCCGCCG CCGCCGCCGAAGAAGAAAUAUAAGAGCCACC
299	(CCG) 9-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGC CGCCGCCGUAAGAAGAAAUAUAAGAGCCACC
300	(CCG) 9-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGCCGCCGCCGCCGCC GCCGCCGGUAAGAAGAAAUAUAAGAGCCACC
301	(CCG) 10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAC CGCCGCCGCCGCCGCCGCCGCCGCCGCCGGCCACC
302	(CCG) 10-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGCCGCCGCCGCCGAGCCACC
303	(CCG) 10-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAACCGC CGCCGCCGCCGCCGCCGCCGCCGCCGGAGCCACC
304	(CCG) 10-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUACCGCC GCCGCCGCCGCCGCCGCCGCCGCCGAGAGCCACC
305	(CCG) 10-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUCCGCCG CCGCCGCCGCCGCCGCCGCCGCCGAAGAGCCACC
306	(CCG) 10-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUACCGCCGC CGCCGCCGCCGCCGCCGCCGCCGUAAGAGCCACC
307	(CCG) 10-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUCCGCCGCC GCCGCCGCCGCCGCCGCCGCCGAUAAGAGCCACC
308	(CCG) 10-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAACCGCCGCCG CCGCCGCCGCCGCCGCCGCCGUAUAAGAGCCACC
309	(CCG) 10-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAACCGCCGCCGC CGCCGCCGCCGCCGCCGCCGAUAUAAGAGCCACC
310	(CCG) 10-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGACCGCCGCCGCC GCCGCCGCCGCCGCCGCCGAAUAUAAGAGCCACC
311	(CCG) 10-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGCCGCCGCCGCCGAAAUAUAAGAGCCACC
312	(CCG) 10-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAACCGCCGCCGCCGC CGCCGCCGCCGCCGCCGGAAAUAUAAGAGCCACC
313	(CCG) 10-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGACCGCCGCCGCCGCC GCCGCCGCCGCCGCCGAGAAAUAUAAGAGCCACC
314	(CCG) 10-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGCCGCCGCCGCCGAAGAAAUAUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
315	(CCG) 10-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAACCGCCGCCGCCGCCGC CGCCGCCGCCGCCGGAAGAAAUAUAAGAGCCACC
316	(CCG) 10-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUACCGCCGCCGCCGCCGCC GCCGCCGCCGCCGAGAAGAAAUAUAAGAGCCACC
317	(CCG) 10-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUCCGCCGCCGCCGCCGCCG CCGCCGCCGCCGAAGAAGAAAUAUAAGAGCCACC
318	(CCG) 10-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGC CGCCGCCGCCGUAAGAAGAAAUAUAAGAGCCACC
319	(CCG) 10-18-UTR	GGGAAAUAAGAGAGAAAAGAAGACCGCCGCCGCCGCCGCCGCC GCCGCCGCCGGUAAGAAGAAAUAUAAGAGCCACC
320	(GCC) 3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCGCCGCCGCCACC
321	(GCC) 3-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCGCCAGCCACC
322	(GCC) 3-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGAGCCACC
323	(GCC) 3-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCGCCAGAGCCACC
324	(GCC) 3-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC GCCAAGAGCCACC
325	(GCC) 3-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCCGCCG CCUAAGAGCCACC
326	(GCC) 3-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCCGCCGC CAUAAGAGCCACC
327	(GCC) 3-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCGCC UAUAAGAGCCACC
328	(GCC) 3-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCCGCCGCCA UAUAAGAGCCACC
329	(GCC) 3-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCCGCCGCCAA UAUAAGAGCCACC
330	(GCC) 3-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCCGCCGCCAAA UAUAAGAGCCACC
331	(GCC) 3-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGAAA UAUAAGAGCCACC
332	(GCC) 3-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCCGCCGCCAGAAA UAUAAGAGCCACC
333	(GCC) 3-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGGCCGCCGCCAAGAAA UAUAAGAGCCACC
334	(GCC) 3-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGAAGAAA UAUAAGAGCCACC
335	(GCC) 3-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCGCCAGAAGAAA UAUAAGAGCCACC
336	(GCC) 3-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCGCCAAGAAGAAA UAUAAGAGCCACC
337	(GCC) 3-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCGCCUAAGAAGAAA UAUAAGAGCCACC
338	(GCC) 3-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGUAAGAAGAAA UAUAAGAGCCACC
339	(GCC) 4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCGCCGCCGCCGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
340	(GCC)4-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCGCCGCCAGCCACC
341	(GCC)4-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGAGCCACC
342	(GCC)4-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCGCCGCCAGAGCCACC
343	(GCC)4-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC GCCGCCAAGAGCCACC
344	(GCC)4-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCCGCCG CCGCCUAAGAGCCACC
345	(GCC)4-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCCGCCGC CGCCAUAAGAGCCACC
346	(GCC)4-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCGCC GCCUAUAAGAGCCACC
347	(GCC)4-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCCGCCGCCG CCAUAUAAGAGCCACC
348	(GCC)4-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCCGCCGCCGC CAAUAUAAGAGCCACC
349	(GCC)4-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCCGCCGCCGCC AAAUAUAAGAGCCACC
350	(GCC)4-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG AAAUAUAAGAGCCACC
351	(GCC)4-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCCGCCGCCGCCAG AAAUAUAAGAGCCACC
352	(GCC)4-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGGCCGCCGCCGCCAAG AAAUAUAAGAGCCACC
353	(GCC)4-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGAAG AAAUAUAAGAGCCACC
354	(GCC)4-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCGCCGCCAGAAG AAAUAUAAGAGCCACC
355	(GCC)4-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCGCCGCCAAGAAG AAAUAUAAGAGCCACC
356	(GCC)4-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCGCCGCCUAAGAAG AAAUAUAAGAGCCACC
357	(GCC)4-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGUAAGAAG AAAUAUAAGAGCCACC
358	(GCC)5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCGCCGCCGCCGCCGCCACC
359	(GCC)5-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCGCCGCCGCCAGCCACC
360	(GCC)5-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGAGCCACC
361	(GCC)5-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCGCCGCCGCCAGAGCCACC
362	(GCC)5-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC GCCGCCGCCAAGAGCCACC
363	(GCC)5-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCCGCCG CCGCCGCCUAAGAGCCACC
364	(GCC)5-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCCGCCGC CGCCGCCAUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
365	(GCC)5-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCGCC GCCGCCUAUAAGAGCCACC
366	(GCC)5-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCCGCCGCCG CCGCCAUAUAAGAGCCACC
367	(GCC)5-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCCGCCGCCGC CGCCAAUAUAAGAGCCACC
368	(GCC)5-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCCGCCGCCGCC GCCAAAUAUAAGAGCCACC
369	(GCC)5-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGAAAUAUAAGAGCCACC
370	(GCC)5-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCCGCCGCCGCCGC CAGAAAUAUAAGAGCCACC
371	(GCC)5-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGGCCGCCGCCGCCGCC AAGAAAUAUAAGAGCCACC
372	(GCC)5-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG AAGAAAUAUAAGAGCCACC
373	(GCC)5-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCGCCGCCGCCAG AAGAAAUAUAAGAGCCACC
374	(GCC)5-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCGCCGCCGCCAAG AAGAAAUAUAAGAGCCACC
375	(GCC)5-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCGCCGCCGCCUAAG AAGAAAUAUAAGAGCCACC
376	(GCC)5-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGUAAG AAGAAAUAUAAGAGCCACC
377	(GCC)6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCGCCGCCGCCGCCGCCGCCACC
378	(GCC)6-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCGCCGCCGCCGCCAGCCACC
379	(GCC)6-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGAGCCACC
380	(GCC)6-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCGCCGCCGCCGCCAGAGCCACC
381	(GCC)6-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC GCCGCCGCCGCCAAGAGCCACC
382	(GCC)6-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCCGCCG CCGCCGCCGCCUAAGAGCCACC
383	(GCC)6-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCCGCCGC CGCCGCCGCCAUAAGAGCCACC
384	(GCC)6-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCGCC GCCGCCGCCUAUAAGAGCCACC
385	(GCC)6-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCCGCCGCCG CCGCCGCCAUAUAAGAGCCACC
386	(GCC)6-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCCGCCGCCGC CGCCGCCAAUAUAAGAGCCACC
387	(GCC)6-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCCGCCGCCGCC GCCGCCAAAUAUAAGAGCCACC
388	(GCC)6-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGAAAUAUAAGAGCCACC
389	(GCC)6-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCCGCCGCCGCCGC CGCCAGAAAUAUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
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391	(GCC)6-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGAAGAAAUAUAAGAGCCACC
392	(GCC)6-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCGCCGCCGCCGC CAGAAGAAAUAUAAGAGCCACC
393	(GCC)6-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCGCCGCCGCCGCC AAGAAGAAAUAUAAGAGCCACC
394	(GCC)6-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCGCCGCCGCCGCCU AAGAAGAAAUAUAAGAGCCACC
395	(GCC)6-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGU AAGAAGAAAUAUAAGAGCCACC
396	(GCC)7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCGCCGCCGCCGCCGCCGCCGCCACC
397	(GCC)7-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCGCCGCCGCCGCCGCCAGCCACC
398	(GCC)7-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGCCGAGCCACC
399	(GCC)7-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCGCCGCCGCCGCCGCCAGAGCCACC
400	(GCC)7-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC GCCGCCGCCGCCGCCAAGAGCCACC
401	(GCC)7-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCCGCCG CCGCCGCCGCCGCCUAAGAGCCACC
402	(GCC)7-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCCGCCGC CGCCGCCGCCGCCAUAAGAGCCACC
403	(GCC)7-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCGCC GCCGCCGCCGCCUAUAAGAGCCACC
404	(GCC)7-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCCGCCGCCG CCGCCGCCGCCAUAUAAGAGCCACC
405	(GCC)7-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCCGCCGCCGC CGCCGCCGCCAAUAUAAGAGCCACC
406	(GCC)7-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCCGCCGCCGCC GCCGCCGCCAAAUAUAAGAGCCACC
407	(GCC)7-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGCCGAAAUAUAAGAGCCACC
408	(GCC)7-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCCGCCGCCGCCGC CGCCGCCAGAAAUAUAAGAGCCACC
409	(GCC)7-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGGCCGCCGCCGCCGCC GCCGCCAAGAAAUAUAAGAGCCACC
410	(GCC)7-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGCCGAAGAAAUAUAAGAGCCACC
411	(GCC)7-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCGCCGCCGCCGC CGCCAGAAGAAAUAUAAGAGCCACC
412	(GCC)7-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCGCCGCCGCCGCC GCCAAGAAGAAAUAUAAGAGCCACC
413	(GCC)7-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCGCCGCCGCCGCCG CCUAAGAAGAAAUAUAAGAGCCACC
414	(GCC)7-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGC CGUAAGAAGAAAUAUAAGAGCCACC

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SEQUENCE LISTING

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416	(GCC)8-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCGCCGCCGCCGCCGCCAGCCACC
417	(GCC)8-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGCCGCCGAGCCACC
418	(GCC)8-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCGCCGCCGCCGCCGCCGCCAGAGCCACC
419	(GCC)8-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC GCCGCCGCCGCCGCCGCCAAGAGCCACC
420	(GCC)8-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCCGCCG CCGCCGCCGCCGCCGCCUAAGAGCCACC
421	(GCC)8-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCCGCCGC CGCCGCCGCCGCCGCCAUAAGAGCCACC
422	(GCC)8-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCGCC GCCGCCGCCGCCGCCUAUAAGAGCCACC
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426	(GCC)8-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGCCGCCGAAAUAUAAGAGCCACC
427	(GCC)8-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCCGCCGCCGCCGC CGCCGCCGCCAGAAAUAUAAGAGCCACC
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429	(GCC)8-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGCCGCCGAAGAAAUAUAAGAGCCACC
430	(GCC)8-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCGCCGCCGCCGC CGCCGCCAGAAGAAAUAUAAGAGCCACC
431	(GCC)8-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCGCCGCCGCCGCC GCCGCCAAGAAGAAAUAUAAGAGCCACC
432	(GCC)8-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCGCCGCCGCCGCCG CCGCCUAAGAAGAAAUAUAAGAGCCACC
433	(GCC)8-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGC CGCCGUAAGAAGAAAUAUAAGAGCCACC
434	(GCC)9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCGCCGCCGCCGCCGCCGCCGCCGCCACC
435	(GCC)9-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCGCCGCCGCCGCCGCCAGCCACC
436	(GCC)9-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGCCGCCGAGCCACC
437	(GCC)9-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCGCCGCCGCCGCCGCCGCCAGAGCCACC
438	(GCC)9-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC GCCGCCGCCGCCGCCGCCAAGAGCCACC
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SEQ ID NO	Description	Sequence
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441	(GCC) 9-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCGCC GCCGCCGCCGCCGCCGCCUAUAAGAGCCACC
442	(GCC) 9-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCCGCCGCCG CCGCCGCCGCCGCCGCCAUAUAAGAGCCACC
443	(GCC) 9-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCCGCCGCCGC CGCCGCCGCCGCCGCCAAUAUAAGAGCCACC
444	(GCC) 9-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCCGCCGCCGCC GCCGCCGCCGCCGCCAAAUAUAAGAGCCACC
445	(GCC) 9-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGCCGCCGCCGAAAUAUAAGAGCCACC
446	(GCC) 9-12-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAGCCGCCGCCGCCGC CGCCGCCGCCGCCAGAAAUAUAAGAGCCACC
447	(GCC) 9-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGGCCGCCGCCGCCGCC GCCGCCGCCGCCAAGAAAUAUAAGAGCCACC
448	(GCC) 9-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGCCGCCGCCGAAGAAAUAUAAGAGCCACC
449	(GCC) 9-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCGCCGCCGCCGC CGCCGCCGCCAGAAGAAAUAUAAGAGCCACC
450	(GCC) 9-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCGCCGCCGCCGCC GCCGCCGCCAAGAAGAAAUAUAAGAGCCACC
451	(GCC) 9-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCGCCGCCGCCGCCG CCGCCGCCUAAGAAGAAAUAUAAGAGCCACC
452	(GCC) 9-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGC CGCCGCCGUAAGAAGAAAUAUAAGAGCCACC
453	(GCC) 10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAG CCGCCGCCGCCGCCGCCGCCGCCGCCGCCACC
454	(GCC) 10-1-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGGC CGCCGCCGCCGCCGCCGCCGCCGCCGCCAGCCACC
455	(GCC) 10-2-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGCC GCCGCCGCCGCCGCCGCCGCCGCCGCCGAGCCACC
456	(GCC) 10-3-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAGCCG CCGCCGCCGCCGCCGCCGCCGCCGCCAGAGCCACC
457	(GCC) 10-4-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUGCCGCC GCCGCCGCCGCCGCCGCCGCCGCCAAGAGCCACC
458	(GCC) 10-5-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAGCCGCCG CCGCCGCCGCCGCCGCCGCCGCCUAAGAGCCACC
459	(GCC) 10-6-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUGCCGCCGC CGCCGCCGCCGCCGCCGCCGCCAUAAGAGCCACC
460	(GCC) 10-7-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAGCCGCCGCC GCCGCCGCCGCCGCCGCCGCCUAUAAGAGCCACC
461	(GCC) 10-8-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAGCCGCCGCCG CCGCCGCCGCCGCCGCCGCCAUAUAAGAGCCACC
462	(GCC) 10-9-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAGCCGCCGCCGC CGCCGCCGCCGCCGCCGCCAAUAUAAGAGCCACC
463	(GCC) 10-10-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGGCCGCCGCCGCC GCCGCCGCCGCCGCCGCCGCCAAAUAUAAGAGCCACC
464	(GCC) 10-11-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGCCGCCGCCGCCG CCGCCGCCGCCGCCGCCGCCGAAAUAUAAGAGCCACC

TABLE 9-continued

SEQUENCE LISTING

SEQ ID NO	Description	Sequence
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466	(GCC)10-13-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGGCCGCCGCCGCCGCC GCCGCCGCCGCCGCCAAGAAAUAUAAGAGCCACC
467	(GCC)10-14-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGCCGCCGCCGCCGCCG CCGCCGCCGCCGCCGAAGAAAUAUAAGAGCCACC
468	(GCC)10-15-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAGCCGCCGCCGCCGCCGC CGCCGCCGCCGCCAGAAGAAAUAUAAGAGCCACC
469	(GCC)10-16-UTR	GGGAAAUAAGAGAGAAAAGAAGAGUGCCGCCGCCGCCGCCGCC GCCGCCGCCGCCAAGAAGAAAUAUAAGAGCCACC
470	(GCC)10-17-UTR	GGGAAAUAAGAGAGAAAAGAAGAGGCCGCCGCCGCCGCCGCCG CCGCCGCCGCCUAAGAAGAAAUAUAAGAGCCACC
471	(GCC)10-18-UTR	GGGAAAUAAGAGAGAAAAGAAGAGCCGCCGCCGCCGCCGCCGC CGCCGCCGCCGUAAGAAGAAAUAUAAGAGCCACC
472	KT1-UTR	GGGCCCGCCGCCAAC
473	KT2-UTR	GGGCCCGCCGCCACC
474	KT3-UTR	GGGCCCGCCGCCGAC
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gggaaauaag agagaaaaga agaguccccg gcgccaagaa gaaauauaag agccacc 57

<210> SEQ ID NO 51
<211> LENGTH: 57
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V1-17-UTR

<400> SEQUENCE: 51

gggaaauaag agagaaaaga agagccccgg cgccuaagaa gaaauauaag agccacc 57

<210> SEQ ID NO 52
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V3-UTR

<400> SEQUENCE: 52

gggaaauaag agagaaaaga agaccccggc gccguaagaa gaaauauaag agccacc 57

<210> SEQ ID NO 53
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V4-UTR

<400> SEQUENCE: 53

gggccccggc gccaaauaag agagaaaaga agaguaagaa gaaauauaag agccacc 57

<210> SEQ ID NO 54
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-UTR

<400> SEQUENCE: 54

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accccggcgc cacc 54

<210> SEQ ID NO 55
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-1-UTR

<400> SEQUENCE: 55

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccccggcagc cacc 54

<210> SEQ ID NO 56
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-2-UTR

<400> SEQUENCE: 56

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cccggcgagc cacc 54

<210> SEQ ID NO 57
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-3-UTR

<400> SEQUENCE: 57

gggaaauaag agagaaaaga agaguaagaa gaaauauacc ccggcagagc cacc 54

<210> SEQ ID NO 58
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-4-UTR

<400> SEQUENCE: 58

gggaaauaag agagaaaaga agaguaagaa gaaauauccc cggcaagagc cacc 54

<210> SEQ ID NO 59
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-5-UTR

<400> SEQUENCE: 59

gggaaauaag agagaaaaga agaguaagaa gaaauacccc ggcuaagagc cacc 54

<210> SEQ ID NO 60
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-6-UTR

<400> SEQUENCE: 60

gggaaauaag agagaaaaga agaguaagaa gaaaucccccg gcauaagagc cacc 54

<210> SEQ ID NO 61
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-7-UTR

<400> SEQUENCE: 61

gggaaauaag agagaaaaga agaguaagaa gaaaccccgg cuauaagagc cacc 54

<210> SEQ ID NO 62
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-8-UTR

<400> SEQUENCE: 62

gggaaauaag agagaaaaga agaguaagaa gaaccccggc auauaagagc cacc 54

<210> SEQ ID NO 63
<211> LENGTH: 54
<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-9-UTR

<400> SEQUENCE: 63

gggaaauaag agagaaaaga agaguaagaa gaccccggca auauaagagc cacc 54

<210> SEQ ID NO 64
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-10-UTR

<400> SEQUENCE: 64

gggaaauaag agagaaaaga agaguaagaa gccccggcaa auauaagagc cacc 54

<210> SEQ ID NO 65
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-11-UTR

<400> SEQUENCE: 65

gggaaauaag agagaaaaga agaguaagaa ccccggcgaa auauaagagc cacc 54

<210> SEQ ID NO 66
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-12-UTR

<400> SEQUENCE: 66

gggaaauaag agagaaaaga agaguaagac cccggcagaa auauaagagc cacc 54

<210> SEQ ID NO 67
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-13-UTR

<400> SEQUENCE: 67

gggaaauaag agagaaaaga agaguaagcc ccggcaagaa auauaagagc cacc 54

<210> SEQ ID NO 68
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<212> TYPE: RNA
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<223> OTHER INFORMATION: Synthetic: V2-14-UTR

<400> SEQUENCE: 68

gggaaauaag agagaaaaga agaguaaccc cggcgaagaa auauaagagc cacc 54

<210> SEQ ID NO 69
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-15-UTR

<400> SEQUENCE: 69

gggaaauaag agagaaaaga agaguacccc ggcagaagaa auauaagagc cacc 54

<210> SEQ ID NO 70
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-16-UTR

<400> SEQUENCE: 70

gggaaauaag agagaaaaga agaguccccg gcaagaagaa auauaagagc cacc 54

<210> SEQ ID NO 71
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-17-UTR

<400> SEQUENCE: 71

gggaaauaag agagaaaaga agagccccgg cuaagaagaa auauaagagc cacc 54

<210> SEQ ID NO 72
<211> LENGTH: 54
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V2-18-UTR

<400> SEQUENCE: 72

gggaaauaag agagaaaaga agaccccggc guaagaagaa auauaagagc cacc 54

<210> SEQ ID NO 73
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-UTR

<400> SEQUENCE: 73

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agcgccccgc ggcgccccgc 60

ggccacc 67

<210> SEQ ID NO 74
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic: CG1-1-UTR

<400> SEQUENCE: 74

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gcgccccgcg gcgccccgcg 60

agccacc 67

<210> SEQ ID NO 75
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic: CG1-2-UTR

<400> SEQUENCE: 75

gggaaauaag agagaaaaga agaguaagaa gaaauauaag cgccccgcgg cgccccgcgg 60

agccacc 67

<210> SEQ ID NO 76
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-3-UTR

<400> SEQUENCE: 76

gggaaauaag agagaaaaga agaguaagaa gaaauauagc gccccgcggc gccccgcgag 60

agccacc 67

<210> SEQ ID NO 77
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-4-UTR

<400> SEQUENCE: 77

gggaaauaag agagaaaaga agaguaagaa gaaauaugcg ccccgcggcg ccccgcgaag 60

agccacc 67

<210> SEQ ID NO 78
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-5-UTR

<400> SEQUENCE: 78

gggaaauaag agagaaaaga agaguaagaa gaaauagcgc cccgcggcgc cccgcguaag 60

agccacc 67

<210> SEQ ID NO 79
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-6-UTR

<400> SEQUENCE: 79

gggaaauaag agagaaaaga agaguaagaa gaaaugcgcc ccgcggcgcc ccgcgauaag 60

agccacc 67

<210> SEQ ID NO 80
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-7-UTR

<400> SEQUENCE: 80

gggaaauaag agagaaaaga agaguaagaa gaaagcgccc cgcggcgccc cgcguauaag 60

agccacc 67

<210> SEQ ID NO 81
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-8-UTR

<400> SEQUENCE: 81

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gggaaauaag agagaaaaga agaguaagaa gaagcgcccc gcggcgcccc gcgauauaag 60

agccacc 67

<210> SEQ ID NO 82
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-9-UTR

<400> SEQUENCE: 82

gggaaauaag agagaaaaga agaguaagaa gagcgccccg cggcgccccg cgaauauaag 60

agccacc 67

<210> SEQ ID NO 83
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-10-UTR

<400> SEQUENCE: 83

gggaaauaag agagaaaaga agaguaagaa ggcgccccgc ggcgccccgc gaaauauaag 60

agccacc 67

<210> SEQ ID NO 84
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-11-UTR

<400> SEQUENCE: 84

gggaaauaag agagaaaaga agaguaagaa gcgccccgcg gcgccccgcg gaaauauaag 60

agccacc 67

<210> SEQ ID NO 85
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-12-UTR

<400> SEQUENCE: 85

gggaaauaag agagaaaaga agaguaagag cgccccgcgg cgccccgcga gaaauauaag 60

agccacc 67

<210> SEQ ID NO 86
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-13-UTR

<400> SEQUENCE: 86

gggaaauaag agagaaaaga agaguaaggc gccccgcggc gccccgcgaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 87
<211> LENGTH: 67
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-14-UTR

<400> SEQUENCE: 87

gggaaauaag agagaaaaga agaguaagcg ccccgcggcg ccccgcggaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 88
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-15-UTR

<400> SEQUENCE: 88

gggaaauaag agagaaaaga agaguagcgc cccgcggcgc cccgcgagaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 89
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-16-UTR

<400> SEQUENCE: 89

gggaaauaag agagaaaaga agagugcgcc ccgcggcgcc ccgcgaagaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 90
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-17-UTR

<400> SEQUENCE: 90

gggaaauaag agagaaaaga agaggcgccc cgcggcgccc cgcguaagaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 91
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG1-18-UTR

<400> SEQUENCE: 91

gggaaauaag agagaaaaga agagcgcccc gcggcgcccc gcgguaagaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 92
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-UTR

<400> SEQUENCE: 92

gggaaauaag agagaaaaga agaguaagaa gaaauauaag acccgcccgc cccgccccgc 60

cgccacc 67

<210> SEQ ID NO 93
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-1-UTR

<400> SEQUENCE: 93

gggaaauaag agagaaaaga agaguaagaa gaaauauaag cccgcccgcc ccgccccgcc 60

agccacc 67

<210> SEQ ID NO 94
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-2-UTR

<400> SEQUENCE: 94

gggaaauaag agagaaaaga agaguaagaa gaaauauaac ccgcccgccc cgccccgccg 60

agccacc 67

<210> SEQ ID NO 95
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-3-UTR

<400> SEQUENCE: 95

gggaaauaag agagaaaaga agaguaagaa gaaauauacc cgcccgcccc gccccgccag 60

agccacc 67

<210> SEQ ID NO 96
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-4-UTR

<400> SEQUENCE: 96

gggaaauaag agagaaaaga agaguaagaa gaaauauccc gcccgccccg ccccgccaag 60

agccacc 67

<210> SEQ ID NO 97
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-5-UTR

<400> SEQUENCE: 97

gggaaauaag agagaaaaga agaguaagaa gaaauacccg cccgccccgc cccgccuaag 60

agccacc 67

<210> SEQ ID NO 98
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-6-UTR

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<400> SEQUENCE: 98

gggaaauaag agagaaaaga agaguaagaa gaaaucccgc ccgccccgcc ccgccauaag 60

agccacc 67

<210> SEQ ID NO 99
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-7-UTR

<400> SEQUENCE: 99

gggaaauaag agagaaaaga agaguaagaa gaaacccgcc cgccccgccc cgccuauaag 60

agccacc 67

<210> SEQ ID NO 100
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-8-UTR

<400> SEQUENCE: 100

gggaaauaag agagaaaaga agaguaagaa gaacccgccc gccccgcccc gccauauaag 60

agccacc 67

<210> SEQ ID NO 101
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-9-UTR

<400> SEQUENCE: 101

gggaaauaag agagaaaaga agaguaagaa gacccgcccg ccccgccccg ccaauauaag 60

agccacc 67

<210> SEQ ID NO 102
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-10-UTR

<400> SEQUENCE: 102

gggaaauaag agagaaaaga agaguaagaa gcccgcccgc cccgccccgc caaauauaag 60

agccacc 67

<210> SEQ ID NO 103
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-11-UTR

<400> SEQUENCE: 103

gggaaauaag agagaaaaga agaguaagaa cccgcccgcc ccgccccgcc gaaauauaag 60

agccacc 67

<210> SEQ ID NO 104
<211> LENGTH: 67

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-12-UTR

<400> SEQUENCE: 104

gggaaauaag agagaaaaga agaguaagac ccgcccgccc cgccccgcca gaaauauaag 60

agccacc 67

<210> SEQ ID NO 105
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-13-UTR

<400> SEQUENCE: 105

gggaaauaag agagaaaaga agaguaagcc cgcccgcccc gccccgccaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 106
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-14-UTR

<400> SEQUENCE: 106

gggaaauaag agagaaaaga agaguaaccc gcccgccccg ccccgccgaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 107
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-15-UTR

<400> SEQUENCE: 107

gggaaauaag agagaaaaga agaguacccg cccgccccgc cccgccagaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 108
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-16-UTR

<400> SEQUENCE: 108

gggaaauaag agagaaaaga agagucccgc ccgccccgcc ccgccaagaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 109
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-17-UTR

<400> SEQUENCE: 109

gggaaauaag agagaaaaga agagcccgcc cgccccgccc cgccuaagaa gaaauauaag 60

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agccacc 67

<210> SEQ ID NO 110
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CG2-18-UTR

<400> SEQUENCE: 110

gggaaauaag agagaaaaga agacccgccc gccccgcccc gccguaagaa gaaauauaag 60

agccacc 67

<210> SEQ ID NO 111
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-UTR

<400> SEQUENCE: 111

gggaaauaag agagaaaaga agaguaagaa gaaauauaag acccgccgcc acc 53

<210> SEQ ID NO 112
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-1-UTR

<400> SEQUENCE: 112

gggaaauaag agagaaaaga agaguaagaa gaaauauaag cccgccagcc acc 53

<210> SEQ ID NO 113
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-2-UTR

<400> SEQUENCE: 113

gggaaauaag agagaaaaga agaguaagaa gaaauauaac ccgccgagcc acc 53

<210> SEQ ID NO 114
<211> LENGTH: 53
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-3-UTR

<400> SEQUENCE: 114

gggaaauaag agagaaaaga agaguaagaa gaaauauacc cgccagagcc acc 53

<210> SEQ ID NO 115
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-4-UTR

<400> SEQUENCE: 115

gggaaauaag agagaaaaga agaguaagaa gaaauauccc gccaagagcc acc 53

<210> SEQ ID NO 116
<211> LENGTH: 53

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-5-UTR

<400> SEQUENCE: 116

gggaaauaag agagaaaaga agaguaagaa gaaauacccg ccuaagagcc acc 53

<210> SEQ ID NO 117
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-6-UTR

<400> SEQUENCE: 117

gggaaauaag agagaaaaga agaguaagaa gaaaucccgc cauaagagcc acc 53

<210> SEQ ID NO 118
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-7-UTR

<400> SEQUENCE: 118

gggaaauaag agagaaaaga agaguaagaa gaaacccgcc uauaagagcc acc 53

<210> SEQ ID NO 119
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-8-UTR

<400> SEQUENCE: 119

gggaaauaag agagaaaaga agaguaagaa gaacccgcca uauaagagcc acc 53

<210> SEQ ID NO 120
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-9-UTR

<400> SEQUENCE: 120

gggaaauaag agagaaaaga agaguaagaa gacccgccaa uauaagagcc acc 53

<210> SEQ ID NO 121
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-10-UTR

<400> SEQUENCE: 121

gggaaauaag agagaaaaga agaguaagaa gcccgccaaa uauaagagcc acc 53

<210> SEQ ID NO 122
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-11-UTR

<400> SEQUENCE: 122

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gggaaauaag agagaaaaga agaguaagaa cccgccgaaa uauaagagcc acc 53

<210> SEQ ID NO 123
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-12-UTR

<400> SEQUENCE: 123

gggaaauaag agagaaaaga agaguaagac ccgccagaaa uauaagagcc acc 53

<210> SEQ ID NO 124
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-13-UTR

<400> SEQUENCE: 124

gggaaauaag agagaaaaga agaguaagcc cgccaagaaa uauaagagcc acc 53

<210> SEQ ID NO 125
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-14-UTR

<400> SEQUENCE: 125

gggaaauaag agagaaaaga agaguaaccc gccgaagaaa uauaagagcc acc 53

<210> SEQ ID NO 126
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-15-UTR

<400> SEQUENCE: 126

gggaaauaag agagaaaaga agaguacccg ccagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 127
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-16-UTR

<400> SEQUENCE: 127

gggaaauaag agagaaaaga agagucccgc caagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 128
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-17-UTR

<400> SEQUENCE: 128

gggaaauaag agagaaaaga agagcccgcc uaagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 129
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK1-18-UTR

<400> SEQUENCE: 129

gggaaauaag agagaaaaga agacccgccg uaagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 130
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-UTR

<400> SEQUENCE: 130

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc acc 53

<210> SEQ ID NO 131
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-1-UTR

<400> SEQUENCE: 131

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccagcc acc 53

<210> SEQ ID NO 132
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-2-UTR

<400> SEQUENCE: 132

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgagcc acc 53

<210> SEQ ID NO 133
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-3-UTR

<400> SEQUENCE: 133

gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccagagcc acc 53

<210> SEQ ID NO 134
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-4-UTR

<400> SEQUENCE: 134

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccaagagcc acc 53

<210> SEQ ID NO 135
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-5-UTR

<400> SEQUENCE: 135

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccuaagagcc acc 53

<210> SEQ ID NO 136
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-6-UTR

<400> SEQUENCE: 136

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cauaagagcc acc 53

<210> SEQ ID NO 137
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-7-UTR

<400> SEQUENCE: 137

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc uauaagagcc acc 53

<210> SEQ ID NO 138
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-8-UTR

<400> SEQUENCE: 138

gggaaauaag agagaaaaga agaguaagaa gaagccgcca uauaagagcc acc 53

<210> SEQ ID NO 139
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-9-UTR

<400> SEQUENCE: 139

gggaaauaag agagaaaaga agaguaagaa gagccgccaa uauaagagcc acc 53

<210> SEQ ID NO 140
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-10-UTR

<400> SEQUENCE: 140

gggaaauaag agagaaaaga agaguaagaa ggccgccaaa uauaagagcc acc 53

<210> SEQ ID NO 141
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-11-UTR

<400> SEQUENCE: 141

gggaaauaag agagaaaaga agaguaagaa gccgccgaaa uauaagagcc acc 53

<210> SEQ ID NO 142
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-12-UTR

<400> SEQUENCE: 142

gggaaauaag agagaaaaga agaguaagag ccgccagaaa uauaagagcc acc 53

<210> SEQ ID NO 143
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-13-UTR

<400> SEQUENCE: 143

gggaaauaag agagaaaaga agaguaaggc cgccaagaaa uauaagagcc acc 53

<210> SEQ ID NO 144
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-14-UTR

<400> SEQUENCE: 144

gggaaauaag agagaaaaga agaguaagcc gccgaagaaa uauaagagcc acc 53

<210> SEQ ID NO 145
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-15-UTR

<400> SEQUENCE: 145

gggaaauaag agagaaaaga agaguagccg ccagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 146
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-16-UTR

<400> SEQUENCE: 146

gggaaauaag agagaaaaga agagugccgc caagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 147
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-17-UTR

<400> SEQUENCE: 147

gggaaauaag agagaaaaga agaggccgcc uaagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 148
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK2-18-UTR

<400> SEQUENCE: 148

gggaaauaag agagaaaaga agagccgccg uaagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 149

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<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-UTR

<400> SEQUENCE: 149

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccggcc acc 53

<210> SEQ ID NO 150
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-1-UTR

<400> SEQUENCE: 150

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgagcc acc 53

<210> SEQ ID NO 151
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-2-UTR

<400> SEQUENCE: 151

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccggagcc acc 53

<210> SEQ ID NO 152
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-3-UTR

<400> SEQUENCE: 152

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgagagcc acc 53

<210> SEQ ID NO 153
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-4-UTR

<400> SEQUENCE: 153

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgaagagcc acc 53

<210> SEQ ID NO 154
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-5-UTR

<400> SEQUENCE: 154

gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cguaagagcc acc 53

<210> SEQ ID NO 155
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-6-UTR

<400> SEQUENCE: 155

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gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gauaagagcc acc 53

<210> SEQ ID NO 156
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-7-UTR

<400> SEQUENCE: 156

gggaaauaag agagaaaaga agaguaagaa gaaaccgccg uauaagagcc acc 53

<210> SEQ ID NO 157
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-8-UTR

<400> SEQUENCE: 157

gggaaauaag agagaaaaga agaguaagaa gaaccgccga uauaagagcc acc 53

<210> SEQ ID NO 158
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-9-UTR

<400> SEQUENCE: 158

gggaaauaag agagaaaaga agaguaagaa gaccgccgaa uauaagagcc acc 53

<210> SEQ ID NO 159
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-10-UTR

<400> SEQUENCE: 159

gggaaauaag agagaaaaga agaguaagaa gccgccgaaa uauaagagcc acc 53

<210> SEQ ID NO 160
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-11-UTR

<400> SEQUENCE: 160

gggaaauaag agagaaaaga agaguaagaa ccgccggaaa uauaagagcc acc 53

<210> SEQ ID NO 161
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-12-UTR

<400> SEQUENCE: 161

gggaaauaag agagaaaaga agaguaagac cgccgagaaa uauaagagcc acc 53

<210> SEQ ID NO 162
<211> LENGTH: 53
<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-13-UTR

<400> SEQUENCE: 162

gggaaauaag agagaaaaga agaguaagcc gccgaagaaa uauaagagcc acc 53

<210> SEQ ID NO 163
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-14-UTR

<400> SEQUENCE: 163

gggaaauaag agagaaaaga agaguaaccg ccggaagaaa uauaagagcc acc 53

<210> SEQ ID NO 164
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-15-UTR

<400> SEQUENCE: 164

gggaaauaag agagaaaaga agaguaccgc cgagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 165
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-16-UTR

<400> SEQUENCE: 165

gggaaauaag agagaaaaga agaguccgcc gaagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 166
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-17-UTR

<400> SEQUENCE: 166

gggaaauaag agagaaaaga agagccgccg uaagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 167
<211> LENGTH: 53
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: EK3-18-UTR

<400> SEQUENCE: 167

gggaaauaag agagaaaaga agaccgccgg uaagaagaaa uauaagagcc acc 53

<210> SEQ ID NO 168
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-UTR

<400> SEQUENCE: 168

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccgccg gccacc 56

<210> SEQ ID NO 169
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-1-UTR

<400> SEQUENCE: 169

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccga gccacc 56

<210> SEQ ID NO 170
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-2-UTR

<400> SEQUENCE: 170

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccgccgga gccacc 56

<210> SEQ ID NO 171
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-3-UTR

<400> SEQUENCE: 171

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgccgaga gccacc 56

<210> SEQ ID NO 172
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-4-UTR

<400> SEQUENCE: 172

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgccgaaga gccacc 56

<210> SEQ ID NO 173
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-5-UTR

<400> SEQUENCE: 173

gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cgccguaaga gccacc 56

<210> SEQ ID NO 174
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-6-UTR

<400> SEQUENCE: 174

gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gccgauaaga gccacc 56

<210> SEQ ID NO 175
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic: (CCG)3-7-UTR

<400> SEQUENCE: 175

gggaaauaag agagaaaaga agaguaagaa gaaaccgccg ccguauaaga gccacc 56

<210> SEQ ID NO 176
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-8-UTR

<400> SEQUENCE: 176

gggaaauaag agagaaaaga agaguaagaa gaaccgccgc cgauauaaga gccacc 56

<210> SEQ ID NO 177
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-9-UTR

<400> SEQUENCE: 177

gggaaauaag agagaaaaga agaguaagaa gaccgccgcc gaauauaaga gccacc 56

<210> SEQ ID NO 178
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-10-UTR

<400> SEQUENCE: 178

gggaaauaag agagaaaaga agaguaagaa gccgccgccg aaauauaaga gccacc 56

<210> SEQ ID NO 179
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-11-UTR

<400> SEQUENCE: 179

gggaaauaag agagaaaaga agaguaagaa ccgccgccgg aaauauaaga gccacc 56

<210> SEQ ID NO 180
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-12-UTR

<400> SEQUENCE: 180

gggaaauaag agagaaaaga agaguaagac cgccgccgag aaauauaaga gccacc 56

<210> SEQ ID NO 181
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-13-UTR

<400> SEQUENCE: 181

gggaaauaag agagaaaaga agaguaagcc gccgccgaag aaauauaaga gccacc 56

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<210> SEQ ID NO 182
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-14-UTR

<400> SEQUENCE: 182

gggaaauaag agagaaaaga agaguaaccg ccgccggaag aaauauaaga gccacc 56

<210> SEQ ID NO 183
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-15-UTR

<400> SEQUENCE: 183

gggaaauaag agagaaaaga agaguaccgc cgccgagaag aaauauaaga gccacc 56

<210> SEQ ID NO 184
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-16-UTR

<400> SEQUENCE: 184

gggaaauaag agagaaaaga agaguccgcc gccgaagaag aaauauaaga gccacc 56

<210> SEQ ID NO 185
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-17-UTR

<400> SEQUENCE: 185

gggaaauaag agagaaaaga agagccgccg ccguaagaag aaauauaaga gccacc 56

<210> SEQ ID NO 186
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)3-18-UTR

<400> SEQUENCE: 186

gggaaauaag agagaaaaga agaccgccgc cgguaagaag aaauauaaga gccacc 56

<210> SEQ ID NO 187
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-UTR

<400> SEQUENCE: 187

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccgccg ccggccacc 59

<210> SEQ ID NO 188
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-1-UTR

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<400> SEQUENCE: 188

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgagccacc 59

<210> SEQ ID NO 189
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-2-UTR

<400> SEQUENCE: 189

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccgccgcc ggagccacc 59

<210> SEQ ID NO 190
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-3-UTR

<400> SEQUENCE: 190

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgccgccg agagccacc 59

<210> SEQ ID NO 191
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-4-UTR

<400> SEQUENCE: 191

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgccgccga agagccacc 59

<210> SEQ ID NO 192
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-5-UTR

<400> SEQUENCE: 192

gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cgccgccgua agagccacc 59

<210> SEQ ID NO 193
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-6-UTR

<400> SEQUENCE: 193

gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gccgccgaua agagccacc 59

<210> SEQ ID NO 194
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-7-UTR

<400> SEQUENCE: 194

gggaaauaag agagaaaaga agaguaagaa gaaaccgccg ccgccguaua agagccacc 59

<210> SEQ ID NO 195
<211> LENGTH: 59

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-8-UTR

<400> SEQUENCE: 195

gggaaauaag agagaaaaga agaguaagaa gaaccgccgc cgccgauaua agagccacc 59

<210> SEQ ID NO 196
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-9-UTR

<400> SEQUENCE: 196

gggaaauaag agagaaaaga agaguaagaa gaccgccgcc gccgaauaua agagccacc 59

<210> SEQ ID NO 197
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-10-UTR

<400> SEQUENCE: 197

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgaaauaua agagccacc 59

<210> SEQ ID NO 198
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-11-UTR

<400> SEQUENCE: 198

gggaaauaag agagaaaaga agaguaagaa ccgccgccgc cggaaauaua agagccacc 59

<210> SEQ ID NO 199
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-12-UTR

<400> SEQUENCE: 199

gggaaauaag agagaaaaga agaguaagac cgccgccgcc gagaaauaua agagccacc 59

<210> SEQ ID NO 200
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-13-UTR

<400> SEQUENCE: 200

gggaaauaag agagaaaaga agaguaagcc gccgccgccg aagaaauaua agagccacc 59

<210> SEQ ID NO 201
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-14-UTR

<400> SEQUENCE: 201

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gggaaauaag agagaaaaga agaguaaccg ccgccgccgg aagaaauaua agagccacc 59

<210> SEQ ID NO 202
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-15-UTR

<400> SEQUENCE: 202

gggaaauaag agagaaaaga agaguaccgc cgccgccgag aagaaauaua agagccacc 59

<210> SEQ ID NO 203
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-16-UTR

<400> SEQUENCE: 203

gggaaauaag agagaaaaga agaguccgcc gccgccgaag aagaaauaua agagccacc 59

<210> SEQ ID NO 204
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-17-UTR

<400> SEQUENCE: 204

gggaaauaag agagaaaaga agagccgccg ccgccguaag aagaaauaua agagccacc 59

<210> SEQ ID NO 205
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)4-18-UTR

<400> SEQUENCE: 205

gggaaauaag agagaaaaga agaccgccgc cgccgguaag aagaaauaua agagccacc 59

<210> SEQ ID NO 206
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-UTR

<400> SEQUENCE: 206

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccgccg ccgccggcca 60

cc 62

<210> SEQ ID NO 207
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-1-UTR

<400> SEQUENCE: 207

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgagcca 60

cc 62

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<210> SEQ ID NO 208
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-2-UTR

<400> SEQUENCE: 208

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccgccgcc gccggagcca 60

cc 62

<210> SEQ ID NO 209
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-3-UTR

<400> SEQUENCE: 209

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgccgccg ccgagagcca 60

cc 62

<210> SEQ ID NO 210
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-4-UTR

<400> SEQUENCE: 210

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgccgccgc cgaagagcca 60

cc 62

<210> SEQ ID NO 211
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-5-UTR

<400> SEQUENCE: 211

gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cgccgccgcc guaagagcca 60

cc 62

<210> SEQ ID NO 212
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-6-UTR

<400> SEQUENCE: 212

gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gccgccgccg auaagagcca 60

cc 62

<210> SEQ ID NO 213
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-7-UTR

<400> SEQUENCE: 213

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gggaaauaag agagaaaaga agaguaagaa gaaaccgccg ccgccgccgu auaagagcca 60

cc 62

<210> SEQ ID NO 214
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-8-UTR

<400> SEQUENCE: 214

gggaaauaag agagaaaaga agaguaagaa gaaccgccgc cgccgccgau auaagagcca 60

cc 62

<210> SEQ ID NO 215
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-9-UTR

<400> SEQUENCE: 215

gggaaauaag agagaaaaga agaguaagaa gaccgccgcc gccgccgaau auaagagcca 60

cc 62

<210> SEQ ID NO 216
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-10-UTR

<400> SEQUENCE: 216

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgaaau auaagagcca 60

cc 62

<210> SEQ ID NO 217
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-11-UTR

<400> SEQUENCE: 217

gggaaauaag agagaaaaga agaguaagaa ccgccgccgc cgccggaaau auaagagcca 60

cc 62

<210> SEQ ID NO 218
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-12-UTR

<400> SEQUENCE: 218

gggaaauaag agagaaaaga agaguaagac cgccgccgcc gccgagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 219
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-13-UTR

<400> SEQUENCE: 219

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 220
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-14-UTR

<400> SEQUENCE: 220

gggaaauaag agagaaaaga agaguaaccg ccgccgccgc cggaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 221
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-15-UTR

<400> SEQUENCE: 221

gggaaauaag agagaaaaga agaguaccgc cgccgccgcc gagaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 222
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-16-UTR

<400> SEQUENCE: 222

gggaaauaag agagaaaaga agaguccgcc gccgccgccg aagaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 223
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-17-UTR

<400> SEQUENCE: 223

gggaaauaag agagaaaaga agagccgccg ccgccgccgu aagaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 224
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)5-18-UTR

<400> SEQUENCE: 224

gggaaauaag agagaaaaga agaccgccgc cgccgccggu aagaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 225
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-UTR

<400> SEQUENCE: 225

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccgccg ccgccgccgg 60

ccacc 65

<210> SEQ ID NO 226
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-1-UTR

<400> SEQUENCE: 226

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgag 60

ccacc 65

<210> SEQ ID NO 227
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-2-UTR

<400> SEQUENCE: 227

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccgccgcc gccgccggag 60

ccacc 65

<210> SEQ ID NO 228
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-3-UTR

<400> SEQUENCE: 228

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgccgccg ccgccgagag 60

ccacc 65

<210> SEQ ID NO 229
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-4-UTR

<400> SEQUENCE: 229

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgccgccgc cgccgaagag 60

ccacc 65

<210> SEQ ID NO 230
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-5-UTR

<400> SEQUENCE: 230

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gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cgccgccgcc gccguaagag 60

ccacc 65

<210> SEQ ID NO 231
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-6-UTR

<400> SEQUENCE: 231

gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gccgccgccg ccgauaagag 60

ccacc 65

<210> SEQ ID NO 232
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-7-UTR

<400> SEQUENCE: 232

gggaaauaag agagaaaaga agaguaagaa gaaaccgccg ccgccgccgc cguauaagag 60

ccacc 65

<210> SEQ ID NO 233
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-8-UTR

<400> SEQUENCE: 233

gggaaauaag agagaaaaga agaguaagaa gaaccgccgc cgccgccgcc gauauaagag 60

ccacc 65

<210> SEQ ID NO 234
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-9-UTR

<400> SEQUENCE: 234

gggaaauaag agagaaaaga agaguaagaa gaccgccgcc gccgccgccg aauauaagag 60

ccacc 65

<210> SEQ ID NO 235
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-10-UTR

<400> SEQUENCE: 235

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccga aauauaagag 60

ccacc 65

<210> SEQ ID NO 236
<211> LENGTH: 65
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-11-UTR

<400> SEQUENCE: 236

gggaaauaag agagaaaaga agaguaagaa ccgccgccgc cgccgccgga aauauaagag 60

ccacc 65

<210> SEQ ID NO 237
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-12-UTR

<400> SEQUENCE: 237

gggaaauaag agagaaaaga agaguaagac cgccgccgcc gccgccgaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 238
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-13-UTR

<400> SEQUENCE: 238

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 239
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-14-UTR

<400> SEQUENCE: 239

gggaaauaag agagaaaaga agaguaaccg ccgccgccgc cgccggaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 240
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-15-UTR

<400> SEQUENCE: 240

gggaaauaag agagaaaaga agaguaccgc cgccgccgcc gccgagaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 241
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-16-UTR

<400> SEQUENCE: 241

gggaaauaag agagaaaaga agaguccgcc gccgccgccg ccgaagaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 242
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-17-UTR

<400> SEQUENCE: 242

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cguaagaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 243
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)6-18-UTR

<400> SEQUENCE: 243

gggaaauaag agagaaaaga agaccgccgc cgccgccgcc gguaagaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 244
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-UTR

<400> SEQUENCE: 244

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccgccg ccgccgccgc 60

cggccacc 68

<210> SEQ ID NO 245
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-1-UTR

<400> SEQUENCE: 245

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgcc 60

gagccacc 68

<210> SEQ ID NO 246
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-2-UTR

<400> SEQUENCE: 246

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccgccgcc gccgccgccg 60

gagccacc 68

<210> SEQ ID NO 247
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-3-UTR

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<400> SEQUENCE: 247

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgccgccg ccgccgccga 60

gagccacc 68

<210> SEQ ID NO 248
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-4-UTR

<400> SEQUENCE: 248

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgccgccgc cgccgccgaa 60

gagccacc 68

<210> SEQ ID NO 249
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-5-UTR

<400> SEQUENCE: 249

gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cgccgccgcc gccgccguaa 60

gagccacc 68

<210> SEQ ID NO 250
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-6-UTR

<400> SEQUENCE: 250

gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gccgccgccg ccgccgauaa 60

gagccacc 68

<210> SEQ ID NO 251
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-7-UTR

<400> SEQUENCE: 251

gggaaauaag agagaaaaga agaguaagaa gaaaccgccg ccgccgccgc cgccguauaa 60

gagccacc 68

<210> SEQ ID NO 252
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-8-UTR

<400> SEQUENCE: 252

gggaaauaag agagaaaaga agaguaagaa gaaccgccgc cgccgccgcc gccgauauaa 60

gagccacc 68

<210> SEQ ID NO 253
<211> LENGTH: 68

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-9-UTR

<400> SEQUENCE: 253

gggaaauaag agagaaaaga agaguaagaa gaccgccgcc gccgccgccg ccgaauauaa 60

gagccacc 68

<210> SEQ ID NO 254
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-10-UTR

<400> SEQUENCE: 254

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccgc cgaaauauaa 60

gagccacc 68

<210> SEQ ID NO 255
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-11-UTR

<400> SEQUENCE: 255

gggaaauaag agagaaaaga agaguaagaa ccgccgccgc cgccgccgcc ggaaauauaa 60

gagccacc 68

<210> SEQ ID NO 256
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-12-UTR

<400> SEQUENCE: 256

gggaaauaag agagaaaaga agaguaagac cgccgccgcc gccgccgccg agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 257
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-13-UTR

<400> SEQUENCE: 257

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgccga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 258
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-14-UTR

<400> SEQUENCE: 258

gggaaauaag agagaaaaga agaguaaccg ccgccgccgc cgccgccgga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 259
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-15-UTR

<400> SEQUENCE: 259

gggaaauaag agagaaaaga agaguaccgc cgccgccgcc gccgccgaga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 260
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-16-UTR

<400> SEQUENCE: 260

gggaaauaag agagaaaaga agaguccgcc gccgccgccg ccgccgaaga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 261
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-17-UTR

<400> SEQUENCE: 261

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cgccguaaga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 262
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)7-18-UTR

<400> SEQUENCE: 262

gggaaauaag agagaaaaga agaccgccgc cgccgccgcc gccgguaaga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 263
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-UTR

<400> SEQUENCE: 263

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccgccg ccgccgccgc 60

cgccggccac c 71

<210> SEQ ID NO 264
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-1-UTR

<400> SEQUENCE: 264

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgcc 60

gccgagccac c 71

<210> SEQ ID NO 265
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-2-UTR

<400> SEQUENCE: 265

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccgccgcc gccgccgccg 60

ccggagccac c 71

<210> SEQ ID NO 266
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-3-UTR

<400> SEQUENCE: 266

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgccgccg ccgccgccgc 60

cgagagccac c 71

<210> SEQ ID NO 267
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-4-UTR

<400> SEQUENCE: 267

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgccgccgc cgccgccgcc 60

gaagagccac c 71

<210> SEQ ID NO 268
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-5-UTR

<400> SEQUENCE: 268

gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cgccgccgcc gccgccgccg 60

uaagagccac c 71

<210> SEQ ID NO 269
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-6-UTR

<400> SEQUENCE: 269

gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gccgccgccg ccgccgccga 60

uaagagccac c 71

<210> SEQ ID NO 270

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<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-7-UTR

<400> SEQUENCE: 270

gggaaauaag agagaaaaga agaguaagaa gaaaccgccg ccgccgccgc cgccgccgua 60

uaagagccac c 71

<210> SEQ ID NO 271
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-8-UTR

<400> SEQUENCE: 271

gggaaauaag agagaaaaga agaguaagaa gaaccgccgc cgccgccgcc gccgccgaua 60

uaagagccac c 71

<210> SEQ ID NO 272
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-9-UTR

<400> SEQUENCE: 272

gggaaauaag agagaaaaga agaguaagaa gaccgccgcc gccgccgccg ccgccgaaua 60

uaagagccac c 71

<210> SEQ ID NO 273
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-10-UTR

<400> SEQUENCE: 273

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccgc cgccgaaaua 60

uaagagccac c 71

<210> SEQ ID NO 274
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-11-UTR

<400> SEQUENCE: 274

gggaaauaag agagaaaaga agaguaagaa ccgccgccgc cgccgccgcc gccggaaaua 60

uaagagccac c 71

<210> SEQ ID NO 275
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-12-UTR

<400> SEQUENCE: 275

gggaaauaag agagaaaaga agaguaagac cgccgccgcc gccgccgccg ccgagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 276
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-13-UTR

<400> SEQUENCE: 276

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgccgc cgaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 277
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-14-UTR

<400> SEQUENCE: 277

gggaaauaag agagaaaaga agaguaaccg ccgccgccgc cgccgccgcc ggaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 278
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-15-UTR

<400> SEQUENCE: 278

gggaaauaag agagaaaaga agaguaccgc cgccgccgcc gccgccgccg agaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 279
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-16-UTR

<400> SEQUENCE: 279

gggaaauaag agagaaaaga agaguccgcc gccgccgccg ccgccgccga agaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 280
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)8-17-UTR

<400> SEQUENCE: 280

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cgccgccgua agaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 281
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic: (CCG)8-18-UTR

<400> SEQUENCE: 281

gggaaauaag agagaaaaga agaccgccgc cgccgccgcc gccgccggua agaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 282
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-UTR

<400> SEQUENCE: 282

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccgccg ccgccgccgc 60

cgccgccggc cacc 74

<210> SEQ ID NO 283
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-1-UTR

<400> SEQUENCE: 283

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgcc 60

gccgccgagc cacc 74

<210> SEQ ID NO 284
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-2-UTR

<400> SEQUENCE: 284

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccgccgcc gccgccgccg 60

ccgccggagc cacc 74

<210> SEQ ID NO 285
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-3-UTR

<400> SEQUENCE: 285

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgccgccg ccgccgccgc 60

cgccgagagc cacc 74

<210> SEQ ID NO 286
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-4-UTR

<400> SEQUENCE: 286

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgccgccgc cgccgccgcc 60

gccgaagagc cacc 74

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<210> SEQ ID NO 287
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-5-UTR

<400> SEQUENCE: 287

gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cgccgccgcc gccgccgccg 60

ccguaagagc cacc 74

<210> SEQ ID NO 288
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-6-UTR

<400> SEQUENCE: 288

gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gccgccgccg ccgccgccgc 60

cgauaagagc cacc 74

<210> SEQ ID NO 289
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-7-UTR

<400> SEQUENCE: 289

gggaaauaag agagaaaaga agaguaagaa gaaaccgccg ccgccgccgc cgccgccgcc 60

guauaagagc cacc 74

<210> SEQ ID NO 290
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-8-UTR

<400> SEQUENCE: 290

gggaaauaag agagaaaaga agaguaagaa gaaccgccgc cgccgccgcc gccgccgccg 60

auauaagagc cacc 74

<210> SEQ ID NO 291
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-9-UTR

<400> SEQUENCE: 291

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auauaagagc cacc 74

<210> SEQ ID NO 292
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-10-UTR

<400> SEQUENCE: 292

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gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccgc cgccgccgaa 60

auauaagagc cacc 74

<210> SEQ ID NO 293
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-11-UTR

<400> SEQUENCE: 293

gggaaauaag agagaaaaga agaguaagaa ccgccgccgc cgccgccgcc gccgccggaa 60

auauaagagc cacc 74

<210> SEQ ID NO 294
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-12-UTR

<400> SEQUENCE: 294

gggaaauaag agagaaaaga agaguaagac cgccgccgcc gccgccgccg ccgccgagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 295
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-13-UTR

<400> SEQUENCE: 295

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgccgc cgccgaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 296
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-14-UTR

<400> SEQUENCE: 296

gggaaauaag agagaaaaga agaguaaccg ccgccgccgc cgccgccgcc gccggaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 297
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-15-UTR

<400> SEQUENCE: 297

gggaaauaag agagaaaaga agaguaccgc cgccgccgcc gccgccgccg ccgagaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 298
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-16-UTR

<400> SEQUENCE: 298

gggaaauaag agagaaaaga agaguccgcc gccgccgccg ccgccgccgc cgaagaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 299
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-17-UTR

<400> SEQUENCE: 299

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cgccgccgcc guaagaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 300
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)9-18-UTR

<400> SEQUENCE: 300

gggaaauaag agagaaaaga agaccgccgc cgccgccgcc gccgccgccg guaagaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 301
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-UTR

<400> SEQUENCE: 301

gggaaauaag agagaaaaga agaguaagaa gaaauauaag accgccgccg ccgccgccgc 60

cgccgccgcc ggccacc 77

<210> SEQ ID NO 302
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-1-UTR

<400> SEQUENCE: 302

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgcc 60

gccgccgccg agccacc 77

<210> SEQ ID NO 303
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-2-UTR

<400> SEQUENCE: 303

gggaaauaag agagaaaaga agaguaagaa gaaauauaac cgccgccgcc gccgccgccg 60

ccgccgccgg agccacc 77

<210> SEQ ID NO 304
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-3-UTR

<400> SEQUENCE: 304

gggaaauaag agagaaaaga agaguaagaa gaaauauacc gccgccgccg ccgccgccgc 60

cgccgccgag agccacc 77

<210> SEQ ID NO 305
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-4-UTR

<400> SEQUENCE: 305

gggaaauaag agagaaaaga agaguaagaa gaaauauccg ccgccgccgc cgccgccgcc 60

gccgccgaag agccacc 77

<210> SEQ ID NO 306
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-5-UTR

<400> SEQUENCE: 306

gggaaauaag agagaaaaga agaguaagaa gaaauaccgc cgccgccgcc gccgccgccg 60

ccgccguaag agccacc 77

<210> SEQ ID NO 307
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-6-UTR

<400> SEQUENCE: 307

gggaaauaag agagaaaaga agaguaagaa gaaauccgcc gccgccgccg ccgccgccgc 60

cgccgauaag agccacc 77

<210> SEQ ID NO 308
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-7-UTR

<400> SEQUENCE: 308

gggaaauaag agagaaaaga agaguaagaa gaaaccgccg ccgccgccgc cgccgccgcc 60

gccguauaag agccacc 77

<210> SEQ ID NO 309
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-8-UTR

<400> SEQUENCE: 309

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gggaaauaag agagaaaaga agaguaagaa gaaccgccgc cgccgccgcc gccgccgccg 60

ccgauauaag agccacc 77

<210> SEQ ID NO 310
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-9-UTR

<400> SEQUENCE: 310

gggaaauaag agagaaaaga agaguaagaa gaccgccgcc gccgccgccg ccgccgccgc 60

cgaauauaag agccacc 77

<210> SEQ ID NO 311
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-10-UTR

<400> SEQUENCE: 311

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccgc cgccgccgcc 60

gaaauauaag agccacc 77

<210> SEQ ID NO 312
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-11-UTR

<400> SEQUENCE: 312

gggaaauaag agagaaaaga agaguaagaa ccgccgccgc cgccgccgcc gccgccgccg 60

gaaauauaag agccacc 77

<210> SEQ ID NO 313
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-12-UTR

<400> SEQUENCE: 313

gggaaauaag agagaaaaga agaguaagac cgccgccgcc gccgccgccg ccgccgccga 60

gaaauauaag agccacc 77

<210> SEQ ID NO 314
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-13-UTR

<400> SEQUENCE: 314

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgccgc cgccgccgaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 315
<211> LENGTH: 77
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-14-UTR

<400> SEQUENCE: 315

gggaaauaag agagaaaaga agaguaaccg ccgccgccgc cgccgccgcc gccgccggaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 316
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-15-UTR

<400> SEQUENCE: 316

gggaaauaag agagaaaaga agaguaccgc cgccgccgcc gccgccgccg ccgccgagaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 317
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-16-UTR

<400> SEQUENCE: 317

gggaaauaag agagaaaaga agaguccgcc gccgccgccg ccgccgccgc cgccgaagaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 318
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-17-UTR

<400> SEQUENCE: 318

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cgccgccgcc gccguaagaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 319
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (CCG)10-18-UTR

<400> SEQUENCE: 319

gggaaauaag agagaaaaga agaccgccgc cgccgccgcc gccgccgccg ccgguaagaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 320
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-UTR

<400> SEQUENCE: 320

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc gccacc 56

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<210> SEQ ID NO 321
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-1-UTR

<400> SEQUENCE: 321

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccgcca gccacc 56

<210> SEQ ID NO 322
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-2-UTR

<400> SEQUENCE: 322

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccga gccacc 56

<210> SEQ ID NO 323
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-3-UTR

<400> SEQUENCE: 323

gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccgccaga gccacc 56

<210> SEQ ID NO 324
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-4-UTR

<400> SEQUENCE: 324

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccgccaaga gccacc 56

<210> SEQ ID NO 325
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-5-UTR

<400> SEQUENCE: 325

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccgccuaaga gccacc 56

<210> SEQ ID NO 326
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-6-UTR

<400> SEQUENCE: 326

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cgccauaaga gccacc 56

<210> SEQ ID NO 327
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-7-UTR

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<400> SEQUENCE: 327

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc gccuauaaga gccacc 56

<210> SEQ ID NO 328
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-8-UTR

<400> SEQUENCE: 328

gggaaauaag agagaaaaga agaguaagaa gaagccgccg ccauauaaga gccacc 56

<210> SEQ ID NO 329
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-9-UTR

<400> SEQUENCE: 329

gggaaauaag agagaaaaga agaguaagaa gagccgccgc caauauaaga gccacc 56

<210> SEQ ID NO 330
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-10-UTR

<400> SEQUENCE: 330

gggaaauaag agagaaaaga agaguaagaa ggccgccgcc aaauauaaga gccacc 56

<210> SEQ ID NO 331
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-11-UTR

<400> SEQUENCE: 331

gggaaauaag agagaaaaga agaguaagaa gccgccgccg aaauauaaga gccacc 56

<210> SEQ ID NO 332
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-12-UTR

<400> SEQUENCE: 332

gggaaauaag agagaaaaga agaguaagag ccgccgccag aaauauaaga gccacc 56

<210> SEQ ID NO 333
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-13-UTR

<400> SEQUENCE: 333

gggaaauaag agagaaaaga agaguaaggc cgccgccaag aaauauaaga gccacc 56

<210> SEQ ID NO 334
<211> LENGTH: 56

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-14-UTR

<400> SEQUENCE: 334

gggaaauaag agagaaaaga agaguaagcc gccgccgaag aaauauaaga gccacc 56

<210> SEQ ID NO 335
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-15-UTR

<400> SEQUENCE: 335

gggaaauaag agagaaaaga agaguagccg ccgccagaag aaauauaaga gccacc 56

<210> SEQ ID NO 336
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-16-UTR

<400> SEQUENCE: 336

gggaaauaag agagaaaaga agagugccgc cgccaagaag aaauauaaga gccacc 56

<210> SEQ ID NO 337
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-17-UTR

<400> SEQUENCE: 337

gggaaauaag agagaaaaga agaggccgcc gccuaagaag aaauauaaga gccacc 56

<210> SEQ ID NO 338
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)3-18-UTR

<400> SEQUENCE: 338

gggaaauaag agagaaaaga agagccgccg ccguaagaag aaauauaaga gccacc 56

<210> SEQ ID NO 339
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-UTR

<400> SEQUENCE: 339

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc gccgccacc 59

<210> SEQ ID NO 340
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-1-UTR

<400> SEQUENCE: 340

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gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccgccg ccagccacc 59

<210> SEQ ID NO 341
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-2-UTR

<400> SEQUENCE: 341

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgagccacc 59

<210> SEQ ID NO 342
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-3-UTR

<400> SEQUENCE: 342

gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccgccgcc agagccacc 59

<210> SEQ ID NO 343
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-4-UTR

<400> SEQUENCE: 343

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccgccgcca agagccacc 59

<210> SEQ ID NO 344
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-5-UTR

<400> SEQUENCE: 344

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccgccgccua agagccacc 59

<210> SEQ ID NO 345
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-6-UTR

<400> SEQUENCE: 345

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cgccgccaua agagccacc 59

<210> SEQ ID NO 346
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-7-UTR

<400> SEQUENCE: 346

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc gccgccuaua agagccacc 59

<210> SEQ ID NO 347
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-8-UTR

<400> SEQUENCE: 347

gggaaauaag agagaaaaga agaguaagaa gaagccgccg ccgccauaua agagccacc 59

<210> SEQ ID NO 348
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-9-UTR

<400> SEQUENCE: 348

gggaaauaag agagaaaaga agaguaagaa gagccgccgc cgccaauaua agagccacc 59

<210> SEQ ID NO 349
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-10-UTR

<400> SEQUENCE: 349

gggaaauaag agagaaaaga agaguaagaa ggccgccgcc gccaaauaua agagccacc 59

<210> SEQ ID NO 350
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-11-UTR

<400> SEQUENCE: 350

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgaaauaua agagccacc 59

<210> SEQ ID NO 351
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-12-UTR

<400> SEQUENCE: 351

gggaaauaag agagaaaaga agaguaagag ccgccgccgc cagaaauaua agagccacc 59

<210> SEQ ID NO 352
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-13-UTR

<400> SEQUENCE: 352

gggaaauaag agagaaaaga agaguaaggc cgccgccgcc aagaaauaua agagccacc 59

<210> SEQ ID NO 353
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-14-UTR

<400> SEQUENCE: 353

gggaaauaag agagaaaaga agaguaagcc gccgccgccg aagaaauaua agagccacc 59

<210> SEQ ID NO 354
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-15-UTR

<400> SEQUENCE: 354

gggaaauaag agagaaaaga agaguagccg ccgccgccag aagaaauaua agagccacc 59

<210> SEQ ID NO 355
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-16-UTR

<400> SEQUENCE: 355

gggaaauaag agagaaaaga agagugccgc cgccgccaag aagaaauaua agagccacc 59

<210> SEQ ID NO 356
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-17-UTR

<400> SEQUENCE: 356

gggaaauaag agagaaaaga agaggccgcc gccgccuaag aagaaauaua agagccacc 59

<210> SEQ ID NO 357
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)4-18-UTR

<400> SEQUENCE: 357

gggaaauaag agagaaaaga agagccgccg ccgccguaag aagaaauaua agagccacc 59

<210> SEQ ID NO 358
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-UTR

<400> SEQUENCE: 358

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc gccgccgcca 60

cc 62

<210> SEQ ID NO 359
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-1-UTR

<400> SEQUENCE: 359

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccgccg ccgccagcca 60

cc 62

<210> SEQ ID NO 360
<211> LENGTH: 62

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-2-UTR

<400> SEQUENCE: 360

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgagcca 60

cc 62

<210> SEQ ID NO 361
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-3-UTR

<400> SEQUENCE: 361

gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccgccgcc gccagagcca 60

cc 62

<210> SEQ ID NO 362
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-4-UTR

<400> SEQUENCE: 362

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccgccgccg ccaagagcca 60

cc 62

<210> SEQ ID NO 363
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-5-UTR

<400> SEQUENCE: 363

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccgccgccgc cuaagagcca 60

cc 62

<210> SEQ ID NO 364
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-6-UTR

<400> SEQUENCE: 364

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cgccgccgcc auaagagcca 60

cc 62

<210> SEQ ID NO 365
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-7-UTR

<400> SEQUENCE: 365

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc gccgccgccu auaagagcca 60

cc 62

<210> SEQ ID NO 366
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-8-UTR

<400> SEQUENCE: 366

gggaaauaag agagaaaaga agaguaagaa gaagccgccg ccgccgccau auaagagcca 60

cc 62

<210> SEQ ID NO 367
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-9-UTR

<400> SEQUENCE: 367

gggaaauaag agagaaaaga agaguaagaa gagccgccgc cgccgccaau auaagagcca 60

cc 62

<210> SEQ ID NO 368
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-10-UTR

<400> SEQUENCE: 368

gggaaauaag agagaaaaga agaguaagaa ggccgccgcc gccgccaaau auaagagcca 60

cc 62

<210> SEQ ID NO 369
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-11-UTR

<400> SEQUENCE: 369

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgaaau auaagagcca 60

cc 62

<210> SEQ ID NO 370
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-12-UTR

<400> SEQUENCE: 370

gggaaauaag agagaaaaga agaguaagag ccgccgccgc cgccagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 371
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-13-UTR

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<400> SEQUENCE: 371

gggaaauaag agagaaaaga agaguaaggc cgccgccgcc gccaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 372
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-14-UTR

<400> SEQUENCE: 372

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 373
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-15-UTR

<400> SEQUENCE: 373

gggaaauaag agagaaaaga agaguagccg ccgccgccgc cagaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 374
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-16-UTR

<400> SEQUENCE: 374

gggaaauaag agagaaaaga agagugccgc cgccgccgcc aagaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 375
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-17-UTR

<400> SEQUENCE: 375

gggaaauaag agagaaaaga agaggccgcc gccgccgccu aagaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 376
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)5-18-UTR

<400> SEQUENCE: 376

gggaaauaag agagaaaaga agagccgccg ccgccgccgu aagaagaaau auaagagcca 60

cc 62

<210> SEQ ID NO 377

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<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-UTR

<400> SEQUENCE: 377

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc gccgccgccg 60

ccacc 65

<210> SEQ ID NO 378
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-1-UTR

<400> SEQUENCE: 378

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccgccg ccgccgccag 60

ccacc 65

<210> SEQ ID NO 379
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-2-UTR

<400> SEQUENCE: 379

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgag 60

ccacc 65

<210> SEQ ID NO 380
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-3-UTR

<400> SEQUENCE: 380

gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccgccgcc gccgccagag 60

ccacc 65

<210> SEQ ID NO 381
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-4-UTR

<400> SEQUENCE: 381

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccgccgccg ccgccaagag 60

ccacc 65

<210> SEQ ID NO 382
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-5-UTR

<400> SEQUENCE: 382

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccgccgccgc cgccuaagag 60

ccacc 65

<210> SEQ ID NO 383
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-6-UTR

<400> SEQUENCE: 383

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cgccgccgcc gccauaagag 60

ccacc 65

<210> SEQ ID NO 384
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-7-UTR

<400> SEQUENCE: 384

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc gccgccgccg ccuauaagag 60

ccacc 65

<210> SEQ ID NO 385
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-8-UTR

<400> SEQUENCE: 385

gggaaauaag agagaaaaga agaguaagaa gaagccgccg ccgccgccgc cauauaagag 60

ccacc 65

<210> SEQ ID NO 386
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-9-UTR

<400> SEQUENCE: 386

gggaaauaag agagaaaaga agaguaagaa gagccgccgc cgccgccgcc aauauaagag 60

ccacc 65

<210> SEQ ID NO 387
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-10-UTR

<400> SEQUENCE: 387

gggaaauaag agagaaaaga agaguaagaa ggccgccgcc gccgccgcca aauauaagag 60

ccacc 65

<210> SEQ ID NO 388
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic: (GCC)6-11-UTR

<400> SEQUENCE: 388

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccga aauauaagag 60

ccacc 65

<210> SEQ ID NO 389
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-12-UTR

<400> SEQUENCE: 389

gggaaauaag agagaaaaga agaguaagag ccgccgccgc cgccgccaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 390
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-13-UTR

<400> SEQUENCE: 390

gggaaauaag agagaaaaga agaguaaggc cgccgccgcc gccgccaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 391
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-14-UTR

<400> SEQUENCE: 391

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 392
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-15-UTR

<400> SEQUENCE: 392

gggaaauaag agagaaaaga agaguagccg ccgccgccgc cgccagaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 393
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-16-UTR

<400> SEQUENCE: 393

gggaaauaag agagaaaaga agagugccgc cgccgccgcc gccaagaaga aauauaagag 60

ccacc 65

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<210> SEQ ID NO 394
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-17-UTR

<400> SEQUENCE: 394

gggaaauaag agagaaaaga agaggccgcc gccgccgccg ccuaagaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 395
<211> LENGTH: 65
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)6-18-UTR

<400> SEQUENCE: 395

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cguaagaaga aauauaagag 60

ccacc 65

<210> SEQ ID NO 396
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-UTR

<400> SEQUENCE: 396

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc gccgccgccg 60

ccgccacc 68

<210> SEQ ID NO 397
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-1-UTR

<400> SEQUENCE: 397

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccgccg ccgccgccgc 60

cagccacc 68

<210> SEQ ID NO 398
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-2-UTR

<400> SEQUENCE: 398

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgcc 60

gagccacc 68

<210> SEQ ID NO 399
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-3-UTR

<400> SEQUENCE: 399

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gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccgccgcc gccgccgcca 60

gagccacc 68

<210> SEQ ID NO 400
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-4-UTR

<400> SEQUENCE: 400

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccgccgccg ccgccgccaa 60

gagccacc 68

<210> SEQ ID NO 401
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-5-UTR

<400> SEQUENCE: 401

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccgccgccgc cgccgccuaa 60

gagccacc 68

<210> SEQ ID NO 402
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-6-UTR

<400> SEQUENCE: 402

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cgccgccgcc gccgccauaa 60

gagccacc 68

<210> SEQ ID NO 403
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-7-UTR

<400> SEQUENCE: 403

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc gccgccgccg ccgccuauaa 60

gagccacc 68

<210> SEQ ID NO 404
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-8-UTR

<400> SEQUENCE: 404

gggaaauaag agagaaaaga agaguaagaa gaagccgccg ccgccgccgc cgccauauaa 60

gagccacc 68

<210> SEQ ID NO 405
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-9-UTR

<400> SEQUENCE: 405

gggaaauaag agagaaaaga agaguaagaa gagccgccgc cgccgccgcc gccaauauaa 60

gagccacc 68

<210> SEQ ID NO 406
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-10-UTR

<400> SEQUENCE: 406

gggaaauaag agagaaaaga agaguaagaa ggccgccgcc gccgccgccg ccaaauauaa 60

gagccacc 68

<210> SEQ ID NO 407
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-11-UTR

<400> SEQUENCE: 407

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccgc cgaaauauaa 60

gagccacc 68

<210> SEQ ID NO 408
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-12-UTR

<400> SEQUENCE: 408

gggaaauaag agagaaaaga agaguaagag ccgccgccgc cgccgccgcc agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 409
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-13-UTR

<400> SEQUENCE: 409

gggaaauaag agagaaaaga agaguaaggc cgccgccgcc gccgccgcca agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 410
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-14-UTR

<400> SEQUENCE: 410

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgccga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 411
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-15-UTR

<400> SEQUENCE: 411

gggaaauaag agagaaaaga agaguagccg ccgccgccgc cgccgccaga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 412
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-16-UTR

<400> SEQUENCE: 412

gggaaauaag agagaaaaga agagugccgc cgccgccgcc gccgccaaga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 413
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-17-UTR

<400> SEQUENCE: 413

gggaaauaag agagaaaaga agaggccgcc gccgccgccg ccgccuaaga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 414
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)7-18-UTR

<400> SEQUENCE: 414

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cgccguaaga agaaauauaa 60

gagccacc 68

<210> SEQ ID NO 415
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-UTR

<400> SEQUENCE: 415

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc gccgccgccg 60

ccgccgccac c 71

<210> SEQ ID NO 416
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-1-UTR

<400> SEQUENCE: 416

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccgccg ccgccgccgc 60

cgccagccac c 71

<210> SEQ ID NO 417
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-2-UTR

<400> SEQUENCE: 417

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgcc 60

gccgagccac c 71

<210> SEQ ID NO 418
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-3-UTR

<400> SEQUENCE: 418

gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccgccgcc gccgccgccg 60

ccagagccac c 71

<210> SEQ ID NO 419
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-4-UTR

<400> SEQUENCE: 419

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccgccgccg ccgccgccgc 60

caagagccac c 71

<210> SEQ ID NO 420
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-5-UTR

<400> SEQUENCE: 420

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccgccgccgc cgccgccgcc 60

uaagagccac c 71

<210> SEQ ID NO 421
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-6-UTR

<400> SEQUENCE: 421

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cgccgccgcc gccgccgcca 60

uaagagccac c 71

<210> SEQ ID NO 422
<211> LENGTH: 71
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-7-UTR

<400> SEQUENCE: 422

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc gccgccgccg ccgccgccua 60

uaagagccac c 71

<210> SEQ ID NO 423
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-8-UTR

<400> SEQUENCE: 423

gggaaauaag agagaaaaga agaguaagaa gaagccgccg ccgccgccgc cgccgccaua 60

uaagagccac c 71

<210> SEQ ID NO 424
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-9-UTR

<400> SEQUENCE: 424

gggaaauaag agagaaaaga agaguaagaa gagccgccgc cgccgccgcc gccgccaaua 60

uaagagccac c 71

<210> SEQ ID NO 425
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-10-UTR

<400> SEQUENCE: 425

gggaaauaag agagaaaaga agaguaagaa ggccgccgcc gccgccgccg ccgccaaaua 60

uaagagccac c 71

<210> SEQ ID NO 426
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-11-UTR

<400> SEQUENCE: 426

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccgc cgccgaaaua 60

uaagagccac c 71

<210> SEQ ID NO 427
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-12-UTR

<400> SEQUENCE: 427

gggaaauaag agagaaaaga agaguaagag ccgccgccgc cgccgccgcc gccagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 428
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-13-UTR

<400> SEQUENCE: 428

gggaaauaag agagaaaaga agaguaaggc cgccgccgcc gccgccgccg ccaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 429
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-14-UTR

<400> SEQUENCE: 429

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgccgc cgaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 430
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-15-UTR

<400> SEQUENCE: 430

gggaaauaag agagaaaaga agaguagccg ccgccgccgc cgccgccgcc agaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 431
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-16-UTR

<400> SEQUENCE: 431

gggaaauaag agagaaaaga agagugccgc cgccgccgcc gccgccgcca agaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 432
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-17-UTR

<400> SEQUENCE: 432

gggaaauaag agagaaaaga agaggccgcc gccgccgccg ccgccgccua agaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 433
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)8-18-UTR

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<400> SEQUENCE: 433

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cgccgccgua agaagaaaua 60

uaagagccac c 71

<210> SEQ ID NO 434
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-UTR

<400> SEQUENCE: 434

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc gccgccgccg 60

ccgccgccgc cacc 74

<210> SEQ ID NO 435
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-1-UTR

<400> SEQUENCE: 435

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccgccg ccgccgccgc 60

cgccgccagc cacc 74

<210> SEQ ID NO 436
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-2-UTR

<400> SEQUENCE: 436

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgcc 60

gccgccgagc cacc 74

<210> SEQ ID NO 437
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-3-UTR

<400> SEQUENCE: 437

gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccgccgcc gccgccgccg 60

ccgccagagc cacc 74

<210> SEQ ID NO 438
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-4-UTR

<400> SEQUENCE: 438

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccgccgccg ccgccgccgc 60

cgccaagagc cacc 74

<210> SEQ ID NO 439
<211> LENGTH: 74

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-5-UTR

<400> SEQUENCE: 439

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccgccgccgc cgccgccgcc 60

gccuaagagc cacc 74

<210> SEQ ID NO 440
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-6-UTR

<400> SEQUENCE: 440

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cgccgccgcc gccgccgccg 60

ccauaagagc cacc 74

<210> SEQ ID NO 441
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-7-UTR

<400> SEQUENCE: 441

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc gccgccgccg ccgccgccgc 60

cuauaagagc cacc 74

<210> SEQ ID NO 442
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-8-UTR

<400> SEQUENCE: 442

gggaaauaag agagaaaaga agaguaagaa gaagccgccg ccgccgccgc cgccgccgcc 60

auauaagagc cacc 74

<210> SEQ ID NO 443
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-9-UTR

<400> SEQUENCE: 443

gggaaauaag agagaaaaga agaguaagaa gagccgccgc cgccgccgcc gccgccgcca 60

auauaagagc cacc 74

<210> SEQ ID NO 444
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-10-UTR

<400> SEQUENCE: 444

gggaaauaag agagaaaaga agaguaagaa ggccgccgcc gccgccgccg ccgccgccaa 60

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auauaagagc cacc 74

<210> SEQ ID NO 445
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-11-UTR

<400> SEQUENCE: 445

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccgc cgccgccgaa 60

auauaagagc cacc 74

<210> SEQ ID NO 446
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-12-UTR

<400> SEQUENCE: 446

gggaaauaag agagaaaaga agaguaagag ccgccgccgc cgccgccgcc gccgccagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 447
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-13-UTR

<400> SEQUENCE: 447

gggaaauaag agagaaaaga agaguaaggc cgccgccgcc gccgccgccg ccgccaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 448
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-14-UTR

<400> SEQUENCE: 448

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgccgc cgccgaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 449
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-15-UTR

<400> SEQUENCE: 449

gggaaauaag agagaaaaga agaguagccg ccgccgccgc cgccgccgcc gccagaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 450
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-16-UTR

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<400> SEQUENCE: 450

gggaaauaag agagaaaaga agagugccgc cgccgccgcc gccgccgccg ccaagaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 451
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-17-UTR

<400> SEQUENCE: 451

gggaaauaag agagaaaaga agaggccgcc gccgccgccg ccgccgccgc cuaagaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 452
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)9-18-UTR

<400> SEQUENCE: 452

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cgccgccgcc guaagaagaa 60

auauaagagc cacc 74

<210> SEQ ID NO 453
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-UTR

<400> SEQUENCE: 453

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccgccgcc gccgccgccg 60

ccgccgccgc cgccacc 77

<210> SEQ ID NO 454
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-1-UTR

<400> SEQUENCE: 454

gggaaauaag agagaaaaga agaguaagaa gaaauauaag gccgccgccg ccgccgccgc 60

cgccgccgcc agccacc 77

<210> SEQ ID NO 455
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-2-UTR

<400> SEQUENCE: 455

gggaaauaag agagaaaaga agaguaagaa gaaauauaag ccgccgccgc cgccgccgcc 60

gccgccgccg agccacc 77

<210> SEQ ID NO 456

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<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-3-UTR

<400> SEQUENCE: 456

gggaaauaag agagaaaaga agaguaagaa gaaauauagc cgccgccgcc gccgccgccg 60

ccgccgccag agccacc 77

<210> SEQ ID NO 457
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-4-UTR

<400> SEQUENCE: 457

gggaaauaag agagaaaaga agaguaagaa gaaauaugcc gccgccgccg ccgccgccgc 60

cgccgccaag agccacc 77

<210> SEQ ID NO 458
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-5-UTR

<400> SEQUENCE: 458

gggaaauaag agagaaaaga agaguaagaa gaaauagccg ccgccgccgc cgccgccgcc 60

gccgccuaag agccacc 77

<210> SEQ ID NO 459
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-6-UTR

<400> SEQUENCE: 459

gggaaauaag agagaaaaga agaguaagaa gaaaugccgc cgccgccgcc gccgccgccg 60

ccgccauaag agccacc 77

<210> SEQ ID NO 460
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-7-UTR

<400> SEQUENCE: 460

gggaaauaag agagaaaaga agaguaagaa gaaagccgcc gccgccgccg ccgccgccgc 60

cgccuauaag agccacc 77

<210> SEQ ID NO 461
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-8-UTR

<400> SEQUENCE: 461

gggaaauaag agagaaaaga agaguaagaa gaagccgccg ccgccgccgc cgccgccgcc 60

gccauauaag agccacc 77

<210> SEQ ID NO 462
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-9-UTR

<400> SEQUENCE: 462

gggaaauaag agagaaaaga agaguaagaa gagccgccgc cgccgccgcc gccgccgccg 60

ccaauauaag agccacc 77

<210> SEQ ID NO 463
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-10-UTR

<400> SEQUENCE: 463

gggaaauaag agagaaaaga agaguaagaa ggccgccgcc gccgccgccg ccgccgccgc 60

caaauauaag agccacc 77

<210> SEQ ID NO 464
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-11-UTR

<400> SEQUENCE: 464

gggaaauaag agagaaaaga agaguaagaa gccgccgccg ccgccgccgc cgccgccgcc 60

gaaauauaag agccacc 77

<210> SEQ ID NO 465
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-12-UTR

<400> SEQUENCE: 465

gggaaauaag agagaaaaga agaguaagag ccgccgccgc cgccgccgcc gccgccgcca 60

gaaauauaag agccacc 77

<210> SEQ ID NO 466
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-13-UTR

<400> SEQUENCE: 466

gggaaauaag agagaaaaga agaguaaggc cgccgccgcc gccgccgccg ccgccgccaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 467
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

<223> OTHER INFORMATION: Synthetic: (GCC)10-14-UTR

<400> SEQUENCE: 467

gggaaauaag agagaaaaga agaguaagcc gccgccgccg ccgccgccgc cgccgccgaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 468
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-15-UTR

<400> SEQUENCE: 468

gggaaauaag agagaaaaga agaguagccg ccgccgccgc cgccgccgcc gccgccagaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 469
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-16-UTR

<400> SEQUENCE: 469

gggaaauaag agagaaaaga agagugccgc cgccgccgcc gccgccgccg ccgccaagaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 470
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-17-UTR

<400> SEQUENCE: 470

gggaaauaag agagaaaaga agaggccgcc gccgccgccg ccgccgccgc cgccuaagaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 471
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: (GCC)10-18-UTR

<400> SEQUENCE: 471

gggaaauaag agagaaaaga agagccgccg ccgccgccgc cgccgccgcc gccguaagaa 60

gaaauauaag agccacc 77

<210> SEQ ID NO 472
<211> LENGTH: 15
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: KT1-UTR

<400> SEQUENCE: 472

gggcccgccg ccaac 15

<210> SEQ ID NO 473
<211> LENGTH: 15

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: KT2-UTR

<400> SEQUENCE: 473

gggcccgccg ccacc 15

<210> SEQ ID NO 474
<211> LENGTH: 15
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: KT3-UTR

<400> SEQUENCE: 474

gggcccgccg ccgac 15

<210> SEQ ID NO 475
<211> LENGTH: 15
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: KT4-UTR

<400> SEQUENCE: 475

gggcccgccg ccgcc 15

<210> SEQ ID NO 476
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-001, Upstream UTR

<400> SEQUENCE: 476

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 477
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-002, Upstream UTR

<400> SEQUENCE: 477

gggagaucag agagaaaaga agaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 478
<211> LENGTH: 145
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-003, Upstream UTR

<400> SEQUENCE: 478

ggaauaaaag ucucaacaca acauauacaa aacaaacgaa ucucaagcaa ucaagcauuc 60

uacuucuauu gcagcaauuu aaaucauuuc uuuuaaagca aaagcaauuu ucugaaaauu 120

uucaccauuu acgaacgaua gcaac 145

<210> SEQ ID NO 479
<211> LENGTH: 42
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic: 5UTR-004, Upstream UTR

<400> SEQUENCE: 479

gggagacaag cuuggcauuc cgguacuguu gguaaagcca cc 42

<210> SEQ ID NO 480
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-005, Upstream UTR

<400> SEQUENCE: 480

gggagaucag agagaaaaga agaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 481
<211> LENGTH: 145
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-006, Upstream UTR

<400> SEQUENCE: 481

ggaauaaaag ucucaacaca acauauacaa aacaaacgaa ucucaagcaa ucaagcauuc 60

uacuucuauu gcagcaauuu aaaucauuuc uuuuaaagca aaagcaauuu ucugaaaauu 120

uucaccauuu acgaacgaua gcaac 145

<210> SEQ ID NO 482
<211> LENGTH: 42
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-007, Upstream UTR

<400> SEQUENCE: 482

gggagacaag cuuggcauuc cgguacuguu gguaaagcca cc 42

<210> SEQ ID NO 483
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-008, Upstream UTR

<400> SEQUENCE: 483

gggaauuaac agagaaaaga agaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 484
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-009, Upstream UTR

<400> SEQUENCE: 484

gggaaauuag acagaaaaga agaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 485
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-010, Upstream UTR

<400> SEQUENCE: 485

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gggaaauaag agaguaaaga acaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 486
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-011, Upstream UTR

<400> SEQUENCE: 486

gggaaaaaag agagaaaaga agacuaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 487
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-012, Upstream UTR

<400> SEQUENCE: 487

gggaaauaag agagaaaaga agaguaagaa gauauauaag agccacc 47

<210> SEQ ID NO 488
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-013, Upstream UTR

<400> SEQUENCE: 488

gggaaauaag agacaaaaca agaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 489
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-014, Upstream UTR

<400> SEQUENCE: 489

gggaaauuag agaguaaaga acaguaagua gaauuaaaag agccacc 47

<210> SEQ ID NO 490
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-015, Upstream UTR

<400> SEQUENCE: 490

gggaaauaag agagaauaga agaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 491
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-016, Upstream UTR

<400> SEQUENCE: 491

gggaaauaag agagaaaaga agaguaagaa gaaaauuaag agccacc 47

<210> SEQ ID NO 492
<211> LENGTH: 47
<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-017, Upstream UTR

<400> SEQUENCE: 492

gggaaauaag agagaaaaga agaguaagaa gaaauuuaag agccacc 47

<210> SEQ ID NO 493
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-018, Upstream UTR

<400> SEQUENCE: 493

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agccacc 47

<210> SEQ ID NO 494
<211> LENGTH: 92
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-019, Upstream UTR

<400> SEQUENCE: 494

ucaagcuuuu ggacccucgu acagaagcua auacgacuca cuauagggaa auaagagaga 60

aaagaagagu aagaagaaau auaagagcca cc 92

<210> SEQ ID NO 495
<211> LENGTH: 140
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-020, Upstream UTR

<400> SEQUENCE: 495

ggacagaucg ccuggagacg ccauccacgc uguuuugacc uccauagaag acaccgggac 60

cgauccagcc uccgcggccg ggaacggugc auuggaacgc ggauuccccg ugccaagagu 120

gacucaccgu ccuugacacg 140

<210> SEQ ID NO 496
<211> LENGTH: 42
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 5UTR-021, Upstream UTR

<400> SEQUENCE: 496

ggcgcugccu acggaggugg cagccaucuc cuucucggca uc 42

<210> SEQ ID NO 497
<211> LENGTH: 371
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-001 - Creatine Kinase

<400> SEQUENCE: 497

gcgccugccc accugccacc gacugcugga acccagccag ugggagggcc uggcccacca 60

gaguccugcu cccucacucc ucgccccgcc cccuguccca gagucccacc ugggggcucu 120

cuccacccuu cucagaguuc caguuucaac cagaguucca accaaugggc uccauccucu 180

ggauucuggc caaugaaaua ucucccuggc aggguccucu ucuuuuccca gagcuccacc 240

-continued

ccaaccagga gcucuaguua auggagagcu cccagcacac ucggagcuug ugcuuugucu 300

ccacgcaaag cgauaaauaa aagcauuggu ggccuuuggu cuuugaauaa agccugagua 360

ggaagucuag a 371

<210> SEQ ID NO 498
<211> LENGTH: 568
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-002 - Myoglobin

<400> SEQUENCE: 498

gccccugccg cucccacccc cacccaucug ggccccgggu ucaagagaga gcggggucug 60

aucucgugua gccauauaga guuugcuucu gagugucugc uuuguuuagu agaggugggc 120

aggaggagcu gaggggcugg ggcuggggug uugaaguugg cuuugcaugc ccagcgaugc 180

gccucccugu gggaugucau cacccuggga accgggagug gcccuuggcu cacuguguuc 240

ugcaugguuu ggaucugaau uaauuguccu uucuucuaaa ucccaaccga acuucuucca 300

accuccaaac uggcuguaac cccaaaucca agccauuaac uacaccugac aguagcaauu 360

gucugauuaa ucacuggccc cuugaagaca gcagaauguc ccuuugcaau gaggaggaga 420

ucugggcugg gcgggccagc uggggaagca uuugacuauc uggaacuugu gugugccucc 480

ucagguaugg cagugacuca ccugguuuua auaaaacaac cugcaacauc ucauggucuu 540

ugaauaaagc cugaguagga agucuaga 568

<210> SEQ ID NO 499
<211> LENGTH: 289
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-003 - alpha-actin

<400> SEQUENCE: 499

acacacucca ccuccagcac gcgacuucuc aggacgacga aucuucucaa ugggggggcg 60

gcugagcucc agccaccccg cagucacuuu cuuuguaaca acuuccguug cugccaucgu 120

aaacugacac aguguuuaua acguguacau acauuaacuu auuaccucau uuuguuauuu 180

uucgaaacaa agcccugugg aagaaaaugg aaaacuugaa gaagcauuaa agucauucug 240

uuaagcugcg uaaauggucu uugaauaaag ccugaguagg aagucuaga 289

<210> SEQ ID NO 500
<211> LENGTH: 379
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-004 - Albumin

<400> SEQUENCE: 500

caucacauuu aaaagcaucu cagccuacca ugagaauaag agaaagaaaa ugaagaucaa 60

aagcuuauuc aucuguuuuu cuuuuucguu gguguaaagc caacacccug ucuaaaaaac 120

auaaauuucu uuaaucauuu ugccucuuuu cucugugcuu caauuaauaa aaaauggaaa 180

gaaucuaaua gagugguaca gcacuguuau uuuucaaaga uguguugcua uccugaaaau 240

ucuguagguu cuguggaagu uccaguguuc ucucuuauuc cacuucggua gaggauuucu 300

aguuucuugu gggcuaauua aauaaaucau uaauacucuu cuaauggucu uugaauaaag 360

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ccugaguagg aagucuaga 379

<210> SEQ ID NO 501
<211> LENGTH: 118
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-005 - alpha-globin

<400> SEQUENCE: 501

gcugccuucu gcggggcuug ccuucuggcc augcccuucu ucucucccuu gcaccuguac 60

cucuuggucu uugaauaaag ccugaguagg aaggcggccg cucgagcaug caucuaga 118

<210> SEQ ID NO 502
<211> LENGTH: 908
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-006 - G-CSF

<400> SEQUENCE: 502

gccaagcccu ccccauccca uguauuuauc ucuauuuaau auuuaugucu auuuaagccu 60

cauauuuaaa gacagggaag agcagaacgg agccccaggc cucugugucc uucccugcau 120

uucugaguuu cauucuccug ccuguagcag ugagaaaaag cuccuguccu cccauccccu 180

ggacugggag guagauaggu aaauaccaag uauuuauuac uaugacugcu ccccagcccu 240

ggcucugcaa ugggcacugg gaugagccgc ugugagcccc ugguccugag gguccccacc 300

ugggacccuu gagaguauca ggucucccac gugggagaca agaaaucccu guuuaauauu 360

uaaacagcag uguucccccau cuggguccuu gcaccccuca cucuggccuc agccgacugc 420

acagcggccc cugcaucccc uuggcuguga ggccccugga caagcagagg uggccagagc 480

ugggaggcau ggcccugggg ucccacgaau uugcugggga aucucguuuu ucuucuuaag 540

acuuuuggga caugguuuga cucccgaaca ucaccgacgc gucuccuguu uuucugggug 600

gccucgggac accugcccug cccccacgag ggucaggacu gugacucuuu uuagggccag 660

gcaggugccu ggacauuugc cuugcuggac ggggacuggg gaugugggag ggagcagaca 720

ggaggaauca ugucaggccu gugugugaaa ggaagcucca cugucacccu ccaccucuuc 780

acccccacu caccaguguc cccuccacug ucacauugua acugaacuuc aggauaauaa 840

aguguuugcc uccauggucu uugaauaaag ccugaguagg aaggcggccg cucgagcaug 900

caucuaga 908

<210> SEQ ID NO 503
<211> LENGTH: 835
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-007 - Col1a2; collagen, type I, alpha 2

<400> SEQUENCE: 503

acucaaucua aauuaaaaaa gaaagaaauu ugaaaaaacu uucucuuugc cauuucuucu 60

ucuucuuuuu uaacugaaag cugaauccuu ccauuucuuc ugcacaucua cuugcuuaaa 120

uuguggggcaa aagagaaaaa gaaggauuga ucagagcauu gugcaauaca guuucauuaa 180

cuccuucccc cgcucccccа aaaauuugaa uuuuuuuuuc aacacucuua caccuguuau 240

ggaaaauguc aaccuuugua agaaaaccaa aauaaaaauu gaaaaauaaa aaccauaaac 300

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auuugcacca cuuguggcuu uugaauaucu uccacagagg gaaguuuaaa acccaaacuu 360

ccaaagguuu aaacuaccuc aaaacacuuu cccaugagug ugauccacau uguuaggugc 420

ugaccuagac agagaugaac ugagguccuu guuuuguuuu guucauaaua caaaggugcu 480

aauuaauagu auuucagaua cuugaagaau guugauggug cuagaagaau uugagaagaa 540

auacuccugu auugaguugu aucguguggu guauuuuuua aaaaauuuga uuuagcauuc 600

auauuuucca ucuuauuccc aauuaaaagu augcagauua uuugcccaaa ucuucuucag 660

auucagcauu uguucuuugc cagucucauu uucaucuucu uccaugguuc cacagaagcu 720

uuguuucuug ggcaagcaga aaaauuaaau uguaccuauu uuguauaugu gagauguuua 780

aauaaauugu gaaaaaaaug aaauaaagca uguuugguuu uccaaaagaa cauau 835

<210> SEQ ID NO 504
<211> LENGTH: 297
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-008 - Col6a2; collagen, type VI, alpha 2

<400> SEQUENCE: 504

cgccgccgcc cgggccccgc agucgagggu cgugagccca ccccguccau ggugcuaagc 60

gggcccgggu cccacacggc cagcaccgcu gcucacucgg acgacgcccu gggccugcac 120

cucuccagcu ccucccacgg ggucccccgua gccccggccc ccgcccagcc ccaggucucc 180

ccaggcccuc cgcaggcugc ccggccuccc ucccccugca gccaucccaa ggcuccugac 240

cuaccuggcc ccugagcucu ggagcaagcc cugacccaau aaaggcuuug aacccau 297

<210> SEQ ID NO 505
<211> LENGTH: 602
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-009 - RPN1; ribophorin I

<400> SEQUENCE: 505

ggggcuagag cccucuccgc acagcgugga gacggggcaa ggaggggggu uauuaggauu 60

gguggguuuug uuuugcuuug uuuaaagccg ugggaaaaug gcacaacuuu accucugugg 120

gagaugcaac acugagagcc aagggguggg aguugggaua auuuuuauau aaaagaaguu 180

uuuccacuuu gaauugcuaa aaguggcauu uuuccuaugu gcagucacuc cucucauuuc 240

uaaaauaggg acguggccag gcacgguggc ucaugccugu aaucccagca cuuugggagg 300

ccgaggcagg cggcucacga ggucaggaga ucgagacuau ccuggcuaac acgguaaaac 360

ccugucucua cuaaaaguac aaaaaauuag cugggcgugg uggugggcac cuguagucc 420

agcuacucgg gaggcugagg caggagaaag gcaugaaucc aagaggcaga gcuugcagug 480

agcugagauc acgccauugc acuccagccu gggcaacagu guuaagacuc ugucucaaau 540

auaaauaaau aaauaaauaa auaaauaaau aaauaaaaau aaagcgagau guugcccuca 600

aa 602

<210> SEQ ID NO 506
<211> LENGTH: 785
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-010 - LRP1; low density lipoprotein receptor-related protein 1

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<400> SEQUENCE: 506

ggcccugccc cgucggacug cccccagaaa gccuccugcc cccugccagu gaaguccuuc 60

agugagcccc uccccagcca gcccuucccu ggccccgccg gauguauaaa uguaaaaaug 120

aaggaauuac auuuuauaug ugagcgagca agccggcaag cgagcacagu auuauuucuc 180

caucccccucc cugccugcuc cuuggcaccc ccaugcugcc uucagggaga caggcaggga 240

gggcuuggggg cugcaccucc uacccuccca ccagaacgca ccccacuggg agagcuggug 300

gugcagccuu ccccucccug uauaagacac uuugccaagg cucucccccuc ucgccccauc 360

ccugcuugcc cgcucccaca gcuuccugag ggcuaauucu gggaagggag aguucuuugc 420

ugccccuguc uggaagacgu ggcucugggu gagguaggcg ggaaaggaug gaguguuuua 480

guucuuggggg gaggccaccc caaaccccag ccccaacucc aggggcaccu augagauggc 540

caugcucaac cccccuccca gacaggcccu cccugucucc agggccccca ccgagguucc 600

cagggcugga gacuuccucu gguaaacauu ccuccagccu ccccuccccu ggggacgcca 660

aggaggugggg ccacacccag gaagggaaag cgggcagccc cguuuugggg acgugaacgu 720

uuuaauaauu uuugcugaau uccuuuacaa cuaaauaaca cagauauugu uauaaauaaa 780

auugu 785

<210> SEQ ID NO 507
<211> LENGTH: 3001
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-011 - Nnt1; cardiotrophin-like cytokine factor 1

<400> SEQUENCE: 507

auauuaagga ucaagcuguu agcuaauaau gccaccucug caguuuuggg aacaggcaaa 60

uaaaguauca guauacaugg ugauguacau cuguagcaaa gcucuuggag aaaaugaaga 120

cugaagaaag caaagcaaaa acuguauaga gagauuuuuc aaaagcagua aucccucaau 180

uuuaaaaaag gauugaaaau ucuaaauguc uuucugugca uauuuuuugu guuaggaauc 240

aaaaguauuu uauaaaagga gaaagaacag ccucauuuua gauguagucc uguuggauuu 300

uuuaugccuc cucaguaacc agaaauguuu uaaaaaacua aguguuuagg auuucaagac 360

aacauuauac auggcucuga aauaucugac acaauguaaa cauugcaggc accugcauuu 420

uauguuuuuu uuuucaacaa augugacuaa uuugaaacuu uuaugaacuu cugagcuguc 480

cccuugcaau ucaaccgcag uuugaauuaa ucauaucaaa ucaguuuuaa uuuuuuaaau 540

uguacuucag agucuauauu ucaagggcac auuuucucac uacuauuuua auacauuaaa 600

ggacuaaaua aucuuucaga gaugcuggaa acaaaucauu ugcuuuauau guuucauuag 660

aauaccaaug aaacauacaa cuugaaaauu aguaauagua uuuuugaaga ucccauuucu 720

aauuggagau cucuuuaauu ucgaucaacu uauaaugugu aguacuauau uaagugcacu 780

ugaguggaau ucaacauuug acuaauaaaa ugaguucauc auguuggcaa gugauguggc 840

aauuaucucu ggugacaaaa gaguaaaauc aaauauuucu gccuguuaca aauaucaagg 900

aagaccugcu acuaugaaau agaugacauu aaucugucuu cacuguuuau aauacggaug 960

gauuuuuuuu caaaucagug uguguuuuga ggucuuaugu aauugaugac auuugagaga 1020

aaugguggcu uuuuuuagcu accucuuugu ucauuuaagc accaguaaag aucaugucuu 1080

uuuauagaag uguagauuuu cuuugugacu uugcuaucgu gccuaaagcu cuaaauauag 1140

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gugaaugugu gaugaauacu cagauuauuu gucucucuau auaauuaguu ugguacuaag 1200

uuucucaaaa aauuauuaac acaugaaaga caaucucuaa accagaaaaa gaaguaguac 1260

aaauuuuguu acuguaaugc ucgcguuuag ugaguuuaaa acacacagua ucuuuugguu 1320

uuauaaucag uuucuauuuu gcugugccug agauuaagau cuguguaugu gugugugugu 1380

gugugugcgu uuguguguua aagcagaaaa gacuuuuuua aaaguuuuaa gugauaaaug 1440

caauuuguua auugaucuua gaucacuagu aaacucaggg cugaauuaua ccauguauau 1500

ucuauuagaa gaaaguaaac accaucuuua uuccugcccu uuuucuucuc ucaaaguagu 1560

uguaguuaua ucuagaaaga agcaauuuug auuucuugaa aagguaguuc cugcacucag 1620

uuuaaacuaa aaauaaucau acuuggauuu uauuuauuuu ugucauagua aaaauuuuaa 1680

uuuauauaua uuuuuauuua guauuaucuu auucuuugcu auuugccaau ccuuugucau 1740

caauuguguu aaaugaauug aaaauucaug cccuguucau uuuauuuuac uuuauugguu 1800

aggauauuua aaggauuuuu guauauauaa uuucuuaaau uaauauucca aaagguuagu 1860

ggacuuagau uauaaauuau ggcaaaaauc uaaaaacaac aaaaaugauu uuuauacauu 1920

cuauuucauu auuccucuuu uuccaauaag ucauacaauu gguagauaug acuuauuuua 1980

uuuuuguauu auucacuaua ucuuuaugau auuuaaguau aaauaauuaa aaaaauuuau 2040

uguaccuuau agucugucac caaaaaaaaa aaauuaucug uagguaguga aaugcuaaug 2100

uugauuuguc uuuaagggcu uguuaacuau ccuuuauuuu cucauuuguc uuaaauuagg 2160

aguuuguguu uaaauuacuc aucuaagcaa aaaauguaua uaaaucccau uacuggguau 2220

auacccaaag gauuauaaau caugcugcua uaaagacaca ugcacacgua uguuuauugc 2280

agcacuauuc acaauagcaa agacuuggaa ccaacccaaa uguccaucaa ugauagacuu 2340

gauuaagaaa augugcacau auacaccaug gaauacuaug cagccauaaa aaaggaugag 2400

uucauguccu uuguagggac auggauaaag cuggaaacca ucauucugag caaacuauug 2460

caaggacaga aaaccaaaca cugcauguuc ucacucauag gugggaauug aacaaugaga 2520

acacuuggac acaagguggg gaacaccaca caccagggcc ugucaugggg ugggggggagu 2580

ggggagggau agcauuagga gauauaccua auguaaauga ugaguuaaug ggugcagcac 2640

accaacaugg cacauguaua cauauguagc aaaccugcac guugugcaca uguacccuag 2700

aacuuaaagu auaauuaaaa aaaaaaagaa aacagaagcu auuuauaaag aaguuauuug 2760

cugaaauaaa ugugaucuuu cccauuaaaa aaauaaagaa auuuuggggu aaaaaaacac 2820

aauauauugu auucuugaaa aauucuaaga gaguggaugu gaaguguucu caccacaaaa 2880

gugauaacua auugagguaa ugcacauauu aauuagaaag auuuugucau uccacaaugu 2940

auauauacuu aaaaauaugu uauacacaau aaauacauac auuaaaaaau aaguaaaugu 3000

a 3001

<210> SEQ ID NO 508
<211> LENGTH: 1037
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-012 - Col6a1; collagen, type VI, alpha 1

<400> SEQUENCE: 508

cccacccugc acgccggcac caaacccugu ccucccaccc cucccacuc aucacuaaac 60

agaguaaaau gugaugcgaa uuuucccgac caaccugauu cgcuagauuu uuuuuaagga 120

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aaagcuugga aagccaggac acaacgcugc ugccugcuuu gugcaggguc cuccggggcu 180

cagcccugag uuggcaucac cugcgcaggg cccucugggg cucagcccug agcuaguguc 240

accugcacag ggcccucuga ggcucagccc ugagcuggcg ucaccugugc agggcccucu 300

ggggcucagc ccugagcugg ccucaccugg guuccccacc ccgggcucuc cugcccugcc 360

cuccugcccg cccucccucc ugccugcgca gcuccuuccc uaggcaccuc ugugcugcau 420

cccaccagcc ugagcaagac gcccucucgg ggccugugcc gcacuagccu cccucuccuc 480

uguccccaua gcugguuuuu cccaccaauc cucaccuaac aguuacuuua caauuaaacu 540

caaagcaagc ucuucuccuc agcuuggggc agccauuggc cucugucucg uuuugggaaa 600

ccaaggucag gaggccguug cagacauaaa ucucggcgac ucggccccgu cuccugaggg 660

uccugcuggu gaccggccug gaccuuggcc cuacagcccu ggaggccgcu gcugaccagc 720

acugaccccg accucagaga guacucgcag gggcgcuggc ugcacucaag acccucgaga 780

uuaacggugc uaaccccguc ugcuccuccc ucccgcagag acuggggccu ggacuggaca 840

ugagagcccc uuggugccac agagggcugu gucuuacuag aaacaacgca aaccucuccu 900

uccucagaau agugaugugu ucgacguuuu aucaaaggcc cccuuucuau guucauguua 960

guuuugcucc uucuguguuu uuuucugaac cauauccaug uugcugacuu uuccaaauaa 1020

agguuuucac uccucuc 1037

<210> SEQ ID NO 509
<211> LENGTH: 577
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-013 - Calr; calreticulin

<400> SEQUENCE: 509

agaggccugc cuccagggcu ggacugaggc cugagcgcuc cugccgcaga gcuggccgcg 60

ccaaauaaug ucucugugag acucgagaac uuucauuuuu uuccaggcug guucggauuu 120

gggguggauu uugguuuugu uccccuccuc cacucucccc cacccccucc ccgcccuuuu 180

uuuuuuuuuu uuuuaaacug guauuuuauc uuugauucuc cuucagcccu cacccccuggu 240

ucucaucuuu cuugaucaac aucuuuucuu gccucugucc ccuucucuca ucucuuagcu 300

ccccuccaac cuggggggca gugguguggga gaagccacag gccugagauu ucaucugcuc 360

uccuuccugg agcccagagg agggcagcag aaggggggugg ugucuccaac cccccagcac 420

ugaggaagaa cggggcucuu cucauuucac cccucccuuu cucccccugcc cccaggacug 480

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caaacaaaau uucuauuaaa uuaaauuuug ugucucc 577

<210> SEQ ID NO 510
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-014 - Colla1; collagen, type I, alpha 1

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gacuuuggaa aauauuuuuu uccuuugcau ucaucucuca aacuuaguuu uuaucuuuga 180

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ccaaccgaac augaccaaaa accaaaagug cauucaaccu uaccaaaaaa aaaaaaaaaa 240

aaagaauaaa uaaauaacuu uuuaaaaaag gaagcuuggu ccacuugcuu gaagacccau 300

gcggggguaa gucccuuucu gcccguuggg cuuaugaaac cccaaugcug cccuuucugc 360

uccuuucucc acaccccccu uggggccucc ccuccacucc uucccaaauc ugucucccca 420

gaagacacag gaaacaaugu auugucugcc cagcaaucaa aggcaaugcu caaacaccca 480

aguggccccc acccucagcc cgcuccugcc cgcccagcac ccccaggccc ugggggaccu 540

gggguucuca gacugccaaa gaagccuugc caucuggcgc ucccauggcu cuugcaacau 600

cuccccuucg uuuuugaggg ggucaugccg ggggagccac cagccccuca cuggguucgg 660

aggagaguca ggaagggcca cgacaaagca gaaacaucgg auuuggggaa cgcgugucaa 720

ucccuugugc cgcagggcug ggcgggagag acuguucugu uccuugugua acuguguugc 780

ugaaagacua ccucguucuu gucuugaugu gucaccgggg caacugccug ggggcgggga 840

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auguuccuuu uuguucaaag ucuauuuuua uuccuugaua uuuuucuuuu uuuuuuuuuu 1020

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gcauguggaa augacccaaa cauaauccgc aguggccucc uaauuuccuu cuuuggaguu 1440

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ugcaccuuuu ccaaauccuc uucucccccug caauaccaua caggcaaucc acgugcacaa 1680

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acugggaggg ugaggggagu ggaacccgug aggaggaccu gggggccucu ccuugaacug 1860

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aggcgugcuc ccugcucgcu guccuggccc ugggggagug agggagacag acaccuggga 2040

gagcuguggg gaaggcacuc gcaccgugcu cuugggaagg aaggagaccu ggcccugcuc 2100

accacggacu gggugccucg accuccugaa uccccagaac acaacccccc ugggcugggg 2160

uggucugggg aaccaucgug cccccgccuc ccgccuacuc cuuuuuaagc uu 2212

<210> SEQ ID NO 511
<211> LENGTH: 729
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-015 - Plod1; procollagen-
lysine, 2-oxoglutarate 5-dioxygenase 1

<400> SEQUENCE: 511

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cucuuggagc caccaaucaa agagauucaa agagauuccu gcaggccaga ggcggaacac 180

accuuuaugg cuggggcucu ccgugguguu cuggacccag ccccuggaga caccauucac 240

uuuuacugcu uuguagugac ucgugcucuc caaccugucu uccugaaaaa ccaaggcccc 300

cuucccccac cucuuccaug gggugagacu ugagcagaac aggggcuucc ccaaguugcc 360

cagaaagacu gucuggguga gaagccaugg ccagagcuuc ucccaggcac agguguugca 420

ccagggacuu cugcuucaag uuuuggggua aagacaccug gaucagacuc caagggcugc 480

ccugagucug ggacuucugc cuccauggcu ggucaugaga gcaaaccgua gucccugga 540

gacagcgacu ccagagaacc ucuugggaga cagaagaggc aucugugcac agcucgaucu 600

ucuacuugcc uguggggagg ggagugacag guccacacac cacacugggu cacccugucc 660

uggaugccuc ugaagagagg gacagaccgu cagaaacugg agaguuucua uuaaagguca 720

uuuaaacca 729

<210> SEQ ID NO 512
<211> LENGTH: 847
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-016 - Nucb1; nucleobindin 1

<400> SEQUENCE: 512

uccuccggga ccccagcccu caggauuccu gaugcuccaa ggcgacugau gggcgcugga 60

ugaaguggca cagucagcuu cccuggggge ugguucaug uugggcuccu ggggcggggg 120

cacggccugg cauuucacgc auugcugcca ccccaggucc accugucucc acuuucacag 180

ccuccaaguc uguggcucuu cccuucuguc cuccgagggg cuugccuucu cucgugucca 240

gugaggugcu cagugaucgg cuuaacuuag agaagcccgc ccccucccu ucuccgucug 300

ucccaagagg gucugcucug agccugcguu ccuagguggc ucggccucag cugccugggu 360

uguggccgcc cuagcauccu guaugcccac agcuacugga aucccgcug cugcuccggg 420

ccaagcuucu gguugauuaa ugagggcaug ggguggucc ucaagaccuu ccccuaccuu 480

uuguggaacc agugaugccu caaagacagu gucccucca cagcugggug ccaggggcag 540

gggauccuca guauagccgg ugaacccuga uaccaggagc cugggccucc cugaaccccu 600

ggcuuccagc caucucaucg ccagccuccu ccuggaccuc uuggccccca gccccuuccc 660

cacacagccc cagaaggguc ccagagcuga ccccacucca ggaccuaggc ccagccccuc 720

agccucaucu ggagccccug aagaccaguc ccacccaccu uucuggccuc aucugacacu 780

gcuccgcauc cugcugugug uccuguucca uguuccgguu ccauccaaau acacuuucug 840

gaacaaa 847

<210> SEQ ID NO 513
<211> LENGTH: 110
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-017 - alpha-globin

<400> SEQUENCE: 513

gcuggagccu cgguggccau gcuucuugcc ccuugggccu ccccccagcc ccuccucccc 60

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uuccugcacc cguacccccg uggucuuuga auaaagucug agugggcggc 110

<210> SEQ ID NO 514
<211> LENGTH: 116
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-018 - Downstream UTR

<400> SEQUENCE: 514

uaauaggcug gagccucggu ggccaugcuu cuugccccuu gggccucccc ccagccccuc 60

cuccccuucc ugcacccgua cccccguggu cuuugaauaa agucugagug ggcggc 116

<210> SEQ ID NO 515
<211> LENGTH: 118
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-019 - Downstream UTR

<400> SEQUENCE: 515

ugauaaauagg cuggagccuc gguggccaug cuucuugccc cuugggccuc cccccagccc 60

cuccucccccu uccugcaccc guacccccug gucuuugaau aaagucugag ugggcggc 118

<210> SEQ ID NO 516
<211> LENGTH: 138
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-018 + miR-122-5p binding site

<400> SEQUENCE: 516

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cuccccuucc ugcacccgua ccccccaaac accauuguca cacuccagug gucuuugaau 120

aaagucugag ugggcggc 138

<210> SEQ ID NO 517
<211> LENGTH: 138
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-018 + miR-122-3p binding site

<400> SEQUENCE: 517

uaauaggcug gagccucggu ggccaugcuu cuugccccuu gggccucccc ccagccccuc 60

cuccccuucc ugcacccgua cccccuauuu agugugauaa uggcguugug gucuuugaau 120

aaagucugag ugggcggc 138

<210> SEQ ID NO 518
<211> LENGTH: 141
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 3UTR-019 + miR-122 binding site

<400> SEQUENCE: 518

ugauaaauagg cuggagccuc gguggccaug cuucuugccc cuugggccuc cccccagccc 60

cuccucccccu uccugcaccc guacccccca aacaccauug ucacacucca guggucuuug 120

aauaaagucu gagugggcgg c 141

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<210> SEQ ID NO 519
<211> LENGTH: 87
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: mmiR-142

<400> SEQUENCE: 519

gacagugcag ucacccauaa aguagaaagc acuacuaaca gcacuggagg guguaguguu 60

uccuacuuua uggaugagug uacugug 87

<210> SEQ ID NO 520
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: mmiR-142-3p

<400> SEQUENCE: 520

uguaguguuu ccuacuuuau gga 23

<210> SEQ ID NO 521
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: mmiR-142-3p binding site

<400> SEQUENCE: 521

uccauaaagu aggaaacacu aca 23

<210> SEQ ID NO 522
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: mmiR-142-5p

<400> SEQUENCE: 522

cauaaaguag aaagcacuac u 21

<210> SEQ ID NO 523
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: mmiR-142-5p binding site

<400> SEQUENCE: 523

aguagugcuu ucuacuuuau g 21

<210> SEQ ID NO 524
<211> LENGTH: 85
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: miR-122

<400> SEQUENCE: 524

ccuuagcaga gcuguggagu gugacaaugg uguuuguguc uaaacuauca aacgccauua 60

ucacacuaaa uagcuacugc uaggc 85

<210> SEQ ID NO 525
<211> LENGTH: 22
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: miR-122-3p

<400> SEQUENCE: 525

aacgccauua ucacacuaaa ua 22

<210> SEQ ID NO 526
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: miR-122-3p binding site

<400> SEQUENCE: 526

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<210> SEQ ID NO 527
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: miR-122-5p

<400> SEQUENCE: 527

uggaguguga caaugguguu ug 22

<210> SEQ ID NO 528
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: miR-122-5p binding site

<400> SEQUENCE: 528

caaacaccau ugucacacuc ca 22

<210> SEQ ID NO 529
<211> LENGTH: 6
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 6nt

<400> SEQUENCE: 529

gggaaa 6

<210> SEQ ID NO 530
<211> LENGTH: 6
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 6nt (TISU)

<400> SEQUENCE: 530

ggcaag 6

<210> SEQ ID NO 531
<211> LENGTH: 262
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Tubulin-like

<400> SEQUENCE: 531

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auucucccgc cucccagccc cggcgcacgc gcgccccgcc cagccugcuu ucccuccgcg 120

cccuccccuc uccuuucucc cucucagaac cuuccugccg ucgcguuugc accucgcugc 180

uccagccucu cgcauuccaa ccuuccagcc ugcgaccugc ggagacuuag ccccauacau 240

accuugaggc gagcuuuuaa cc 262

<210> SEQ ID NO 532
<211> LENGTH: 57
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: GC Scramble #1-UTR

<400> SEQUENCE: 532

gggaaauaag agagaaaaga agaguaagaa gaaauauaag aggggcgccc ggccacc 57

<210> SEQ ID NO 533
<211> LENGTH: 57
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: GC Scramble #2-UTR

<400> SEQUENCE: 533

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agcccgcccg cgccacc 57

<210> SEQ ID NO 534
<211> LENGTH: 57
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: GC Scramble #3-UTR

<400> SEQUENCE: 534

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agcgccccgc ggccacc 57

<210> SEQ ID NO 535
<211> LENGTH: 67
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: GC1-UTR

<400> SEQUENCE: 535

gggaaauaag agagaaaaga agaguaagaa gaaauauaag agcgccccgc ggcgccccgc 60

ggccacc 67

<210> SEQ ID NO 536
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Standard 5'UTR construct

<400> SEQUENCE: 536

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<210> SEQ ID NO 537
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 6nt 5'UTR construct

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<400> SEQUENCE: 537

gggaaaatgg 10

<210> SEQ ID NO 538
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 6nt (TISU) 5'UTR construct

<400> SEQUENCE: 538

ggcaagatgg 10

<210> SEQ ID NO 539
<211> LENGTH: 266
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Tubulin-like 5'UTR construct

<400> SEQUENCE: 539

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ccctcccctc tcctttctcc ctctcagaac cttcctgccg tcgcgtttgc acctcgctgc 180

tccagcctct cgcattccaa ccttccagcc tgcgacctgc ggagacttag ccccatacat 240

accttgaggc gagcttttaa ccatgg 266

<210> SEQ ID NO 540
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Standard UTR

<400> SEQUENCE: 540

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<210> SEQ ID NO 541
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: V1-1-UTR

<400> SEQUENCE: 541

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<210> SEQ ID NO 542
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: GC Scramble #1-UTR

<400> SEQUENCE: 542

gggaaataag agagaaaaga agagtaagaa gaaatataag aggggcgccc ggccacc 57

<210> SEQ ID NO 543
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: GC Scramble #2-UTR

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<400> SEQUENCE: 543

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<210> SEQ ID NO 544
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: GC Scramble #3-UTR

<400> SEQUENCE: 544

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<210> SEQ ID NO 545
<211> LENGTH: 67
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: GC1-UTR

<400> SEQUENCE: 545

gggaaataag agagaaaaga agagtaagaa gaaatataag agcgccccgc ggcgccccgc 60

ggccacc 67

What is claimed is:

1. A messenger RNA (mRNA) comprising

(i) a 5' untranslated region (UTR) comprising at least one RNA element that provides a translational regulatory activity;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR,

wherein the at least one RNA element is a GC-rich RNA element comprising guanine (G) and cytosine (C) nucleobases and, optionally, adenine (A) and uracil (U) nucleobases, or derivatives or analogs thereof, wherein the GC-rich RNA element is at least 50% or greater cytosine (C) nucleobases and is at least 6 and up to 30 nucleotides in length, wherein the GC-rich RNA element is located about 15-20, about 10-15, about 5-10 or less than 5 nucleotides upstream a Kozak consensus sequence in the 5' UTR, and wherein the mRNA comprises one or more chemically modified nucleotides.

2. The mRNA of claim **1**, wherein the GC-rich RNA element is about 50%-55% cytosine, about 55%-60% cytosine, about 60%-65% cytosine, about 65%-70% cytosine, about 70%-75% cytosine or about 75%-80% cytosine.

3. The mRNA of claim **1**, wherein the GC-rich RNA element does not comprise adenine (A) or uracil (U) nucleobases.

4. The mRNA of claim **1**, wherein the GC-rich RNA element comprises a nucleotide sequence of about 6-30 guanine (G) and cytosine (C) nucleotides, or derivatives or analogues thereof, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases, and wherein the GC-rich RNA element comprises a repeating sequence motif.

5. The mRNA of claim **4**, wherein the repeating sequence motif is (i) $[CCG]_n$, wherein n=2 to 10, 2 to 5, 4, 3 or 2; or (ii) $[GCC]_n$, wherein n=2 to 10, 2 to 5, 4, 3 or 2.

6. The mRNA of claim **1**, comprising a second RNA element that provides a translational regulatory activity, wherein the second RNA element comprises a stable RNA secondary structure.

7. The mRNA of claim **1**, wherein the initiation codon comprises at least one modified nucleotide, and wherein the at least one modified nucleotide is selected from the group consisting of 2-thiouridine, 2'-O-methyl-2-thiouridine, 2-selenouridine, 2'-O-methyl ribose, a modified nucleotide in which the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon, inosine, 2-methyl-guanosine, 6-methyl-adenosine, a deoxyribonucleotide.

8. The mRNA of claim **7**, wherein the mRNA comprises:

a first polynucleotide, wherein the first polynucleotide is chemically synthesized, wherein the first polynucleotide comprises a 5' UTR; and

(ii) a second polynucleotide, wherein the second polynucleotide is synthesized by in vitro transcription, and wherein the second polynucleotide comprises a full open reading frame encoding a polypeptide, and a 3' UTR, and wherein (i) and (ii) are chemically cross-linked or enzymatically ligated.

9. The mRNA of claim **1**, wherein the mRNA comprises a poly A tail, optionally wherein the poly A tail is about 100 nucleotides in length.

10. The mRNA of claim **1**, wherein the mRNA comprises a 5' Cap 1 structure.

11. The mRNA of claim **1**, wherein the one or more chemically modified nucleotides is selected from the group consisting of pseudouridine or a pseudouridine analog.

12. The mRNA of claim **1**, wherein the one or more chemically modified nucleotides is N1-methylpseudouridine.

13. The mRNA of claim **12**, wherein the mRNA is fully modified with N1-methylpseudouridine.

14. A composition comprising the mRNA of claim **1** and a pharmaceutically acceptable carrier.

15. A lipid nanoparticle comprising the mRNA of claim **1**.

16. A pharmaceutical composition comprising the lipid nanoparticle of claim 15, and a pharmaceutically acceptable carrier.

17. The mRNA of claim 1, wherein the GC-rich RNA element is located about 20, about 15, about 10, about 5, about 4, about 3, about 2, or about 1 nucleotide(s) upstream of the Kozak consensus sequence in the 5' UTR.

18. The mRNA of claim 1, wherein the translational regulatory activity is selected from the group consisting of:

(a) inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome;

(b) increases an amount of a polypeptide translated from the full open reading frame;

(c) increases initiation of polypeptide synthesis at or from the initiation codon;

(d) inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon;

(e) inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame;

inhibits or reduces translation of truncated or aberrant translation products from the mRNA; and

(g) a combination of any of (a)-(f).

19. A messenger RNA (mRNA) comprising

(i) a 5' untranslated region (UTR) comprising at least one RNA element that provides a translational regulatory activity;

(ii) a full open reading frame comprising an initiation codon and encoding a polypeptide; and

(iii) a 3' UTR,

wherein the at least one RNA element is a GC-rich RNA element comprising guanine (G) and cytosine (C) nucleobases and, optionally, adenine (A) and uracil (U) nucleobases, or derivatives or analogs thereof, wherein the GC-rich RNA element is at least 50% or greater cytosine (C) nucleobases and is at least 6 and up to 30 nucleotides in length, wherein the GC-rich RNA element is located upstream of and immediately adjacent to a Kozak consensus sequence in the 5' UTR, and wherein the mRNA comprises one or more chemically modified nucleotides.

20. The mRNA of claim 19, wherein the GC-rich RNA element is about 50%-55% cytosine, about 55%-60% cytosine, about 60%-65% cytosine, about 65%-70% cytosine, about 70%-75% cytosine or about 75%-80% cytosine.

21. The mRNA of claim 19, wherein the GC-rich RNA element does not comprise adenine (A) or uracil (U) nucleobases.

22. The mRNA of claim 19, wherein the GC-rich RNA element comprises a nucleotide sequence of about 6-30 guanine (G) and cytosine (C) nucleotides, or derivatives or analogues thereof, wherein the sequence is >50% cytosine, >60% cytosine or >70% cytosine nucleobases, and wherein the GC-rich RNA element comprises a repeating sequence motif.

23. The mRNA of claim 22, wherein the repeating sequence motif is (i) $[CCG]_n$, wherein n=2 to 10, 2 to 5, 4, 3 or 2; or (ii) $[GCC]_n$, wherein n=2 to 10, 2 to 5, 4, 3 or 2.

24. The mRNA of claim 19, wherein the mRNA comprises a poly A tail, optionally wherein the poly A tail is about 100 nucleotides in length.

25. The mRNA of claim 19, wherein the mRNA comprises a 5' Cap 1 structure.

26. The mRNA of claim 19, wherein the one or more chemically modified nucleotides is N1-methylpseudouridine, optionally wherein the mRNA is fully modified with N1-methylpseudouridine.

27. The mRNA of claim 19, wherein the translational regulatory activity is selected from the group consisting of:

(a) inhibits or reduces leaky scanning of the mRNA by the PIC or ribosome;

(b) increases an amount of a polypeptide translated from the full open reading frame;

(c) increases initiation of polypeptide synthesis at or from the initiation codon;

(d) inhibits or reduces initiation of polypeptide synthesis at any codon within the mRNA other than the initiation codon;

(e) inhibits or reduces an amount of polypeptide translated from any open reading frame within the mRNA other than the full open reading frame;

(f) inhibits or reduces translation of truncated or aberrant translation products from the mRNA; and

(g) a combination of any of (a)-(f).

28. A composition comprising the mRNA of claim 19 and a pharmaceutically acceptable carrier.

29. A lipid nanoparticle comprising the mRNA of claim 19.

30. A pharmaceutical composition comprising the lipid nanoparticle of claim 29, and a pharmaceutically acceptable carrier.

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