COMPOSITIONS AND METHODS COMPRISING A TTR GUIDE RNA AND A POLYNUCLEOTIDE ENCODING AN RNA-GUIDED DNA BINDING AGENT

[0001] This patent application claims priority to United States provisional application 62/825,637 filed March 28, 2019, the content of which is incorporated herein by reference in its entirety for all purposes.

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 24, 2020, is named 2020-03-24_01155-0028-00PCT_ST25.txt and is 451 KB in size.

[0003] Transthyretin (TTR) is a protein produced by the *TTR* gene that normally functions to transport retinol and thyroxine throughout the body. TTR is predominantly synthesized in the liver, with small fractions being produced in the choroid plexus and retina. TTR normally circulates as a soluble tetrameric protein in the blood.

[0004] Pathogenic variants of TTR, which may disrupt tetramer stability, can be encoded by mutant alleles of the *TTR* gene. Mutant TTR may result in misfolded TTR, which may generate amyloids (i.e., aggregates of misfolded TTR protein). In some cases, pathogenic variants of TTR can lead to amyloidosis, or disease resulting from build-up of amyloids. For example, misfolded TTR monomers can polymerize into amyloid fibrils within tissues, such as the peripheral nerves, heart, and gastrointestinal tract. Amyloid plaques can also comprise wild-type TTR that has deposited on misfolded TTR.

[0005] Misfolding and deposition of wild-type TTR has also been observed in males aged 60 or more and is associated with heart rhythm problems, heart failure, and carpal tunnel.

[0006] Amyloidosis characterized by deposition of TTR may be referred to as "ATTR," "TTR-related amyloidosis," "TTR amyloidosis," or "ATTR amyloidosis," "ATTR familial amyloidosis" (when associated with a genetic mutation in a family), or "ATTRwt" or "wild-type ATTR" (when arising from misfolding and deposition of wild-type TTR).

[0007] ATTR can present with a wide spectrum of symptoms, and patients with different classes of ATTR may have different characteristics and prognoses. Some classes of ATTR include familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and wild-type TTR amyloidosis (wt-TTR amyloidosis). FAP commonly presents with sensorimotor neuropathy, while FAC and wt-TTR amyloidosis commonly present with congestive heart failure. FAP and FAC are usually associated with a genetic mutation in the *TTR* gene, and more than 100 different mutations in the *TTR* gene have been associated with

ATTR. In contrast, wt-TTR amyloidosis is associated with aging and not with a genetic mutation in *TTR*. It is estimated that approximately 50,000 patients worldwide may be affected by FAP and FAC.

[0008] While more than 100 mutations in *TTR* are associated with ATTR, certain mutations have been more closely associated with neuropathy and/or cardiomyopathy. For example, mutations at T60 of TTR are associated with both cardiomyopathy and neuropathy; mutations at V30 are more associated with neuropathy; and mutations at V122 are more associated with cardiomyopathy.

[0009] A range of treatment approaches have been studied for treatment of ATTR, but there are no approved drugs that stop disease progression and improve quality of life. While liver transplant has been studied for treatment of ATTR, its use is declining as it involves significant risk and disease progression sometimes continues after transplantation. Small molecule stabilizers, such as diflunisal and tafamidis, appear to slow ATTR progression, but these agents do not halt disease progression.

[0010] Approaches using small interfering RNA (siRNA) knockdown, antisense knockdown, or a monoclonal antibody targeting amyloid fibrils for destruction are also currently being investigated, but while results on short-term suppression of TTR expression show encouraging preliminary data, a need exists for treatments that can produce long-lasting suppression of TTR.

[0011] Accordingly, the following embodiments are provided. In some embodiments, the present invention provides compositions and methods using a guide RNA with an RNA-guided DNA binding agent such as the CRISPR/Cas system to substantially reduce or knockout expression of the *TTR* gene, thereby substantially reducing or eliminating the production of TTR protein associated with ATTR. The substantial reduction or elimination of the production of TTR protein associated with ATTR through alteration of the *TTR* gene can be a long-term reduction or elimination.

SUMMARY

[0012] The following embodiments are provided herein.

[0013] Embodiment 1 is a composition comprising:

(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 93% identity to SEQ ID NO: 311; and/or

b. the open reading frame has at least 93% identity to SEQ ID NO: 311 over at least its first 50, 200, 250, or 300 nucleotides, or at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides; and/or

c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5; and/or

d. the open reading frame has an adenine content ranging from its minimum adenine content to 123% of the minimum adenine content; and/or

e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82.

[0014] Embodiment 2 is a method of modifying the *TTR* gene and/or inducing a double-stranded break (DSB) within the *TTR* gene, comprising delivering a composition to a cell, wherein the composition comprises:

(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 93% identity to SEQ ID NO:311; and/or

b. the open reading frame has at least 93% identity to SEQ ID NO: 311over at least its first 50, 200, 250, or 300 nucleotides, or at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides; and/or

c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5; and/or

d. the open reading frame has an adenine content ranging from its minimum adenine content to 123% of the minimum adenine content; and/or

e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82.

[0015] Embodiment 3 is a method of reducing TTR serum concentration, treating amyloidosis associated with TTR (ATTR), and/or reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, comprising administering a composition to a subject in need thereof, wherein the composition comprises:
(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 95% identity to SEQ ID NO:311; and/or
b. the open reading frame has at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides; and/or
c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5; and/or
d. the open reading frame has an adenine content ranging from its minimum adenine content to 150% of the minimum adenine content; and/or
e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82, thereby reducing TTR serum concentration, treating amyloidosis associated with TTR (ATTR), and/or reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in the subject.

[0016] Embodiment 4 is the composition or method of any one of the preceding embodiments, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 22, 23, 27, 29, 30, 35, 36, 37, 38, 55, 61, 63, 65, 66, 68, or 69.

[0017] Embodiment 5 is the composition of embodiment 1 or 4, for use in inducing a double-stranded break (DSB) within the *TTR* gene in a cell or subject.

[0018] Embodiment 6 is the composition of embodiment 1, 4, or 5 for use in modifying the *TTR* gene in a cell or subject.

[0019] Embodiment 7 is the composition of embodiment 1, 4, 5, or 6 for use in treating amyloidosis associated with TTR (ATTR) in a subject.

[0020] Embodiment 8 is the composition of embodiment 1, 4, 5, 6, or 7 for use in reducing TTR serum concentration in a subject.

[0021] Embodiment 9 is the composition of embodiment 1, 4, 5, 6, 7, or 8, for use in reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

[0022] Embodiment 10 is the composition for use or method of any one of embodiments 2-9, wherein the method comprises administering the composition by infusion for more than 30 minutes.

[0023] Embodiment 11 is the method or composition for use of embodiment 10, wherein the composition is administered by infusion for about 45-75 minutes, 75-105 minutes, 105-135 minutes, 135-165 minutes, 165-195 minutes, 195-225 minutes, 225-255 minutes, 255-285 minutes, 285-315 minutes, 315-345 minutes, or 345-375 minutes.

[0024] Embodiment 12 is the method or composition for use of embodiment 10 or 11, wherein the composition is administered by infusion for about 1.5-6 hours.

[0025] Embodiment 13 is the method or composition for use of embodiment 10, wherein the composition is administered by infusion for about 60 minutes, about 90 minutes, about 120 minutes, about 150 minutes, about 180 minutes, or about 240 minutes.

[0026] Embodiment 14 is the method or composition for use of embodiment 10, wherein the composition is administered by infusion for about 120 minutes.

[0027] Embodiment 15 is the method or composition for use of any one of embodiments 2-14, wherein the composition reduces serum TTR levels.

[0028] Embodiment 16 is the method or composition for use of embodiment 15, wherein the serum TTR levels are reduced by at least 50% as compared to serum TTR levels before administration of the composition.

[0029] Embodiment 17 is the method or composition for use of embodiment 151, wherein the serum TTR levels are reduced by 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, 95-98%, 98-99%, or 99-100% as compared to serum TTR levels before administration of the composition.

[0030] Embodiment 18 is the method or composition for use of any one of embodiments 2-17, wherein the composition results in editing of the TTR gene.

[0031] Embodiment 19 is the method or composition for use of embodiment 18, wherein the editing is calculated as a percentage of the population that is edited (percent editing).

[0032] Embodiment 20 is the method or composition for use of embodiment 19, wherein the percent editing is between 30 and 99% of the population.

[0033] Embodiment 21 is the method or composition for use of embodiment 19, wherein the percent editing is between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and

55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the population.

[0034] Embodiment 22 is the method or the composition for use of any one of embodiments of any one of embodiments 2-21, wherein the composition reduces amyloid deposition in at least one tissue.

[0035] Embodiment 23 is the method or composition for use of embodiment 22, wherein the at least one tissue comprises one or more of stomach, colon, sciatic nerve, or dorsal root ganglion.

[0036] Embodiment 24 is the method or composition for use of embodiment 22 or 23, wherein amyloid deposition is measured 8 weeks after administration of the composition.

[0037] Embodiment 25 is the method or composition for use of any one of embodiments 22-24, wherein amyloid deposition is compared to a negative control or a level measured before administration of the composition.

[0038] Embodiment 26 is the method or composition for use of any one of embodiments 22-25, wherein amyloid deposition is measured in a biopsy sample and/or by immunostaining.

[0039] Embodiment 27 is the method or composition for use of any one of embodiments 22-26, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen in a negative control.

[0040] Embodiment 28 is the method or composition for use of any one of embodiments 22-27, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen before administration of the composition.

[0041] Embodiment 29 is the method or composition for use of any one of embodiments 2-28, wherein the composition is administered or delivered at least two times.

[0042] Embodiment 30 is The method or composition for use of embodiment 29, wherein the composition is administered or delivered at least three times.

[0043] Embodiment 31 is the method or composition for use of embodiment 29, wherein the composition is administered or delivered at least four times.

[0044] Embodiment 32 is the method or composition for use of embodiment 29, wherein the composition is administered or delivered up to five, six, seven, eight, nine, or ten times.

[0045] Embodiment 33 is the method or composition for use of any one of embodiments 29-32, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days.

[0046] Embodiment 34 is the method or composition for use of any one of embodiments 29-32, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks.

[0047] Embodiment 35 is the method or composition for use of any one of embodiments 29-32, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 months.

[0048] Embodiment 36 is the method or composition of any one of the preceding embodiments, wherein the guide RNA comprises a crRNA that comprises the guide sequence and further comprises a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end.

[0049] Embodiment 37 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is a dual guide (dgRNA).

[0050] Embodiment 38 is the method or composition of embodiment 37, wherein the dual guide RNA comprises a crRNA comprising a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end, and a trRNA.

[0051] Embodiment 39 is the method or composition of any one of embodiments 1-36, wherein the guide RNA is a single guide (sgRNA).

[0052] Embodiment 40 is the method or composition of embodiment 39, wherein the sgRNA comprises a guide sequence that has the pattern of SEQ ID NO: 3.

[0053] Embodiment 41 is the method or composition of embodiment 39, wherein the sgRNA comprises the sequence of SEQ ID NO: 3.

[0054] Embodiment 42 is the method or composition of any one of embodiments 39-41, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82 and the nucleotides of SEQ ID NO: 126.

[0055] Embodiment 43 is the method or composition of any one of embodiments 39-42, wherein the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID Nos: 87-113, 115-120, and 122-124.

[0056] Embodiment 44 is the method or composition of embodiment 39, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-113, 115-120, and 122-124.

[0057] Embodiment 45 is the method or composition of any one of the preceding embodiments, wherein the guide RNA comprises at least one modification.

[0058] Embodiment 46 is the method or composition of embodiment 45, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

[0059] Embodiment 47 is the method or composition of embodiment 45 or 46, wherein the at least one modification includes a phosphorothioate (PS) bond between nucleotides.

[0060] Embodiment 48 is the method or composition of any one of embodiments 45-47, wherein the at least one modification includes a 2'-fluoro (2'-F) modified nucleotide.

[0061] Embodiment 49 is the method or composition of any one of embodiments 45-48, wherein the at least one modification includes a modification at one or more of the first five nucleotides at the 5' end.

[0062] Embodiment 50 is the method or composition of any one of embodiments 45-49, wherein the at least one modification includes a modification at one or more of the last five nucleotides at the 3' end.

[0063] Embodiment 51 is the method or composition of any one of embodiments 45-50, wherein the at least one modification includes PS bonds between the first four nucleotides.

[0064] Embodiment 52 is the method or composition of any one of embodiments 45-51, wherein the at least one modification includes PS bonds between the last four nucleotides.

[0065] Embodiment 53 is the method or composition of any one of embodiments 45-52, wherein the at least one modification includes 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

[0066] Embodiment 54 is The method or composition of any one of embodiments 45-53, wherein the at least one modification includes 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

[0067] Embodiment 55 is the method or composition of any one of embodiments 45-54, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

[0068] Embodiment 56 is the method or composition of any one of embodiments 1-55, wherein the composition further comprises a pharmaceutically acceptable excipient.

[0069] Embodiment 57 is the method or composition of any one of embodiments 1-56, wherein the guide RNA and the nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent are associated with a lipid nanoparticle (LNP).

[0070] Embodiment 58 is the method or composition of embodiment 57, wherein the LNP comprises a CCD lipid.

[0071] Embodiment 59 is the method or composition of embodiment 58, wherein the CCD lipid is Lipid A or Lipid B, optionally wherein the CCD lipid is lipid A.

[0072] Embodiment 60 is the method or composition of any one of embodiments 57-59, wherein the LNP comprises a helper lipid.

[0073] Embodiment 61 is the method or composition of embodiment 60, wherein the helper lipid is cholesterol.

[0074] Embodiment 62 is the method or composition of any one of embodiments 57-61, wherein the LNP comprises a stealth lipid (e.g., a PEG lipid).

[0075] Embodiment 63 is the method or composition of embodiment 62, wherein the stealth lipid is PEG2k-DMG.

[0076] Embodiment 64 is the method or composition of any one of embodiments 57-63, wherein:

(i) the LNP comprises a lipid component and the lipid component comprises: about 50-60 mol-% amine lipid such as Lipid A, about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 6;

(ii) the LNP comprises about 50-60 mol-% amine lipid such as Lipid A; about 27-39.5 mol-% helper lipid; about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% stealth lipid (e.g., a PEG lipid), wherein the N/P ratio of the LNP composition is about 5-7 (e.g., about 6);

(iii) the LNP comprises a lipid component and the lipid component comprises: about 50-60 mol-% amine lipid such as Lipid A; about 5-15 mol-% neutral lipid; and about 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-10;

(iv) the LNP comprises a lipid component and the lipid component comprises: about 40-60 mol-% amine lipid such as Lipid A; about 5-15 mol-% neutral lipid; and about 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 6;

(v) the LNP comprises a lipid component and the lipid component comprises: about 50-60 mol-% amine lipid such as Lipid A; about 5-15 mol-% neutral lipid; and about 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 6;

(vi) the LNP comprises a lipid component and the lipid component comprises: about 40-60 mol-% amine lipid such as Lipid A; about 0-10 mol-% neutral lipid; and about 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid,

and wherein the N/P ratio of the LNP composition is about 3-10;

(vii) the LNP comprises a lipid component and the lipid component comprises: about 40-60 mol-% amine lipid such as Lipid A; less than about 1 mol-% neutral lipid; and about 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-10;

(viii) the LNP comprises a lipid component and the lipid component comprises: about 40-60 mol-% amine lipid such as Lipid A; and about 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, wherein the N/P ratio of the LNP composition is about 3-10, and wherein the LNP composition is essentially free of or free of neutral phospholipid; or

(ix) the LNP comprises a lipid component and the lipid component comprises: about 50-60 mol-% amine lipid such as Lipid A; about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-7.

[0077] Embodiment 64a is the method or composition of embodiment 64, wherein the mol-% PEG lipid is about 3.

[0078] Embodiment 64b is the method or composition of embodiment 64 or 64a, wherein the mol-% amine lipid is about 50.

[0079] Embodiment 64c is the method or composition of any one of embodiments 64-64b, wherein the mol-% amine lipid is about 55.

[0080] Embodiment 64d is the method or composition of any one of embodiments 64-64c, wherein the mol-% amine lipid is ± 3 mol-%.

[0081] Embodiment 64e is the method or composition of any one of embodiments 64-64d, wherein the mol-% amine lipid is ± 2 mol-%.

[0082] Embodiment 64f is the method or composition of any one of embodiments 64-64e, wherein the mol-% amine lipid is 47-53 mol-%.

[0083] Embodiment 64g is the method or composition of any one of embodiments 64-64f, wherein the mol-% amine lipid is 48-53 mol-%.

[0084] Embodiment 64h is the method or composition of any one of embodiments 64-64g, wherein the mol-% amine lipid is 53-57 mol-%.

[0085] Embodiment 64i is the method or composition of any one of embodiments 64-64h, wherein the N/P ratio is 6 ± 1.

[0086] Embodiment 64j is the method or composition of any one of embodiments 64-64i, wherein the N/P ratio is 6 ± 0.5.

[0087] Embodiment 64k is the method or composition of any one of embodiments 64-64j, wherein the amine lipid is Lipid A.

[0088] Embodiment 64l is the method or composition of any one of embodiments 64-64l, wherein the amine lipid is an analog of Lipid A.

[0089] Embodiment 64m is the method or composition of embodiment 64l, wherein the analog is an acetal analog.

[0090] Embodiment 64n is the method or composition of embodiment 64m, wherein the acetal analog is a C4-C12 acetal analog.

[0091] Embodiment 64o is the method or composition of embodiment 64m, wherein the acetal analog is a C5-C12 acetal analog.

[0092] Embodiment 64p is the method or composition of embodiment 64m, wherein the acetal analog is a C5-C10 acetal analog.

[0093] Embodiment 64q is the method or composition of embodiment 64m, wherein the acetal analog is chosen from a C4, C5, C6, C7, C9, C10, C11, and C12 analog.

[0094] Embodiment 64r is the method or composition of any one of embodiments 64-64q, wherein the helper lipid is cholesterol.

[0095] Embodiment 64s is the method or composition of any one of embodiments 64-64r, wherein the neutral lipid is DSPC.

[0096] Embodiment 64t is the method or composition of any one of embodiments 64-64s, wherein the neutral lipid is DPPC.

[0097] Embodiment 64u is the method or composition of any one of embodiments 64-64t, wherein the PEG lipid comprises dimyristoylglycerol (DMG).

[0098] Embodiment 64v is the method or composition of any one of embodiments 64-64u, wherein the PEG lipid comprises a PEG-2k.

[0099] Embodiment 64w is the method or composition of any one of embodiments 64-64v, wherein the PEG lipid is a PEG-DMG.

[00100] Embodiment 64x is the method or composition of embodiment 64w, wherein the PEG-DMG is a PEG2k-DMG.

[00101] Embodiment 64y is the method or composition of any one of embodiments 64-64x, wherein the LNP composition is essentially free of neutral lipid.

[00102] Embodiment 64z is the method or composition of embodiment 64y, wherein the neutral lipid is a phospholipid.

[00103] Embodiment 65 is the method or composition of any one of embodiments 57-64z, wherein the LNP comprises a neutral lipid, optionally wherein the neutral lipid is DSPC.

[00104] Embodiment 66 is the method or composition of any one of embodiments 64-65, wherein the amine lipid is present at about 50 mol-%.

[00105] Embodiment 67 is the method or composition of any one of embodiments 64-66, wherein the neutral lipid is present at about 9 mol-%.

[00106] Embodiment 68 is the method or composition of any one of embodiments 62-67, wherein the stealth lipid is present at about 3 mol-%.

[00107] Embodiment 69 is the method or composition of any one of embodiments 60-68, wherein the helper lipid is present at about 38 mol-%.

[00108] Embodiment 70 is the method or composition of any one of the preceding embodiments, wherein the LNP has an N/P ratio of about 6.

[00109] Embodiment 71 is the method or composition of embodiment 70, wherein the LNP comprises a lipid component and the lipid component comprises: about 50 mol-% amine lipid such as Lipid A; about 9 mol-% neutral lipd such as DSPC; about 3 mol-% of stealth lipid such as a PEG lipid, such as PEG2k-DMG, and the remainder of the lipid component is helper lipid such as cholesterol wherein the N/P ratio of the LNP composition is about 6.

[00110] Embodiment 72 is the method or composition of any one of embodiments 64-71, wherein the amine lipid is Lipid A.

[00111] Embodiment 73 is the method or composition of any one of embodiments 64-72, wherein the neutral lipid is DSPC.

[00112] Embodiment 74 is the method or composition of any one of embodiments 62-73, wherein the stealth lipid is PEG2k-DMG.

[00113] Embodiment 75 is the method or composition of any one of embodiments 60-74, wherein the helper lipid is cholesterol.

[00114] Embodiment 76 is the method or composition of any one of embodiments 70, wherein the LNP comprises a lipid component and the lipid component comprises: about 50 mol-% Lipid A; about 9 mol-% DSPC; about 3 mol-% of PEG2k-DMG, and the remainder of the lipid component is cholesterol wherein the N/P ratio of the LNP composition is about 6.

[00115] Embodiment 77 is the method or composition of any one of the preceding embodiments, wherein the RNA-guided DNA binding agent is a Cas cleavase.

[00116] Embodiment 78 is the method or composition of embodiment 77, wherein the RNA-guided DNA binding agent is Cas9.

[00117] Embodiment 79 is the method or composition of any one of the preceding embodiments, wherein the RNA-guided DNA binding agent is modified.

[00118] Embodiment 80 is the method or composition of embodiment 79, wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

[00119] Embodiment 81 is the method or composition of any one of the preceding embodiments, wherein the RNA-guided DNA binding agent is a Cas from a Type-II CRISPR/Cas system.

[00120] Embodiment 82 is the method or composition of any one of the preceding embodiments, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

[00121] Embodiment 83 is the method or composition for use of any one of embodiments 2-82, wherein the composition reduces or prevents amyloids or amyloid fibrils comprising TTR.

[00122] Embodiment 84 is the method or composition for use of embodiment 83, wherein the amyloids or amyloid fibrils are in the nerves, heart, or gastrointestinal track.

[00123] Embodiment 85 is the method or composition for use of any one of embodiments 2-84, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the *TTR* gene.

[00124] Embodiment 86 is the method or composition for use of embodiment 85, wherein NHEJ leads to a deletion or insertion of a nucleotide(s) during repair of a DSB in the *TTR* gene.

[00125] Embodiment 87 is the method or composition for use of embodiment 86, wherein the deletion or insertion of a nucleotide(s) induces a frame shift or nonsense mutation in the *TTR* gene.

[00126] Embodiment 88 is the method or composition for use of embodiment 86, wherein a frame shift or nonsense mutation is induced in the *TTR* gene of at least 50% of liver cells.

[00127] Embodiment 89 is the method or composition for use of embodiment 88, wherein a frame shift or nonsense mutation is induced in the *TTR* gene of 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100% of liver cells.

[00128] Embodiment 90 is the method or composition for use of any one of embodiments 86-89, wherein a deletion or insertion of a nucleotide(s) occurs in the *TTR* gene at least 50-fold or more than in off-target sites.

[00129] Embodiment 91 is the method or composition for use of embodiment 90, wherein the deletion or insertion of a nucleotide(s) occurs in the *TTR* gene 50-fold to 150-fold, 150-fold to 500-fold, 500-fold to 1500-fold, 1500-fold to 5000-fold, 5000-fold to 15000-fold, 15000-fold to 30000-fold, or 30000-fold to 60000-fold more than in off-target sites.

[00130] Embodiment 92 is the method or composition for use of any one of embodiments 86-91, wherein the deletion or insertion of a nucleotide(s) occurs at less than or equal to 3, 2, 1, or 0 off-target site(s) in primary human hepatocytes, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[00131] Embodiment 93 is the method or composition for use of embodiment 92, wherein the deletion or insertion of a nucleotide(s) occurs at a number of off-target sites in primary human hepatocytes that is less than the number of off-target sites at which a deletion or insertion of a nucleotide(s) occurs in Cas9-overexpressing cells, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[00132] Embodiment 94 is the method or composition for use of embodiment 93, wherein the Cas9-overexpressing cells are HEK293 cells stably expressing Cas9.

[00133] Embodiment 95 is the method or composition for use of any one of embodiments 92-94, wherein the number of off-target sites in primary human hepatocytes is determined by analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA and the guide RNA, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[00134] Embodiment 96 is the method or composition for use of any one of embodiments 92-94, wherein the number of off-target sites in primary human hepatocytes is determined by an oligonucleotide insertion assay comprising analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[00135] Embodiment 97 is the method or composition of any one of embodiments 1-36 or 39-96, wherein the sequence of the guide RNA is:

a) SEQ ID NO: 92 or 104;

b) SEQ ID NO: 87, 89, 96, or 113;

c) SEQ ID NO: 100, 102, 106, 111, or 112; or

d) SEQ ID NO: 88, 90, 91, 93, 94, 95, 97, 101, 103, 108, or 109.

[00136] Embodiment 98 is the method or composition of embodiment 97, wherein the guide RNA does not produce indels at off-target site(s) that occur in a protein coding region in the genome of primary human hepatocytes.

[00137] Embodiment 99 is the method or composition for use of any one of embodiments 2-98, wherein administering the composition reduces levels of TTR in the subject.

[00138] Embodiment 100 is the method or composition for use of embodiment 99, wherein the levels of TTR are reduced by at least 50%.

[00139] Embodiment 101 is the method or composition for use of embodiment 100, wherein the levels of TTR are reduced by 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100%.

[00140] Embodiment 102 is the method or composition for use of embodiment 100 or 101, wherein the levels of TTR are measured in serum, plasma, blood, cerebral spinal fluid, or sputum.

[00141] Embodiment 103 is the method or composition for use of embodiment 100 or 101, wherein the levels of TTR are measured in liver, choroid plexus, and/or retina.

[00142] Embodiment 104 is the method or composition for use of any one of embodiments 99-103, wherein the levels of TTR are measured via enzyme-linked immunosorbent assay (ELISA).

[00143] Embodiment 105 is the method or composition for use of any one of embodiments 2-104, wherein the subject has ATTR.

[00144] Embodiment 106 is the method or composition for use of any one of embodiments 2-105, wherein the subject is human.

[00145] Embodiment 107 is the method or composition for use of embodiment 105 or 106, wherein the subject has ATTRwt.

[00146] Embodiment 108 is the method or composition for use of embodiment 105 or 106, wherein the subject has hereditary ATTR.

[00147] Embodiment 109 is the method or composition for use of any one of embodiments 2-106 or 108, wherein the subject has a family history of ATTR.

[00148] Embodiment 110 is the method or composition for use of any one of embodiments 2-106 or 108-109, wherein the subject has familial amyloid polyneuropathy.

[00149] Embodiment 111 is the method or composition for use of any one of embodiments 2-110, wherein the subject has only or predominantly nerve symptoms of ATTR.

[00150] Embodiment 112 is the method or composition for use of any one of embodiments 2-111, wherein the subject has familial amyloid cardiomyopathy.

[00151] Embodiment 113 is the method or composition for use of any one of embodiments 2-110 or 112, wherein the subject has only or predominantly cardiac symptoms of ATTR.

[00152] Embodiment 114 is the method or composition for use of any one of embodiments 2-113, wherein the subject expresses TTR having a V30 mutation.

[00153] Embodiment 115 is the method or composition for use of embodiment 114, wherein the V30 mutation is V30A, V30G, V30L, or V30M.

[00154] Embodiment 116 is the method or composition for use of embodiment any one of embodiments 2-113, wherein the subject expresses TTR having a T60 mutation.

[00155] Embodiment 117 is the method or composition for use of embodiment 116, wherein the T60 mutation is T60A.

[00156] Embodiment 118 is the method or composition for use of embodiment any one of embodiments 2-113, wherein the subject expresses TTR having a V122 mutation.

[00157] Embodiment 119 is the method or composition for use of embodiment 118, wherein the V122 mutation is V122A, V122I, or V122(-).

[00158] Embodiment 120 is the method or composition for use of any one of embodiments 2-113, wherein the subject expresses wild-type TTR.

[00159] Embodiment 121 is the method or composition for use of any one of embodiments 2-107, or 120, wherein the subject does not express TTR having a V30, T60, or V122 mutation.

[00160] Embodiment 122 is the method or composition for use of any one of embodiments 2-107, or 120-121, wherein the subject does not express TTR having a pathological mutation.

[00161] Embodiment 123 is the method or composition for use of embodiment 122, wherein the subject is homozygous for wild-type *TTR*.

[00162] Embodiment 124 is the method or composition for use of any one of embodiments 2-123, wherein after administration the subject has an improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy.

[00163] Embodiment 125 is the method or composition for use of embodiment 124, wherein the improvement, stabilization, or slowing of change in sensory neuropathy is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

[00164] Embodiment 126 is the method or composition for use of any one of embodiments 2-125, wherein the subject has an improvement, stabilization, or slowing of change in symptoms of congestive heart failure.

[00165] Embodiment 127 is the method or composition for use of embodiment 126, wherein the improvement, stabilization, or slowing of change in congestive heart failure is measured using cardiac biomarker tests, lung function tests, chest x-rays, or electrocardiography.

[00166] Embodiment 128 is the method or composition for use of any one of embodiments 2-127, wherein the composition or pharmaceutical formulation is administered via a viral vector.

[00167] Embodiment 129 is the method or composition for use of any one of embodiments 2-127, wherein the composition or pharmaceutical formulation is administered via lipid nanoparticles.

[00168] Embodiment 130 is the method or composition for use of any one of embodiments 2-129, wherein the subject is tested for specific mutations in the *TTR* gene before administering the composition or formulation.

[00169] Embodiment 131 is the method or composition of any one of the preceding embodiments, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 5, 6, 9, 13, 14, 15, 16, 17, 22, 23, 27, 30, 35, 36, 37, 38, 55, 63, 65, 66, 68, or 69.

[00170] Embodiment 132 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 5.

[00171] Embodiment 133 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 6.

[00172] Embodiment 134 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 7.

[00173] Embodiment 135 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 8.

[00174] Embodiment 136 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 9.

[00175] Embodiment 137 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 10.

[00176] Embodiment 138 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 11.

[00177] Embodiment 139 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 12.

[00178] Embodiment 140 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 13.

[00179] Embodiment 141 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 14.

[00180] Embodiment 142 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 15.

[00181] Embodiment 143 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 16.

[00182] Embodiment 144 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 17.

[00183] Embodiment 145 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 18.

[00184] Embodiment 146 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 19.

[00185] Embodiment 147 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 20.

[00186] Embodiment 148 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 21.

[00187] Embodiment 149 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 22.

[00188] Embodiment 150 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 23.

[00189] Embodiment 151 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 24.

[00190] Embodiment 152 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 25.

[00191] Embodiment 153 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 26.

[00192] Embodiment 154 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 27.

[00193] Embodiment 155 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 28.

[00194] Embodiment 156 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 29.

[00195] Embodiment 157 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 30.

[00196] Embodiment 158 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 31.

[00197] Embodiment 159 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 32.

[00198] Embodiment 160 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 33.

[00199] Embodiment 161 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 34.

[00200] Embodiment 162 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 35.

[00201] Embodiment 163 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 36.

[00202] Embodiment 164 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 37.

[00203] Embodiment 165 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 38.

[00204] Embodiment 166 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 39.

[00205] Embodiment 167 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 40.

[00206] Embodiment 168 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 41.

[00207] Embodiment 169 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 42.

[00208] Embodiment 170 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 43.

[00209] Embodiment 171 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 44.

[00210] Embodiment 172 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 45.

[00211] Embodiment 173 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 46.

[00212] Embodiment 174 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 47.

[00213] Embodiment 175 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 48.

[00214] Embodiment 176 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 49.

[00215] Embodiment 177 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 50.

[00216] Embodiment 178 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 51.

[00217] Embodiment 179 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 52.

[00218] Embodiment 180 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 53.

[00219] Embodiment 181 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 54.

[00220] Embodiment 182 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 55.

[00221] Embodiment 183 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 56.

[00222] Embodiment 184 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 57.

[00223] Embodiment 185 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 58.

[00224] Embodiment 186 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 59.

[00225] Embodiment 187 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 60.

[00226] Embodiment 188 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 61.

[00227] Embodiment 189 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 62.

[00228] Embodiment 190 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 63.

[00229] Embodiment 191 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 64.

[00230] Embodiment 192 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 65.

[00231] Embodiment 193 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 66.

[00232] Embodiment 194 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 67.

[00233] Embodiment 195 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 68.

[00234] Embodiment 196 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 69.

[00235] Embodiment 197 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 70.

[00236] Embodiment 198 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 71.

[00237] Embodiment 199 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 72.

[00238] Embodiment 200 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 74.

[00239] Embodiment 201 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 75.

[00240] Embodiment 202 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 76.

[00241] Embodiment 203 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 77.

[00242] Embodiment 204 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 78.

[00243] Embodiment 205 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 80.

[00244] Embodiment 206 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 81.

[00245] Embodiment 207 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 82.

[00246] Embodiment 208 is the composition or method of any one of the preceding embodiments, wherein the open reading frame has at least 95% identity to SEQ ID NO: 311 over at least its first 10%, 12%, 15%, 20%, 25%, 30%, or 35% of its sequence.

[00247] Embodiment 209 is the composition or method of any one of the preceding embodiments, wherein the open reading frame comprises a sequence with at least 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 311.

[00248] Embodiment 210 is the composition or method of any one of the preceding embodiments, wherein at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons of the open reading frame are codons listed in Table 4, Table 5, or Table 7.

[00249] Embodiment 211 is the composition or method of embodiment 210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons listed in Table 4.

[00250] Embodiment 212 is the composition or method of embodiment 210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons of the Low U codon set of Table 5.

[00251] Embodiment 213 is the composition or method of embodiment 210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons of the Low A codon set of Table 5.

[00252] Embodiment 214 is the composition or method of embodiment 210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons of the Low A/U codon set of Table 5.

[00253] Embodiment 215 is the composition or method of embodiment 210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons listed in Table 7.

[00254] Embodiment 216 is the composition or method of any one of the preceding embodiments, wherein the open reading frame has an adenine content ranging from its minimum adenine content to 101%, 102%, 103%, 105%, 110%, 115%, 120%, or 123% of the minimum adenine content.

[00255] Embodiment 217 is the composition or method of any one of the preceding embodiments, wherein the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 101%, 102%, 103%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, or 150% of the minimum adenine dinucleotide content.

[00256] Embodiment 218 is the composition or method of any one of the preceding embodiments, wherein the nucleic acid comprises a 5' UTR with at least 90% identity to any one of SEQ ID NOs: 232, 234, 236, 238, 241, or 275-277.

[00257] Embodiment 219 is the composition or method of any one of the preceding embodiments, wherein the nucleic acid comprises a 3' UTR with at least 90% identity to any one of SEQ ID NOs: 233, 235, 237, 239, or 240.

[00258] Embodiment 220 is the composition or method of any one of the preceding embodiments, wherein the nucleic acid comprises a 5' UTR and a 3' UTR from the same source.

[00259] Embodiment 221 is the composition or method of any one of the preceding embodiments, wherein the nucleic acid is an mRNA comprising a 5' cap selected from Cap0, Cap1, and Cap2.

[00260] Embodiment 222 is the composition or method of any one of the preceding embodiments, wherein the open reading frame comprises a sequence with at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 377.

[00261] Embodiment 223 is the composition or method of any of the preceding embodiments, wherein the nucleic acid is an mRNA in which at least 10% of the uridine is substituted with a modified uridine.

[00262] Embodiment 224 is the composition or method of embodiment 223, wherein the modified uridine is one or more of N1-methyl-pseudouridine, pseudouridine, 5-methoxyuridine, or 5-iodouridine.

[00263] Embodiment 225 is the composition or method of embodiment 223, wherein the modified uridine is one or both of N1-methyl-pseudouridine or 5-methoxyuridine.

[00264] Embodiment 226 is the composition or method of embodiment 223, wherein the modified uridine is N1-methyl-pseudouridine.

[00265] Embodiment 227 is the composition or method of embodiment 223, wherein the modified uridine is 5-methoxyuridine.

[00266] Embodiment 228 is the composition or method of any one of embodiments 223-210, wherein 15% to 45% of the uridine in the mRNA is substituted with the modified uridine.

[00267] Embodiment 229 is the composition or method of any one of embodiments 223-211, wherein at least 20% or at least 30% of the uridine in the mRNA is substituted with the modified uridine.

[00268] Embodiment 230 is the composition or method of embodiment 229, wherein at least 80% or at least 90% of the uridine in the mRNA is substituted with the modified uridine.

[00269] Embodiment 231 is the composition or method of embodiment 229, wherein 100% of the uridine in the mRNA is substituted with the modified uridine.

[00270] Embodiment 232 is a use of a composition or formulation of any of embodiments 1 or 4-231 for the preparation of a medicament for treating a human subject having ATTR.

BRIEF DESCRIPTION OF THE DRAWINGS

[00271] FIGs. 1A-1B show % Editing in primary human hepatocytes from two donors as described in Example 2.

[00272] FIG. 2 shows % Editing in primary cyno hepatocytes as described in Example 2.

[00273] FIGS. 3A-C show serum TTR levels (FIG. 3A), liver TTR editing (FIG 3B), and circulating ALT levels (FIG. 3C) in an *in vivo* study in nonhuman primates comparing 30' administration of LNPs to a long dosing protocol.

DETAILED DESCRIPTION

[00274] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention will be described in conjunction with the illustrated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the invention as defined by the appended claims.

[00275] Before describing the present teachings in detail, it is to be understood that the disclosure is not limited to specific compositions or process steps, as such may vary. It should be noted that, as used in this specification and the appended claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a conjugate" includes a plurality of conjugates and reference to "a cell" includes a plurality of cells and the like.

[00276] Numeric ranges are inclusive of the numbers defining the range. Measured and measureable values are understood to be approximate, taking into account significant digits and the error associated with the measurement. Also, the use of "comprise", "comprises", "comprising", "contain", "contains", "containing", "include", "includes", and "including" are not intended to be limiting. It is to be understood that both the foregoing general description and detailed description are exemplary and explanatory only and are not restrictive of the teachings.

[00277] Unless specifically noted in the above specification, embodiments in the specification that recite "comprising" various components are also contemplated as "consisting of" or "consisting essentially of" the recited components; embodiments in the specification that recite "consisting of" various components are also contemplated as "comprising" or "consisting essentially of" the recited components; and embodiments in the specification that recite "consisting essentially of" various components are also contemplated as "consisting of" or "comprising" the recited components (this interchangeability does not apply to the use of these terms in the claims). The term "or" is used in an inclusive sense, i.e., equivalent to "and/or," unless the context clearly indicates otherwise.

[00278] The section headings used herein are for organizational purposes only and are not to be construed as limiting the desired subject matter in any way. In the event that any material incorporated by reference contradicts any term defined in this specification or any other express content of this specification, this specification controls. While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

I. Definitions

[00279] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[00280] "Polynucleotide" and "nucleic acid" are used herein to refer to a multimeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases or base analogs linked together along a backbone, including conventional RNA, DNA, mixed RNA-DNA, and polymers that are analogs thereof. A nucleic acid "backbone" can be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds ("peptide nucleic acids" or PNA; PCT No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of a nucleic acid can be ribose, deoxyribose, or similar compounds with substitutions, e.g., 2' methoxy or 2' halide substitutions. Nitrogenous bases can be conventional bases (A, G, C, T, U), analogs thereof (e.g., modified uridines such as 5-methoxyuridine, pseudouridine, or N1-methylpseudouridine, or others); inosine; derivatives of purines or pyrimidines (e.g., N^4-methyl deoxyguanosine, deaza- or aza-purines, deaza- or aza-pyrimidines, pyrimidine bases with substituent groups at the 5 or 6 position (e.g., 5-methylcytosine), purine bases with a substituent at the 2, 6, or 8 positions, 2-amino-6-methylaminopurine, O^6-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O^4-alkyl-pyrimidines; US Pat. No. 5,378,825 and PCT No. WO 93/13121). For general discussion see *The Biochemistry of the Nucleic Acids* 5-36, Adams et al., ed., 11th ed., 1992). Nucleic acids can include one or more "abasic" residues where the backbone includes no nitrogenous base for position(s) of the polymer (US Pat. No. 5,585,481). A nucleic acid can comprise only conventional RNA or DNA sugars, bases and linkages, or can include both conventional components and substitutions (e.g., conventional bases with 2' methoxy linkages, or polymers containing both conventional bases and one or

more base analogs). Nucleic acid includes "locked nucleic acid" (LNA), an analogue containing one or more LNA nucleotide monomers with a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhance hybridization affinity toward complementary RNA and DNA sequences (Vester and Wengel, 2004, *Biochemistry* 43(42):13233-41). RNA and DNA have different sugar moieties and can differ by the presence of uracil or analogs thereof in RNA and thymine or analogs thereof in DNA.

[00281] "Polypeptide" as used herein refers to a multimeric compound comprising amino acid residues that can adopt a three-dimensional conformation. Polypeptides include but are not limited to enzymes, enzyme precursor proteins, regulatory proteins, structural proteins, receptors, nucleic acid binding proteins, antibodies, etc. Polypeptides may, but do not necessarily, comprise post-translational modifications, non-natural amino acids, prosthetic groups, and the like.

[00282] "Guide RNA", "gRNA", and "guide" are used herein interchangeably to refer to either a crRNA (also known as CRISPR RNA), or the combination of a crRNA and a trRNA (also known as tracrRNA). The crRNA and trRNA may be associated as a single RNA molecule (single guide RNA, sgRNA) or in two separate RNA molecules (dual guide RNA, dgRNA). "Guide RNA" or "gRNA" refers to each type. The trRNA may be a naturally-occurring sequence, or a trRNA sequence with modifications or variations compared to naturally-occurring sequences. Guide RNAs can include modified RNAs as described herein.

[00283] As used herein, a "guide sequence" refers to a sequence within a guide RNA that is complementary to a target sequence and functions to direct a guide RNA to a target sequence for binding or modification (e.g., cleavage) by an RNA-guided DNA binding agent. A "guide sequence" may also be referred to as a "targeting sequence," or a "spacer sequence." A guide sequence can be 20 base pairs in length, e.g., in the case of Streptococcus pyogenes (i.e., Spy Cas9) and related Cas9 homologs/orthologs. Shorter or longer sequences can also be used as guides, e.g., 15-, 16-, 17-, 18-, 19-, 21-, 22-, 23-, 24-, or 25-nucleotides in length. In some embodiments, the guide sequence and the target region may be 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch. For example, the guide sequence and the target sequence may contain 1, 2, 3, or 4 mismatches, where the total length of the target sequence is at least 17, 18, 19, 20 or more base pairs. In some embodiments, the guide sequence and the target region may contain 1-4 mismatches where the guide sequence comprises at least 17, 18, 19, 20 or more nucleotides. In some embodiments, the guide

sequence and the target region may contain 1, 2, 3, or 4 mismatches where the guide sequence comprises 20 nucleotides.

[00284] Target sequences for Cas proteins include both the positive and negative strands of genomic DNA (i.e., the sequence given and the sequence's reverse compliment), as a nucleic acid substrate for a Cas protein is a double stranded nucleic acid. Accordingly, where a guide sequence is said to be "complementary to a target sequence", it is to be understood that the guide sequence may direct a guide RNA to bind to the reverse complement of a target sequence. Thus, in some embodiments, where the guide sequence binds the reverse complement of a target sequence, the guide sequence is identical to certain nucleotides of the target sequence (e.g., the target sequence not including the PAM) except for the substitution of U for T in the guide sequence.

[00285] As used herein, an "RNA-guided DNA binding agent" means a polypeptide or complex of polypeptides having RNA and DNA binding activity, or a DNA-binding subunit of such a complex, wherein the DNA binding activity is sequence-specific and depends on the sequence of the RNA. Exemplary RNA-guided DNA binding agents include Cas cleavases/nickases and inactivated forms thereof ("dCas DNA binding agents"). "Cas nuclease", also called "Cas protein", as used herein, encompasses Cas cleavases, Cas nickases, and dCas DNA binding agents. Cas cleavases/nickases and dCas DNA binding agents include a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases. As used herein, a "Class 2 Cas nuclease" is a single-chain polypeptide with RNA-guided DNA binding activity, such as a Cas9 nuclease or a Cpf1 nuclease. Class 2 Cas nucleases include Class 2 Cas cleavases and Class 2 Cas nickases (e.g., H840A, D10A, or N863A variants), which further have RNA-guided DNA cleavases or nickase activity, and Class 2 dCas DNA binding agents, in which cleavase/nickase activity is inactivated. Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g, K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., *Cell*, 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like nuclease domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. *See, e.g.*, Zetsche, Tables S1 and S3. "Cas9" encompasses Spy Cas9, the variants of Cas9 listed herein, and equivalents thereof. See, *e.g.*, Makarova et al., *Nat Rev Microbiol*, 13(11): 722-36 (2015); Shmakov et al., *Molecular Cell,* 60:385-397 (2015).

[00286] "Modified uridine" is used herein to refer to a nucleoside other than thymidine with the same hydrogen bond acceptors as uridine and one or more structural differences from uridine. In some embodiments, a modified uridine is a substituted uridine, i.e., a uridine in which one or more non-proton substituents (e.g., alkoxy, such as methoxy) takes the place of a proton. In some embodiments, a modified uridine is pseudouridine. In some embodiments, a modified uridine is a substituted pseudouridine, i.e., a pseudouridine in which one or more non-proton substituents (e.g., alkyl, such as methyl) takes the place of a proton, e.g., N1-methyl pseudouridine. In some embodiments, a modified uridine is any of a substituted uridine, pseudouridine, or a substituted pseudouridine.

[00287] "Uridine position" as used herein refers to a position in a polynucleotide occupied by a uridine or a modified uridine. Thus, for example, a polynucleotide in which "100% of the uridine positions are modified uridines" contains a modified uridine at every position that would be a uridine in a conventional RNA (where all bases are standard A, U, C, or G bases) of the same sequence. Unless otherwise indicated, a U in a polynucleotide sequence of a sequence table or sequence listing in, or accompanying, this disclosure can be a uridine or a modified uridine.

[00288] As used herein, a first sequence is considered to "comprise a sequence with at least X% identity to" a second sequence if an alignment of the first sequence to the second sequence shows that X% or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenosine for all of thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine or modified guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar

length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server is generally appropriate.

[00289] "mRNA" is used herein to refer to a polynucleotide that is RNA or modified RNA and comprises an open reading frame that can be translated into a polypeptide (i.e., can serve as a substrate for translation by a ribosome and amino-acylated tRNAs). mRNA can comprise a phosphate-sugar backbone including ribose residues or analogs thereof, e.g., 2'-methoxy ribose residues. In some embodiments, the sugars of a nucleic acid phosphate-sugar backbone consist essentially of ribose residues, 2'-methoxy ribose residues, or a combination thereof. In general, mRNAs do not contain a substantial quantity of thymidine residues (e.g., 0 residues or fewer than 30, 20, 10, 5, 4, 3, or 2 thymidine residues; or less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1% thymidine content). An mRNA can contain modified uridines at some or all of its uridine positions.

[00290] As used herein, the "minimum uridine content" of a given ORF is the uridine content of an ORF that (a) uses a minimal uridine codon at every position and (b) encodes the same amino acid sequence as the given ORF. The minimal uridine codon(s) for a given amino acid is the codon(s) with the fewest uridines (usually 0 or 1 except for a codon for phenylalanine, where the minimal uridine codon has 2 uridines). Modified uridine residues are considered equivalent to uridines for the purpose of evaluating minimum uridine content.

[00291] As used herein, the "minimum uridine dinucleotide content" of a given ORF is the lowest possible uridine dinucleotide (UU) content of an ORF that (a) uses a minimal uridine codon (as discussed above) at every position and (b) encodes the same amino acid sequence as the given ORF. The uridine dinucleotide (UU) content can be expressed in absolute terms as the enumeration of UU dinucleotides in an ORF or on a rate basis as the percentage of positions occupied by the uridines of uridine dinucleotides (for example, AUUAU would have a uridine dinucleotide content of 40% because 2 of 5 positions are occupied by the uridines of a uridine dinucleotide). Modified uridine residues are considered equivalent to uridines for the purpose of evaluating minimum uridine dinucleotide content.As used herein, the "minimum adenine content" of a given open reading frame (ORF) is the adenine content of an ORF that (a) uses a minimal adenine codon at every position and (b) encodes the same amino acid sequence as the given ORF. The minimal adenine codon(s) for a given amino acid is the codon(s) with the fewest adenines (usually 0 or 1 except for a codon for lysine and asparagine, where the minimal adenine codon has 2 adenines). Modified adenine residues are considered equivalent to adenines for the purpose of evaluating minimum adenine content.

[00292] As used herein, the "minimum adenine dinucleotide content" of a given open reading frame (ORF) is the lowest possible adenine dinucleotide (AA) content of an ORF that (a) uses a minimal adenine codon (as discussed above) at every position and (b) encodes the same amino acid sequence as the given ORF. The adenine dinucleotide (AA) content can be expressed in absolute terms as the enumeration of AA dinucleotides in an ORF or on a rate basis as the percentage of positions occupied by the adenines of adenine dinucleotides (for example, UAAUA would have an adenine dinucleotide content of 40% because 2 of 5 positions are occupied by the adenines of an adenine dinucleotide). Modified adenine residues are considered equivalent to adenines for the purpose of evaluating minimum adenine dinucleotide content.

[00293] As used herein, "TTR" refers to transthyretin, which is the gene product of a *TTR* gene.

[00294] As used herein, "amyloid" refers to abnormal aggregates of proteins or peptides that are normally soluble. Amyloids are insoluble, and amyloids can create proteinaceous deposits in organs and tissues. Proteins or peptides in amyloids may be misfolded into a form that allows many copies of the protein to stick together to form fibrils. While some forms of amyloid may have normal functions in the human body, "amyloids" as used herein refers to abnormal or pathologic aggregates of protein. Amyloids may comprise a single protein or peptide, such as TTR, or they may comprise multiple proteins or peptides, such as TTR and additional proteins.

[00295] As used herein, "amyloid fibrils" refers to insoluble fibers of amyloid that are resistant to degradation. Amyloid fibrils can produce symptoms based on the specific protein or peptide and the tissue and cell type in which it has aggregated.

[00296] As used herein, "amyloidosis" refers to a disease characterized by symptoms caused by deposition of amyloid or amyloid fibrils. Amyloidosis can affect numerous organs including the heart, kidney, liver, spleen, nervous system, and digestive track.

[00297] As used herein, "ATTR," "TTR-related amyloidosis," "TTR amyloidosis," "ATTR amyloidosis," or "amyloidosis associated with TTR" refers to amyloidosis associated with deposition of TTR.

[00298] As used herein, "familial amyloid cardiomyopathy" or "FAC" refers to a hereditary transthyretin amyloidosis (ATTR) characterized primarily by restrictive cardiomyopathy. Congestive heart failure is common in FAC. Average age of onset is approximately 60-70 years of age, with an estimated life expectancy of 4-5 years after diagnosis.

[00299] As used herein, "familial amyloid polyneuropathy" or "FAP" refers to a hereditary transthyretin amyloidosis (ATTR) characterized primarily by sensorimotor neuropathy. Autonomic neuropathy is common in FAP. While neuropathy is a primary feature, symptoms of FAP may also include cachexia, renal failure, and cardiac disease. Average age of onset of FAP is approximately 30-50 years of age, with an estimated life expectancy of 5-15 after diagnosis.

[00300] As used herein, "wild-type ATTR" and "ATTRwt" refer to ATTR not associated with a pathological TTR mutation such as T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(-). ATTRwt has also been referred to as senile systemic amyloidosis. Onset typically occurs in men aged 60 or higher with the most common symptoms being congestive heart failure and abnormal heart rhythm such as atrial fibrillation. Additional symptoms include consequences of poor heart function such as shortness of breath, fatigue, dizziness, swelling (especially in the legs), nausea, angina, disrupted sleep, and weight loss. A history of carpal tunnel syndrome indicates increased risk for ATTRwt and may in some cases be indicative of early-stage disease. ATTRwt generally leads to decreasing heart function over time but can have a better prognosis than hereditary ATTR because wild-type TTR deposits accumulate more slowly. Existing treatments are similar to other forms of ATTR (other than liver transplantation) and are generally directed to supporting or improving heart function, ranging from diuretics and limited fluid and salt intake to anticoagulants, and in severe cases, heart transplants. Nonetheless, like FAC, ATTRwt can result in death from heart failure, sometimes within 3-5 years of diagnosis.

[00301] Guide sequences useful in the guide RNA compositions and methods described herein are shown in Table 1 and throughout the application.

[00302] As used herein, "hereditary ATTR" refers to ATTR that is associated with a mutation in the sequence of the *TTR* gene. Known mutations in the *TTR* gene associated with ATTR include those resulting in TTR with substitutions of T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(-).

[00303] As used herein, "indels" refer to insertion/deletion mutations consisting of a number of nucleotides that are either inserted or deleted at the site of double-stranded breaks (DSBs) in a target nucleic acid.

[00304] As used herein, "knockdown" refers to a decrease in expression of a particular gene product (e.g., protein, mRNA, or both). Knockdown of a protein can be measured either by detecting protein secreted by tissue or population of cells (e.g., in serum or cell media) or by detecting total cellular amount of the protein from a tissue or cell population of interest.

Methods for measuring knockdown of mRNA are known, and include sequencing of mRNA isolated from a tissue or cell population of interest. In some embodiments, "knockdown" may refer to some loss of expression of a particular gene product, for example a decrease in the amount of of mRNA transcribed or a decrease in the amount of protein expressed or secreted by a population of cells (including *in vivo* populations such as those found in tissues).

[00305] As used herein, "mutant TTR" refers to a gene product of *TTR* (i.e., the TTR protein) having a change in the amino acid sequence of TTR compared to the wildtype amino acid sequence of TTR. The human wild-type TTR sequence is available at NCBI Gene ID: 7276; Ensembl: Ensembl: ENSG00000118271. Mutants forms of TTR associated with ATTR, e.g., in humans, include T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(-).

[00306] As used herein, "ribonucleoprotein" (RNP) or "RNP complex" refers to a guide RNA together with an RNA-guided DNA binding agent, such as a Cas nuclease, e.g., a Cas cleavase, Cas nickase, or dCas DNA binding agent (e.g., Cas9). In some embodiments, the guide RNA guides the RNA-guided DNA binding agent such as Cas9 to a target sequence, and the guide RNA hybridizes with and the agent binds to the target sequence; in cases where the agent is a cleavase or nickase, binding can be followed by cleaving or nicking.

[00307] As used herein, a "target sequence" refers to a sequence of nucleic acid in a target gene that has complementarity to the guide sequence of the gRNA. The interaction of the target sequence and the guide sequence directs an RNA-guided DNA binding agent to bind, and potentially nick or cleave (depending on the activity of the agent), within the target sequence.

[00308] As used herein, "treatment" refers to any administration or application of a therapeutic for disease or disorder in a subject, and includes inhibiting the disease, arresting its development, relieving one or more symptoms of the disease, curing the disease, or preventing reoccurrence of one or more symptoms of the disease. For example, treatment of ATTR may comprise alleviating symptoms of ATTR.

[00309] As used herein, the term "pathological mutation" refers to a mutation that renders a gene product, such as TTR, more likely to cause, promote, contribute to, or fail to inhibit the development of a disease, such as ATTR.

[00310] As used herein, the term "lipid nanoparticle" (LNP) refers to a particle that comprises a plurality of (i.e., more than one) lipid molecules physically associated with each other by intermolecular forces. The LNPs may be, e.g., microspheres (including unilamellar and multilamellar vesicles, e.g., "liposomes"—lamellar phase lipid bilayers that, in some

embodiments, are substantially spherical—and, in more particular embodiments, can comprise an aqueous core, e.g., comprising a substantial portion of RNA molecules), a dispersed phase in an emulsion, micelles, or an internal phase in a suspension. See also, e.g., WO2017173054A1 and WO2019067992A1, the contents of which are hereby incorporated by reference in their entirety. Any LNP known to those of skill in the art to be capable of delivering nucleotides to subjects may be utilized with the guide RNAs and the nucleic acid encoding an RNA-guided DNA binding agent described herein.

[00311] As used herein, the terms "donor oligonucleotide" or "donor template" refers to a oligonucleotide that includes a desired nucleic acid sequence to be inserted into a target site (e.g., a target sit of a genomic DNA). A donor oligonucleotide may be a single-strand oligonucleotide or a double-strand oligonucleotide. In some embodiments, a donor oligonucleotide may be delivered with a guide RNA and a nucleic acid sequence encoding an RNA-guided DNA binding agent (e.g., Cas9) via use of LNP or transfection.

[00312] As used herein, the terms "nuclear localization signal" (NLS) or "nuclear localization sequence" refers to an amino acid sequence which induces transport of molecules comprising such sequences or linked to such sequences into the nucleus of eukaryotic cells. The nuclear localization signal may form part of the molecule to be transported. In some embodiments, the NLS may be linked to the remaining parts of the molecule by covalent bonds, hydrogen bonds or ionic interactions.

[00313] As used herein, the phrase "pharmaceutically acceptable" means that which is useful in preparing a pharmaceutical composition that is generally non-toxic and is not biologically undesirable and that are not otherwise unacceptable for pharmaceutical use.

[00314] As used herein, "infusion" refers to an active administration of one or more agents with an infusion time of, for example, between approximately 30 minutes and 12 hours. In some embodiments, the one or more agents comprise an LNP, e.g., comprising an mRNA encoding an RNA-guided DNA binding agent (such as Cas9) described herein and a gRNA described herein.

[00315] As used herein, "infusion prophylaxis" refers to a regimen administered to a subject before treatment (e.g., comprising administration of an LNP) comprising one or more, or all, of an intravenous corticosteroid (e.g., dexamethasone 10 mg or equivalent), an antipyretic (e.g. oral acetaminophen or paracetamol 500 mg), an intravenous H1 blocker (e.g., diphenhydramine 50 mg or equivalent), and an intravenous H2 blocker (e.g., ranitidine 50 mg or equivalent). Infusion prophylaxis is optionally combined with advance administration of an oral corticosteroid (e.g., dexamethasone 8 mg or equivalent). In some embodiments, the

oral corticosteroid is administered 8-24 hours prior to treatment. In some embodiments, one or more, or all, of an intravenous corticosteroid (e.g., dexamethasone 10 mg or equivalent), oral acetaminophen 500 mg, an intravenous H1 blocker (e.g., diphenhydramine 50 mg or equivalent), an intravenous H2 blocker (e.g., ranitidine 50 mg or equivalent) are administered 1-2 hours before treatment. In some embodiments, an H1 blocker and/or an H2 blocker are administered orally.

[00316] The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined.

II. Methods and Compositions Targeting the *TTR* gene

[00317] Disclosed herein are methods for inducing a double-stranded break (DSB) within the *TTR* gene in a subject, modifying the *TTR* gene in a cell or subject, treating amyloidosis associated with TTR (ATTR) in a subject, reducing TTR serum concentration in a subject, and/or reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject, and related compositions, including compositions for use in such methods. In general, the disclosed compositions comprise a guide RNA targeting TTR and a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent (e.g., a CRISPR/Cas system). The subjects treated with such methods and compositions may have wild-type or non-wild type *TTR* gene sequences, such as, for example, subjects with ATTR, which may be ATTR wt or a hereditary or familial form of ATTR.

[00318] In some embodiments, the composition is administered by infusion for longer than 30 minutes. In some embodiments, the composition is administered by 30 minute infusion. In some embodiments, the composition is administered by infusion for longer than 60 minutes. In some embodiments, the composition is administered by infusion for longer than 90 minutes. In some embodiments, the composition is administered by infusion for longer than 120 minutes, longer than 150 minutes, longer than 180 minutes, longer than 240 minutes, longer than 300 minutes, or longer than 360 minutes. In some embodiments, the composition is administered by infusion for at least 1 hour, at least 2 hours, at least 4 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours or at least 12 hours. In some embodiments, the composition is administered by infusion for 0.5-1.5 hours, 1.5-2.5 hours, 2.5-3.5 hours, 3.5-4.5 hours, 4.5-5.5 hours, 5.5-6.5 hours, 6.5-7.5 hours, 7.5-8.5 hours, 8.5-9.5 hours, 9.5-10.5 hours, 10.5-11.5 hours, or 11.5-12.5 hours. In some embodiments, the composition is administered by infusion for about 60 minutes, about

90 minutes, about 120 minutes, about 150 minutes, about 180 minutes, about 240 minutes, about 300 minutes, or about 360 minutes. In some embodiments, the composition is administered by infusion for about 45-75 minutes, 75-105 minutes, 105-135 minutes, 135-165 minutes, 165-195 minutes, 195-225 minutes, 225-255 minutes, 255-285 minutes, 285-315 minutes, 315-345 minutes, or 345-375 minutes. In some embodiments, the composition is administered by infusion for about 1.5-6 hours..

A. Guide RNA (gRNAs)

[00319] The guide RNA used in the disclosed methods and compositions comprises a guide sequence targeting the *TTR* gene. Exemplary guide sequences targeting the *TTR* gene are shown in Table 1 at SEQ ID Nos: 5-82.

Table 1: *TTR* targeted guide sequences, nomenclature, chromosomal coordinates, and sequence.

SEQ ID No.	Guide ID	Description	Species	Chromosomal Location	Guide Sequences*
5	CR003335	TTR (Exon 1)	Human	chr18:31591917-31591937	CUGCUCCUCCUCUGCCUUGC
6	CR003336	TTR (Exon 1)	Human	chr18:31591922-31591942	CCUCCUCUGCCUUGCUGGAC
7	CR003337	TTR (Exon 1)	Human	chr18:31591925-31591945	CCAGUCCAGCAAGGCAGAGG
8	CR003338	TTR (Exon 1)	Human	chr18:31591928-31591948	AUACCAGUCCAGCAAGGCAG
9	CR003339	TTR (Exon 1)	Human	chr18:31591934-31591954	ACACAAAUACCAGUCCAGCA
10	CR003340	TTR (Exon 1)	Human	chr18:31591937-31591957	UGGACUGGUAUUUGUGUCUG
11	CR003341	TTR (Exon 1)	Human	chr18:31591941-31591961	CUGGUAUUUGUGUCUGAGGC
12	CR003342	TTR (Exon 2)	Human	chr18:31592880-31592900	CUUCUCUACACCCAGGGCAC
13	CR003343	TTR (Exon 2)	Human	chr18:31592902-31592922	CAGAGGACACUUGGAUUCAC
14	CR003344	TTR (Exon 2)	Human	chr18:31592911-31592931	UUUGACCAUCAGAGGACACU
15	CR003345	TTR (Exon 2)	Human	chr18:31592919-31592939	UCUAGAACUUUGACCAUCAG
16	CR003346	TTR (Exon 2)	Human	chr18:31592928-31592948	AAAGUUCUAGAUGCUGUCCG
17	CR003347	TTR (Exon 2)	Human	chr18:31592948-31592968	CAUUGAUGGCAGGACUGCCU
18	CR003348	TTR (Exon 2)	Human	chr18:31592948-31592968	AGGCAGUCCUGCCAUCAAUG
19	CR003349	TTR (Exon 2)	Human	chr18:31592958-31592978	UGCACGGCCACAUUGAUGGC
20	CR003350	TTR (Exon 2)	Human	chr18:31592962-31592982	CACAUGCACGGCCACAUUGA
21	CR003351	TTR	Human	chr18:315929	AGCCUUUCUGAACACAUGCA

		(Exon 2)		74-31592994	
22	CR003352	TTR (Exon 2)	Human	chr18:31592986-31593006	GAAAGGCUGCUGAUGACACC
23	CR003353	TTR (Exon 2)	Human	chr18:31592987-31593007	AAAGGCUGCUGAUGACACCU
24	CR003354	TTR (Exon 2)	Human	chr18:31593003-31593023	ACCUGGGAGCCAUUUGCCUC
25	CR003355	TTR (Exon 2)	Human	chr18:31593007-31593027	CCCAGAGGCAAAUGGCUCCC
26	CR003356	TTR (Exon 2)	Human	chr18:31593015-31593035	GCAACUUACCCAGAGGCAAA
27	CR003357	TTR (Exon 2)	Human	chr18:31593022-31593042	UUCUUUGGCAACUUACCCAG
28	CR003358	TTR (Exon 3)	Human	chr18:31595127-31595147	AUGCAGCUCUCCAGACUCAC
29	CR003359	TTR (Exon 3)	Human	chr18:31595126-31595146	AGUGAGUCUGGAGAGCUGCA
30	CR003360	TTR (Exon 3)	Human	chr18:31595127-31595147	GUGAGUCUGGAGAGCUGCAU
31	CR003361	TTR (Exon 3)	Human	chr18:31595140-31595160	GCUGCAUGGGCUCACAACUG
32	CR003362	TTR (Exon 3)	Human	chr18:31595143-31595163	GCAUGGGCUCACAACUGAGG
33	CR003363	TTR (Exon 3)	Human	chr18:31595156-31595176	ACUGAGGAGGAAUUUGUAGA
34	CR003364	TTR (Exon 3)	Human	chr18:31595157-31595177	CUGAGGAGGAAUUUGUAGAA
35	CR003365	TTR (Exon 3)	Human	chr18:31595170-31595190	UGUAGAAGGGAUAUACAAAG
36	CR003366	TTR (Exon 3)	Human	chr18:31595193-31595213	AAAUAGACACCAAAUCUUAC
37	CR003367	TTR (Exon 3)	Human	chr18:31595197-31595217	AGACACCAAAUCUUACUGGA
38	CR003368	TTR (Exon 3)	Human	chr18:31595205-31595225	AAGUGCCUUCCAGUAAGAUU
39	CR003369	TTR (Exon 3)	Human	chr18:31595235-31595255	CUCUGCAUGCUCAUGGAAUG
40	CR003370	TTR (Exon 3)	Human	chr18:31595236-31595256	CCUCUGCAUGCUCAUGGAAU
41	CR003371	TTR (Exon 3)	Human	chr18:31595237-31595257	ACCUCUGCAUGCUCAUGGAA
42	CR003372	TTR (Exon 3)	Human	chr18:31595242-31595262	UACUCACCUCUGCAUGCUCA
43	CR003373	TTR (Exon 4)	Human	chr18:31598570-31598590	GUAUUCACAGCCAACGACUC
44	CR003374	TTR (Exon 4)	Human	chr18:31598583-31598603	GCGGCGGGGGCCGGAGUCGU
45	CR003375	TTR (Exon 4)	Human	chr18:31598592-31598612	AAUGGUGUAGCGGCGGGGGC
46	CR003376	TTR (Exon 4)	Human	chr18:31598596-31598616	CGGCAAUGGUGUAGCGGCGG
47	CR003377	TTR (Exon 4)	Human	chr18:31598597-31598617	GCGGCAAUGGUGUAGCGGCG
48	CR003378	TTR (Exon 4)	Human	chr18:31598598-31598618	GGCGGCAAUGGUGUAGCGGC
49	CR003379	TTR (Exon 4)	Human	chr18:31598599-31598619	GGGCGGCAAUGGUGUAGCGG
50	CR003380	TTR (Exon 4)	Human	chr18:31598602-31598622	GCAGGGCGGCAAUGGUGUAG

51	CR003381	TTR (Exon 4)	Human	chr18:31598610-31598630	GGGGCUCAGCAGGGCGGCAA
52	CR003382	TTR (Exon 4)	Human	chr18:31598616-31598636	GGAGUAGGGGCUCAGCAGGG
53	CR003383	TTR (Exon 4)	Human	chr18:31598619-31598639	AUAGGAGUAGGGGCUCAGCA
54	CR003384	TTR (Exon 4)	Human	chr18:31598620-31598640	AAUAGGAGUAGGGGCUCAGC
55	CR003385	TTR (Exon 4)	Human	chr18:31598626-31598646	CCCCUACUCCUAUUCCACCA
56	CR003386	TTR (Exon 4)	Human	chr18:31598629-31598649	CCGUGGUGGAAUAGGAGUAG
57	CR003387	TTR (Exon 4)	Human	chr18:31598630-31598650	GCCGUGGUGGAAUAGGAGUA
58	CR003388	TTR (Exon 4)	Human	chr18:31598637-31598657	GACGACAGCCGUGGUGGAAU
59	CR003389	TTR (Exon 4)	Human	chr18:31598643-31598663	AUUGGUGACGACAGCCGUGG
60	CR003390	TTR (Exon 4)	Human	chr18:31598646-31598666	GGGAUUGGUGACGACAGCCG
61	CR003391	TTR (Exon 4)	Human	chr18:31598647-31598667	GGCUGUCGUCACCAAUCCCA
62	CR003392	TTR (Exon 4)	Human	chr18:31598661-31598681	AGUCCCUCAUUCCUUGGGAU
63	CR005298	TTR (Exon 1)	Human	chr18:31591883-31591903	UCCACUCAUUCUUGGCAGGA
64	CR005299	TTR (Exon 4)	Human	chr18:31598631-31598651	AGCCGUGGUGGAAUAGGAGU
65	CR005300	TTR (Exon 1)	Human	chr18:31591967-31591987	UCACAGAAACACUCACCGUA
66	CR005301	TTR (Exon 1)	Human	chr18:31591968-31591988	GUCACAGAAACACUCACCGU
67	CR005302	TTR (Exon 2)	Human	chr18:31592874-31592894	ACGUGUCUUCUCUACACCCA
68	CR005303	TTR (Exon 2)	Human	chr18:31592903-31592923	UGAAUCCAAGUGUCCUCUGA
69	CR005304	TTR (Exon 2)	Human	chr18:31592969-31592989	GGCCGUGCAUGUGUUCAGAA
70	CR005305	TTR (Exon 3)	Human	chr18:31595114-31595134	UAUAGGAAAACCAGUGAGUC
71	CR005306	TTR (Exon 3)	Human	chr18:31595204-31595224	AAAUCUUACUGGAAGGCACU
72	CR005307	TTR (Exon 4)	Human	chr18:31598548-31598568	UGUCUGUCUUCUCUCAUAGG
73	CR000689	TTR	Cyno	chr18:50681533-50681553	ACACAAAUACCAGUCCAGCG
74	CR005364	TTR	Cyno	chr18:50680481-50680501	AAAGGCUGCUGAUGAGACCU
75	CR005365	TTR	Cyno	chr18:50680520-50680540	CAUUGACAGCAGGACUGCCU
76	CR005366	TTR	Cyno	chr18:50681539-50681559	AUACCAGUCCAGCGAGGCAG
77	CR005367	TTR	Cyno	chr18:50681542-50681562	CCAGUCCAGCGAGGCAGAGG
78	CR005368	TTR	Cyno	chr18:50681545-50681565	CCUCCUCUGCCUCGCUGGAC
79	CR005369	TTR	Cyno	chr18:50680540-50680560	AAAGUUCUAGAUGCCGUCCG
80	CR005370	TTR	Cyno	chr18:50680594-50680614	ACUUGUCUUCUCUAUACCCA

81	CR005371	TTR	Cyno	chr18:50678216-50678236	AAGUGACUUCCAGUAAGAUU
82	CR005372	TTR	Cyno	chr18:50680482-50680502	AAAAGGCUGCUGAUGAGACC

[00320] Each of the Guide Sequences above may further comprise additional nucleotides to form a crRNA, e.g., with the following exemplary nucleotide sequence following the Guide Sequence at its 3' end: GUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 126). In the case of a sgRNA, the above Guide Sequences may further comprise additional nucleotides to form a sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the Guide Sequence: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 125) in 5' to 3' orientation.

[00321] In some embodiments, the sgRNA is modified. In some embodiments, the sgRNA comprises the modification pattern shown below in SEQ ID NO: 3, where N is any natural or non-natural nucleotide, and where the totality of the N's comprise a guide sequence as described herein and the modified sgRNA comprises the following sequence: mN*mN*mN*NNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmU mAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAm AmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU (SEQ ID NO: 3), where "N" may be any natural or non-natural nucleotide. For example, encompassed herein is SEQ ID NO: 3, where the N's are replaced with any of the guide sequences disclosed herein. The modifications remain as shown in SEQ ID NO: 3 despite the substitution of N's for the nucleotides of a guide. That is, although the nucleotides of the guide replace the "N's", the first three nucleotides are 2'OMe modified and there are phosphorothioate linkages between the first and second nucleotides, the second and third nucleotides and the third and fourth nucleotides.

[00322] In some embodiments, any one of the sequences recited in Table 2 is encompassed.

Table 2: TTR targeted sgRNA sequences

SEQ ID No.	Guide ID	Target and Description	Species	Sequence
87	G000480	TTR sgRNA modified sequence	Human	mA*mA*mA*GGCUGCUGAUGACACCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm

				GmGmUmGmCmU*mU*mU*mU
88	G000481	TTR sgRNA modified sequence	Human	mU*mC*mU*AGAACUUUGACCAUCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
89	G000482	TTR sgRNA modified sequence	Human	mU*mG*mU*AGAAGGGAUAUACAAAGG UUUUAGAmGmCmUmAmGmAmAmAmUm AmGmCAAGUUAAAAUAAGGCUAGUCCG UUAUCAmAmCmUmUmGmAmAmAmAmA mGmUmGmGmCmAmCmCmGmAmGmUmC mGmGmUmGmCmU*mU*mU*mU
90	G000483	TTR sgRNA modified sequence	Human	mU*mC*mC*ACUCAUUCUUGGCAGGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
91	G000484	TTR sgRNA modified sequence	Human	mA*mG*mA*CACCAAAUCUUACUGGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
92	G000485	TTR sgRNA modified sequence	Human	mC*mC*mU*CCUCUGCCUUGCUGGACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
93	G000486	TTR sgRNA modified sequence	Human	mA*mC*mA*CAAAUACCAGUCCAGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
94	G000487	TTR sgRNA modified sequence	Human	mU*mU*mC*UUUGGCAACUUACCCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

95	G000488	TTR sgRNA modified sequence	Human	mA*mA*mA*GUUCUAGAUGCUGUCCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
96	G000489	TTR sgRNA modified sequence	Human	mU*mU*mU*GACCAUCAGAGGACACUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
97	G000490	TTR sgRNA modified sequence	Human	mA*mA*mA*UAGACACCAAAUCUUACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
98	G000491	TTR sgRNA modified sequence	Human	mA*mU*mA*CCAGUCCAGCAAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
99	G000492	TTR sgRNA modified sequence	Human	mC*mU*mU*CUCUACACCCAGGGCACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
100	G000493	TTR sgRNA modified sequence	Human	mA*mA*mG*UGCCUUCCAGUAAGAUUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
101	G000494	TTR sgRNA modified sequence	Human	mG*mU*mG*AGUCUGGAGAGCUGCAUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
102	G000495	TTR sgRNA modified sequence	Human	mC*mA*mG*AGGACACUUGGAUUCACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

103	G000496	TTR sgRNA modified sequence	Human	mG*mG*mC*CGUGCAUGUGUUCAGAAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
104	G000497	TTR sgRNA modified sequence	Human	mC*mU*mG*CUCCUCCUCUGCCUUGCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
105	G000498	TTR sgRNA modified sequence	Human	mA*mG*mU*GAGUCUGGAGAGCUGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
106	G000499	TTR sgRNA modified sequence	Human	mU*mG*mA*AUCCAAGUGUCCUCUGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
107	G000500	TTR sgRNA modified sequence	Human	mC*mC*mA*GUCCAGCAAGGCAGAGGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
108	G000501	TTR sgRNA modified sequence	Human	mU*mC*mA*CAGAAACACUCACCGUAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
109	G000567	TTR sgRNA modified sequence	Human	mG*mA*mA*AGGCUGCUGAUGACACCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
110	G000568	TTR sgRNA modified sequence	Human	mG*mG*mC*UGUCGUCACCAAUCCCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

111	G000570	TTR sgRNA modified sequence	Human	mC*mA*mU*UGAUGGCAGGACUGCCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
112	G000571	TTR sgRNA modified sequence	Human	mG*mU*mC*ACAGAAACACUCACCGUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
113	G000572	TTR sgRNA modified sequence	Human	mC*mC*mC*CUACUCCUAUUCCACCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
114	G000502	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mC*mA*CAAAUACCAGUCCAGCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
115	G000503	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*AGGCUGCUGAUGAGACCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
116	G000504	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*GGCUGCUGAUGAGACCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
117	G000505	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mA*mU*UGACAGCAGGACUGCCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
118	G000506	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mU*mA*CCAGUCCAGCGAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

119	G000507	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mC*mA*GUCCAGCGAGGCAGAGGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
120	G000508	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mC*mU*CCUCUGCCUCGCUGGACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
121	G000509	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*GUUCUAGAUGCCGUCCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
122	G000510	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mC*mU*UGUCUUCUCUAUACCCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
123	G000511	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mG*UGACUUCCAGUAAGAUUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
124	G000282	TTR	Mouse	mU*mU*mA*CAGCCACGUCUACAGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

* = PS linkage; 'm' = 2'-O-Me nucleotide

[00323] An alignment mapping of the Guide IDs with the corresponding sgRNA IDs as well as homology to the cyno genome and cyno matched guide IDs are provided in Table 3.

Table 3: TTR targeted guide sequence ID mapping and Cyno Homology

Description	Human Dual Guide ID	Human Single Guide ID	Number Mismatches to Cyno Genome	Cyno Matched dgRNA ID	Cyno Matched sgRNA ID
TTR	CR003335	G000497	1		
TTR	CR003336	G000485	1	CR005368	G000508
TTR	CR003337	G000500	1	CR005367	G000507

TTR	CR003338	G000491	1	CR005366	G000506
TTR	CR003339	G000486	1	CR000689	G000502
TTR	CR003340		0		
TTR	CR003341		0		
TTR	CR003342	G000492	no PAM in cyno		
TTR	CR003343	G000495	no PAM in cyno		
TTR	CR003344	G000489	0		
TTR	CR003345	G000481	0		
TTR	CR003346	G000488	1	CR005369	G000509
TTR	CR003347	G000570	2	CR005365	G000505
TTR	CR003348		2		
TTR	CR003349		>3		
TTR	CR003350		no PAM in cyno		
TTR	CR003351		no PAM in cyno		
TTR	CR003352	G000567	2	CR005372	G000503
TTR	CR003353	G000480	1	CR005364	G000504
TTR	CR003354		1		
TTR	CR003355		1		
TTR	CR003356		3		
TTR	CR003357	G000487	>3		
TTR	CR003358		0		
TTR	CR003359	G000498	0		
TTR	CR003360	G000494	0		
TTR	CR003361		0		
TTR	CR003362		0		
TTR	CR003363		0		
TTR	CR003364		0		
TTR	CR003365	G000482	0		
TTR	CR003366	G000490	0		
TTR	CR003367	G000484	no PAM in cyno		
TTR	CR003368	G000493	1	CR005371	G000511
TTR	CR003369		0		
TTR	CR003370		0		
TTR	CR003371		0		
TTR	CR003372		0		
TTR	CR003373		1		
TTR	CR003374		2		
TTR	CR003375		2		
TTR	CR003376		2		
TTR	CR003377		2		
TTR	CR003378		2		
TTR	CR003379		2		
TTR	CR003380		1		
TTR	CR003381		1		
TTR	CR003382		0		
TTR	CR003383		0		

TTR	CR003384		0		
TTR	CR003385	G000572	0		
TTR	CR003386		0		
TTR	CR003387		0		
TTR	CR003388		0		
TTR	CR003389	G000569	0		
TTR	CR003390		0		
TTR	CR003391	G000568	0		
TTR	CR003392		0		
TTR	CR005298	G000483	1		
TTR	CR005299		0		
TTR	CR005300	G000501	no PAM in cyno		
TTR	CR005301	G000571	0		
TTR	CR005302		2	CR005370	G000510
TTR	CR005303	G000499	0		
TTR	CR005304	G000496	>3		
TTR	CR005305		0		
TTR	CR005306		1		
TTR	CR005307		0		

[00324] In some embodiments, the gRNA comprises a guide sequence that direct an RNA-guided DNA binding agent, which can be a nuclease (e.g., a Cas nuclease such as Cas9), to a target DNA sequence in *TTR*. The gRNA may comprise a crRNA comprising a guide sequence shown in Table 1. The gRNA may comprise a crRNA comprising 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the gRNA comprises a crRNA comprising a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the gRNA comprises a crRNA comprising a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a guide sequence shown in Table 1. The gRNA may further comprise a trRNA. In each composition and method embodiment described herein, the crRNA and trRNA may be associated as a single RNA (sgRNA), or may be on separate RNAs (dgRNA). In the context of sgRNAs, the crRNA and trRNA components may be covalently linked, e.g., via a phosphodiester bond or other covalent bond.

[00325] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise two RNA molecules as a "dual guide RNA" or "dgRNA". The dgRNA comprises a first RNA molecule comprising a crRNA comprising, e.g., a guide sequence shown in Table 1, and a second RNA molecule comprising a trRNA. The first and

second RNA molecules may not be covalently linked, but may form a RNA duplex via the base pairing between portions of the crRNA and the trRNA.

[00326] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise a single RNA molecule as a "single guide RNA" or "sgRNA". The sgRNA may comprise a crRNA (or a portion thereof) comprising a guide sequence shown in Table 1 covalently linked to a trRNA. The sgRNA may comprise 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the crRNA and the trRNA are covalently linked via a linker. In some embodiments, the sgRNA forms a stem-loop structure via the base pairing between portions of the crRNA and the trRNA. In some embodiments, the crRNA and the trRNA are covalently linked via one or more bonds that are not a phosphodiester bond.

[00327] In some embodiments, the trRNA may comprise all or a portion of a trRNA sequence derived from a naturally-occurring CRISPR/Cas system. In some embodiments, the trRNA comprises a truncated or modified wild type trRNA. The length of the trRNA depends on the CRISPR/Cas system used. In some embodiments, the trRNA comprises or consists of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more than 100 nucleotides. In some embodiments, the trRNA may comprise certain secondary structures, such as, for example, one or more hairpin or stem-loop structures, or one or more bulge structures.

[00328] In some embodiments, the composition comprises one or more guide RNAs comprising a guide sequence selected from SEQ ID NOs: 5-82.

[00329] In some embodiments, the composition comprises a gRNA that comprises a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[00330] In some embodiments, the composition comprises one or more guide RNAs comprising a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82. In some embodiments, the composition comprises a gRNA that comprises a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82. In some embodiments, the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NOs: 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 22, 23, 27, 29, 30, 35, 36, 37, 38, 55, 61, 63, 65, 66, 68, or 69. In some embodiments, the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 5, 6, 9, 13, 14, 15, 16, 17, 22, 23, 27, 30, 35, 36, 37, 38, 55, 63, 65, 66, 68, or 69. In particular, the guide RNA comprising a guide sequence selected from SEQ ID NOs: 5-72, 74-

78, and 80-82 may be an sgRNA. The guide RNA comprising a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 may be a chemically modified sgRNA, such as an end modified RNA. The guide RNA comprising a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 may be dgRNA, such as a chemically modified dgRNA.

[00331] In other embodiments, the composition comprises at least one, e.g., at least two gRNAs comprising guide sequences selected from any two or more of the guide sequences of SEQ ID NOs: 5-82. In some embodiments, the composition comprises at least two gRNAs that each comprise a guide sequence at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 5-82.

[00332] In other embodiments, the composition comprises at least one, e.g., at least two gRNAs comprising guide sequences selected from any two or more of the guide sequences selected from SEQ ID NOs: 5-72, 74-78, and 80-82. In some embodiments, the composition comprises at least two gRNAs that each comprise a guide sequence at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the sequences selected from SEQ ID NOs: 5-72, 74-78, and 80-82. In some embodiments, the sequences selected from SEQ ID NOs: 5-72, 74-78, and 80-82 comprise a sequence, or two sequences, selected from SEQ ID NOs: 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 22, 23, 27, 29, 30, 35, 36, 37, 38, 55, 61, 63, 65, 66, 68, or 69. In some embodiments, the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 comprise a sequence, or two sequences, selected from SEQ ID NO: 5, 6, 9, 13, 14, 15, 16, 17, 22, 23, 27, 30, 35, 36, 37, 38, 55, 63, 65, 66, 68, or 69.

[00333] In some embodiments, the gRNA is a sgRNA comprising any one of the sequences shown in Table 2 (SEQ ID Nos. 87-124). In some embodiments, the gRNA is a sgRNA comprising any one of the sequences shown in Table 2 (SEQ ID Nos. 87-124, but without the modifications as shown (i.e., unmodified SEQ ID Nos. 87-124). In some embodiments, the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID Nos. 87-124. In some embodiments, the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID Nos. 87-124, but without the modifications as shown (i.e., unmodified SEQ ID Nos. 87-124). In some embodiments, the sgRNA comprises any one of the guide sequences shown in Table 1 in place of the guide sequences shown in the sgRNA sequences of Table 2 at SEQ ID Nos: 87-124, with or without the modifications.

[00334] In some embodiments, the gRNA is a sgRNA comprising any one of SEQ ID Nos. 87-113, 115-120, or 122-124. In some embodiments, the gRNA is a sgRNA comprising any

one of SEQ ID Nos. 87-113, 115-120, or 122-124, but without the modifications as shown in Table 2 (i.e., unmodified SEQ ID Nos. 87-113, 115-120, or 122-124). In some embodiments, the gRNA is a sgRNA comprising any one of SEQ ID Nos. 87-113, 115-120, or 122-124, but with at least one chemical modification and without the modification pattern as shown in Table 2 (i.e., chemically modified SEQ ID Nos. 87-113, 115-120, or 122-124). The chemically modified guide RNAs may comprise one or more of the modifications as shown in Table 2. In some embodiments, the chemically modified SEQ ID Nos. 87-113, 115-120, or 122-124 without the modification pattern as shown in Table 2 comprise 5' and/or 3' end modifications. In some embodiments, the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID Nos. 87-113, 115-120, or 122-124. In some embodiments, the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID Nos. 87-113, 115-120, or 122-124, but without the modifications as shown (i.e., unmodified SEQ ID Nos. 87-113, 115-120, or 122-124). In some embodiments, the sgRNA comprises any one of the guide sequences shown in Table 1 in place of the guide sequences shown in the sgRNA sequences of Table 2 at SEQ ID Nos: 87-113, 115-120, or 122-124, with or without the modifications.

[00335] The guide RNAs provided herein can be useful for recognizing (e.g., hybridizing to) a target sequence in the *TTR* gene. For example, the *TTR* target sequence may be recognized and cleaved by a provided Cas cleavase comprising a guide RNA. Thus, an RNA-guided DNA binding agent, such as a Cas cleavase, may be directed by a guide RNA to a target sequence of the *TTR* gene, where the guide sequence of the guide RNA hybridizes with the target sequence and the RNA-guided DNA binding agent, such as a Cas cleavase, cleaves the target sequence.

[00336] In some embodiments, the selection of the one or more guide RNAs is determined based on target sequences within the *TTR* gene.

[00337] Without being bound by any particular theory, mutations (e.g., frameshift mutations resulting from indels occurring as a result of a nuclease-mediated DSB) in certain regions of the gene may be less tolerable than mutations in other regions of the gene, thus the location of a DSB is an important factor in the amount or type of protein knockdown that may result. In some embodiments, a gRNA complementary or having complementarity to a target sequence within *TTR* is used to direct the RNA-guided DNA binding agent to a particular location in the *TTR* gene. In some embodiments, gRNAs are designed to have

guide sequences that are complementary or have complementarity to target sequences in exon 1, exon 2, exon 3, or exon 4 of *TTR*.

B. Modifications of gRNAs

[00338] In some embodiments, the gRNA is chemically modified. A gRNA comprising one or more modified nucleosides or nucleotides is called a "modified" gRNA or "chemically modified" gRNA, to describe the presence of one or more non-naturally and/or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. In some embodiments, a modified gRNA is synthesized with a non-canonical nucleoside or nucleotide, is here called "modified." Modified nucleosides and nucleotides can include one or more of: (i) alteration, *e.g.*, replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (ii) alteration, *e.g.*, replacement, of a constituent of the ribose sugar, *e.g.*, of the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (iii) wholesale replacement of the phosphate moiety with "dephospho" linkers (an exemplary backbone modification); (iv) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (v) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (vi) modification of the 3' end or 5' end of the oligonucleotide, *e.g.*, removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, cap or linker (such 3' or 5' cap modifications may comprise a sugar and/or backbone modification); and (vii) modification or replacement of the sugar (an exemplary sugar modification). Chemical modifications such as those listed above can be combined to provide modified gRNAs comprising nucleosides and nucleotides (collectively "residues") that can have two, three, four, or more modifications. For example, a modified residue can have a modified sugar and a modified nucleobase. In some embodiments, every base of a gRNA is modified, *e.g.*, all bases have a modified phosphate group, such as a phosphorothioate group. In certain embodiments, all, or substantially all, of the phosphate groups of an gRNA molecule are replaced with phosphorothioate groups. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 5' end of the RNA. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 3' end of the RNA.

[00339] In some embodiments, the gRNA comprises one, two, three or more modified residues. In some embodiments, at least 5% (*e.g.*, at least 5%, at least 10%, at least 15%, at

least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the positions in a modified gRNA are modified nucleosides or nucleotides.

[00340] Unmodified nucleic acids can be prone to degradation by, *e.g.*, intracellular nucleases or those found in serum. For example, nucleases can hydrolyze nucleic acid phosphodiester bonds. Accordingly, in one aspect the gRNAs described herein can contain one or more modified nucleosides or nucleotides, *e.g.*, to introduce stability toward intracellular or serum-based nucleases. In some embodiments, the modified gRNA molecules described herein can exhibit a reduced innate immune response when introduced into a population of cells, both *in vivo* and *ex vivo*. The term "innate immune response" includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, which involves the induction of cytokine expression and release, particularly the interferons, and cell death.

[00341] In some embodiments of a backbone modification, the phosphate group of a modified residue can be modified by replacing one or more of the oxygens with a different substituent. Further, the modified residue, *e.g.*, modified residue present in a modified nucleic acid, can include the wholesale replacement of an unmodified phosphate moiety with a modified phosphate group as described herein. In some embodiments, the backbone modification of the phosphate backbone can include alterations that result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[00342] Examples of modified phosphate groups include, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. The phosphorous atom in an unmodified phosphate group is achiral. However, replacement of one of the non-bridging oxygens with one of the above atoms or groups of atoms can render the phosphorous atom chiral. The stereogenic phosphorous atom can possess either the "R" configuration (herein Rp) or the "S" configuration (herein Sp). The backbone can also be modified by replacement of a bridging oxygen, (*i.e.*, the oxygen that links the phosphate to the nucleoside), with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylenephosphonates). The replacement can occur at either linking oxygen or at both of the linking oxygens.

[00343] The phosphate group can be replaced by non-phosphorus containing connectors in certain backbone modifications. In some embodiments, the charged phosphate group can be

replaced by a neutral moiety. Examples of moieties which can replace the phosphate group can include, without limitation, *e.g.*, methyl phosphonate, hydroxylamino, siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino, methylenehydrazo, methylenedimethylhydrazo and methyleneoxymethylimino.
Scaffolds that can mimic nucleic acids can also be constructed wherein the phosphate linker and ribose sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates. Such modifications may comprise backbone and sugar modifications. In some embodiments, the nucleobases can be tethered by a surrogate backbone. Examples can include, without limitation, the morpholino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates.

[00344] The modified nucleosides and modified nucleotides can include one or more modifications to the sugar group, *i.e.* at sugar modification. For example, the 2' hydroxyl group (OH) can be modified, *e.g.* replaced with a number of different "oxy" or "deoxy" substituents. In some embodiments, modifications to the 2' hydroxyl group can enhance the stability of the nucleic acid since the hydroxyl can no longer be deprotonated to form a 2'-alkoxide ion.

[00345] Examples of 2' hydroxyl group modifications can include alkoxy or aryloxy (OR, wherein "R" can be, *e.g.*, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or a sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$ wherein R can be, *e.g.*, H or optionally substituted alkyl, and n can be an integer from 0 to 20 (*e.g.*, from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20). In some embodiments, the 2' hydroxyl group modification can be 2'-O-Me. In some embodiments, the 2' hydroxyl group modification can be a 2'-fluoro modification, which replaces the 2' hydroxyl group with a fluoride. In some embodiments, the 2' hydroxyl group modification can include "locked" nucleic acids (LNA) in which the 2' hydroxyl can be connected, *e.g.*, by a C_{1-6} alkylene or C_{1-6} heteroalkylene bridge, to the 4' carbon of the same ribose sugar, where exemplary bridges can include methylene, propylene, ether, or amino bridges; O-amino (wherein amino can be, *e.g.*, NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino) and aminoalkoxy, $O(CH_2)_n$-amino, (wherein amino can be, *e.g.*, NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino). In

some embodiments, the 2' hydroxyl group modification can included "unlocked" nucleic acids (UNA) in which the ribose ring lacks the C2'-C3' bond. In some embodiments, the 2' hydroxyl group modification can include the methoxyethyl group (MOE), ($OCH_2CH_2OCH_3$, *e.g.*, a PEG derivative).

[00346] "Deoxy" 2' modifications can include hydrogen (*i.e.* deoxyribose sugars, *e.g.*, at the overhang portions of partially dsRNA); halo (*e.g.*, bromo, chloro, fluoro, or iodo); amino (wherein amino can be, *e.g.*, NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, diheteroarylamino, or amino acid); $NH(CH_2CH_2NH)_nCH2CH_2$- amino (wherein amino can be, *e.g.*, as described herein), -NHC(O)R (wherein R can be, *e.g.*, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with *e.g.*, an amino as described herein.

The sugar modification can comprise a sugar group which may also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified nucleic acid can include nucleotides containing *e.g.*, arabinose, as the sugar. The modified nucleic acids can also include abasic sugars. These abasic sugars can also be further modified at one or more of the constituent sugar atoms. The modified nucleic acids can also include one or more sugars that are in the L form, *e.g.* L-nucleosides.

[00347] The modified nucleosides and modified nucleotides described herein, which can be incorporated into a modified nucleic acid, can include a modified base, also called a nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C), and uracil (U). These nucleobases can be modified or wholly replaced to provide modified residues that can be incorporated into modified nucleic acids. The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine analog, or pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

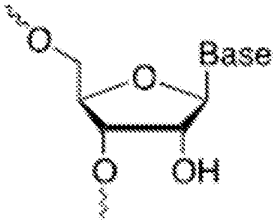
In embodiments employing a dual guide RNA, each of the crRNA and the tracr RNA can contain modifications. Such modifications may be at one or both ends of the crRNA and/or tracr RNA. In embodiments comprising an sgRNA, one or more residues at one or both ends of the sgRNA may be chemically modified, or the entire sgRNA may be chemically modified. Certain embodiments comprise a 5' end modification. Certain embodiments comprise a 3' end modification. In certain embodiments, one or more or all of the nucleotides in single stranded overhang of a guide RNA molecule are deoxynucleotides.

[00348] In some embodiments, the guide RNAs disclosed herein comprise one of the modification patterns disclosed in US 62/431,756, filed December 8, 2016, titled "Chemically Modified Guide RNAs," the contents of which are hereby incorporated by reference in their entirety.

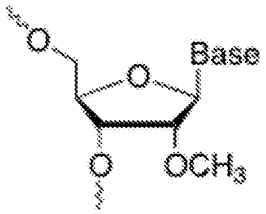
In some embodiments, the invention comprises a gRNA comprising one or more modifications. In some embodiments, the modification comprises a 2'-O-methyl (2'-O-Me) modified nucleotide. In some embodiments, the modification comprises a phosphorothioate (PS) bond between nucleotides.

[00349] The terms "mA," "mC," "mU," or "mG" may be used to denote a nucleotide that has been modified with 2'-O-Me.

[00350] Modification of 2'-*O*-methyl can be depicted as follows:



RNA

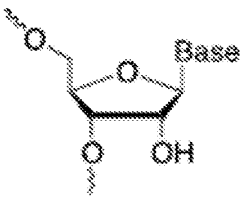


2'-O-Me

[00351] Another chemical modification that has been shown to influence nucleotide sugar rings is halogen substitution. For example, 2'-fluoro (2'-F) substitution on nucleotide sugar rings can increase oligonucleotide binding affinity and nuclease stability.

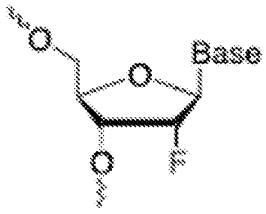
[00352] In this application, the terms "fA," "fC," "fU," or "fG" may be used to denote a nucleotide that has been substituted with 2'-F.

[00353] Substitution of 2'-F can be depicted as follows:



RNA

Natural composition of RNA



2'F-RNA

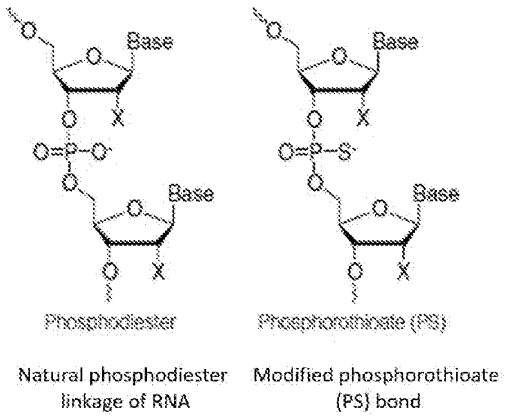
2'F substitution

[00354] Phosphorothioate (PS) linkage or bond refers to a bond where a sulfur is substituted for one nonbridging phosphate oxygen in a phosphodiester linkage, for example in the bonds between nucleotides bases. When phosphorothioates are used to generate oligonucleotides, the modified oligonucleotides may also be referred to as S-oligos.

[00355] A "*" may be used to depict a PS modification. In this application, the terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (e.g., 3') nucleotide with a PS bond.

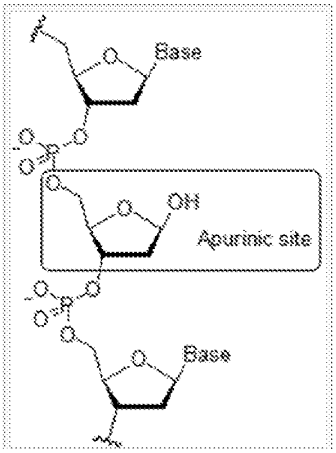
[00356] In this application, the terms "mA*," "mC*," "mU*," or "mG*" may be used to denote a nucleotide that has been substituted with 2'-O-Me and that is linked to the next (e.g., 3') nucleotide with a PS bond.

[00357] The diagram below shows the substitution of S- into a nonbridging phosphate oxygen, generating a PS bond in lieu of a phosphodiester

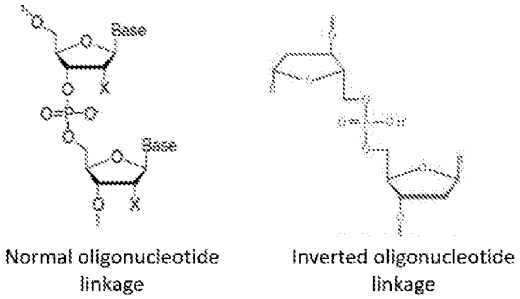


bond:

[00358] Abasic nucleotides refer to those which lack nitrogenous bases. The figure below depicts an oligonucleotide with an abasic (also known as apurinic) site that lacks a base:



[00359] Inverted bases refer to those with linkages that are inverted from the normal 5' to 3' linkage (i.e., either a 5' to 5' linkage or a 3' to 3' linkage). For example:



[00360] An abasic nucleotide can be attached with an inverted linkage. For example, an abasic nucleotide may be attached to the terminal 5' nucleotide via a 5' to 5' linkage, or an abasic nucleotide may be attached to the terminal 3' nucleotide via a 3' to 3' linkage. An inverted abasic nucleotide at either the terminal 5' or 3' nucleotide may also be called an inverted abasic end cap.

[00361] In some embodiments, one or more of the first three, four, or five nucleotides at the 5' terminus, and one or more of the last three, four, or five nucleotides at the 3' terminus are modified. In some embodiments, the modification is a 2'-O-Me, 2'-F, inverted abasic nucleotide, PS bond, or other nucleotide modification well known in the art to increase stability and/or performance.

[00362] In some embodiments, the first four nucleotides at the 5' terminus, and the last four nucleotides at the 3' terminus are linked with phosphorothioate (PS) bonds.

[00363] In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-O-methyl (2'-O-Me) modified nucleotide. In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-fluoro (2'-F) modified nucleotide. In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise an inverted abasic nucleotide.

[00364] In some embodiments, the guide RNA comprises a modified sgRNA. In some embodiments, the sgRNA comprises the modification pattern shown in SEQ ID No: 3, where N is any natural or non-natural nucleotide, and where the totality of the N's comprise a guide sequence that directs a nuclease to a target sequence.

[00365] In some embodiments, the guide RNA comprises a sgRNA shown in any one of SEQ ID No: 87-124. In some embodiments, the guide RNA comprises a sgRNA comprising any one of the guide sequences of SEQ ID No: 5-82 and the nucleotides of SEQ ID No: 125, wherein the nucleotides of SEQ ID No: 125 are on the 3' end of the guide sequence, and wherein the guide sequence may be modified as shown in SEQ ID No: 3.

[00366] In some embodiments, the guide RNA comprises a sgRNA comprising a guide sequence selected from SEQ ID Nos: 5-72, 74-78, and 80-82 and the nucleotides of SEQ ID No: 125, wherein the nucleotides of SEQ ID No: 125 are on the 3' end of the guide sequence, and wherein the guide sequence may be modified as shown in SEQ ID No: 3.

C. Nucleic Acid Comprising an Open Reading Frame Encoding an RNA-Guided DNA Binding Agent

[00367] Any nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, disclosed herein may be combined in a composition or method with any of the gRNAs disclosed herein. In any of the embodiments set forth herein, the nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent may be an mRNA.

1. ORFs with low adenine content

[00368] In some embodiments, the ORF encoding the RNA-guided DNA-binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, has an adenine content ranging from its minimum adenine content to about 150% of its minimum adenine content. In some embodiments, the adenine content of the ORF is less than or equal to about 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of its minimum adenine content. In some embodiments, the ORF has an adenine content equal to

its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 150% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 145% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 140% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 135% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 130% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 125% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 120% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 115% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 110% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 105% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 104% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 103% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 102% of its minimum adenine content. In some embodiments, the ORF has an adenine content less than or equal to about 101% of its minimum adenine content.

[00369] In some embodiments, the ORF has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 200% of its minimum adenine dinucleotide content. In some embodiments, the adenine dinucleotide content of the ORF is less than or equal to about 195%, 190%, 185%, 180%, 175%, 170%, 165%, 160%, 155%, 150%, 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content equal to its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 200% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 195% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 190% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 185% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 180% of its minimum adenine dinucleotide

content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 175% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 170% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 165% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 160% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 155% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content equal to its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 150% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 145% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 140% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 135% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 130% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 125% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 120% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 115% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 110% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 105% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 104% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 103% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 102% of its minimum adenine dinucleotide content. In some embodiments, the ORF has an adenine dinucleotide content less than or equal to about 101% of its minimum adenine dinucleotide content.

[00370] In some embodiments, the ORF has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to the adenine dinucleotide content that is 90% or lower of the maximum adenine dinucleotide content of a reference sequence that encodes the same protein as the mRNA in question. In some embodiments, the adenine dinucleotide content of the ORF is less than or equal to about 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% of the maximum adenine dinucleotide content of a reference sequence that encodes the same protein as the mRNA in question.

[00371] In some embodiments, the ORF has an adenine trinucleotide content ranging from 0 adenine trinucleotides to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, or 50 adenine trinucleotides (where a longer run of adenines counts as the number of unique three-adenine segments within it, e.g., an adenine tetranucleotide contains two adenine trinucleotides, an adenine pentanucleotide contains three adenine trinucleotides, etc.). In some embodiments, the ORF has an adenine trinucleotide content ranging from 0% adenine trinucleotides to 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, or 2% adenine trinucleotides, where the percentage content of adenine trinucleotides is calculated as the percentage of positions in a sequence that are occupied by adenines that form part of an adenine trinucleotide (or longer run of adenines), such that the sequences UUUAAA and UUUUAAAA would each have an adenine trinucleotide content of 50%. For example, in some embodiments, the ORF has an adenine trinucleotide content less than or equal to 2%. For example, in some embodiments, the ORF has an adenine trinucleotide content less than or equal to 1.5%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 1%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.9%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.8%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.7%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.6%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.5%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.4%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.3%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.2%. In some embodiments, the ORF has an adenine trinucleotide content less than or equal to 0.1%. In some embodiments, a nucleic acid is provided that encodes an RNA-guided DNA-binding agent comprising an ORF containing no adenine trinucleotides.

[00372] In some embodiments, the ORF has an adenine trinucleotide content ranging from its minimum adenine trinucleotide content to the adenine trinucleotide content that is 90% or lower of the maximum adenine trinucleotide content of a reference sequence that encodes the same protein as the mRNA in question. In some embodiments, the adenine trinucleotide content of the ORF is less than or equal to about 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% of the maximum adenine trinucleotide content of a reference sequence that encodes the same protein as the mRNA in question.

[00373] A given ORF can be reduced in adenine content or adenine dinucleotide content or adenine trinucleotide content, for example, by using minimal adenine codons in a sufficient fraction of the ORF. For example, an amino acid sequence for an RNA-guided DNA-binding agent can be back-translated into an ORF sequence by converting amino acids to codons, wherein some or all of the ORF uses the exemplary minimal adenine codons shown below. In some embodiments, at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons in the ORF are codons listed in Table 4.

Table 4. Exemplary minimal adenine codons

	Amino Acid	**Minimal adenine codon**
A	Alanine	GCU or GCC or GCG
G	Glycine	GGU or GGC or GGG
V	Valine	GUC or GUU or GUG
D	Aspartic acid	GAC or GAU
E	Glutamic acid	GAG
I	Isoleucine	AUC or AUU
T	Threonine	ACU or ACC or ACG
N	Asparagine	AAC or AAU
K	Lysine	AAG
S	Serine	UCU or UCC or UCG
R	Arginine	CGU or CGC or CGG
L	Leucine	CUG or CUC or CUU
P	Proline	CCG or CCU or CCC
H	Histidine	CAC or CAU
Q	Glutamine	CAG
F	Phenylalanine	UUC or UUU
Y	Tyrosine	UAC or UAU
C	Cysteine	UGC or UGU
W	Tryptophan	UGG
M	Methionine	AUG

[00374] In some embodiments, a nucleic acid is provided that encodes an RNA-guided DNA-binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, comprising an ORF consisting of a set of codons of which at least about 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons are codons listed in Table 4. In some embodiments, the ORF has minimal nucleotide homopolymers, e.g., repetitive strings of the same nucleotides. For example, in some embodiments, when selecting a minimal uridine codon from the codons listed in Table 4, a nucleic acid is constructed by selecting the minimal adenine codons that reduce the number and length of nucleotide homopolymers, e.g., selecting GCG instead of GCC for alanine or selecting GGC instead of GGG for glycine.

[00375] In any of the foregoing embodiments, the nucleic acid may be an mRNA.

2. Codons that increase translation and/or that correspond to highly expressed tRNAs; exemplary codon sets

[00376] In some embodiments, the nucleic acid comprises an ORF having codons that increase translation in a mammal, such as a human. In further embodiments, the nucleic acid comprises an ORF having codons that increase translation in an organ, such as the liver, of the mammal, e.g., a human. In further embodiments, the nucleic acid comprises an ORF having codons that increase translation in a cell type, such as a hepatocyte, of the mammal, e.g., a human. An increase in translation in a mammal, cell type, organ of a mammal, human, organ of a human, etc., can be determined relative to the extent of translation wild-type sequence of the ORF, or relative to an ORF having a codon distribution matching the codon distribution of the organism from which the ORF was derived or the organism that contains the most similar ORF at the amino acid level, such as *S. pyogenes*, *S. aureus*, or another prokaryote as the case may be for prokaryotically-derived Cas nucleases, such as the Cas nucleases from other prokaryotes described below. Alternatively, in some embodiments, an increase in translation for a Cas9 sequence in a mammal, cell type, organ of a mammal, human, organ of a human, etc., is determined relative to translation of an ORF with the sequence of SEQ ID NO: 205 with all else equal, including any applicable point mutations, heterologous domains, and the like. Codons useful for increasing expression in a human, including the human liver and human hepatocytes, can be codons corresponding to highly expressed tRNAs in the human liver/hepatocytes, which are discussed in Dittmar KA, *PLos Genetics* 2(12): e221 (2006). In some embodiments, at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in an ORF are codons corresponding to highly expressed tRNAs (e.g., the highest-expressed tRNA for each amino acid) in a

mammal, such as a human. In some embodiments, at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in an ORF are codons corresponding to highly expressed tRNAs (e.g., the highest-expressed tRNA for each amino acid) in a mammalian organ, such as a human organ. In some embodiments, at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in an ORF are codons corresponding to highly expressed tRNAs (e.g., the highest-expressed tRNA for each amino acid) in a mammalian liver, such as a human liver. In some embodiments, at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in an ORF are codons corresponding to highly expressed tRNAs (e.g., the highest-expressed tRNA for each amino acid) in a mammalian hepatocyte, such as a human hepatocyte.

[00377] Alternatively, codons corresponding to highly expressed tRNAs in an organism (e.g., human) in general may be used.

[00378] Any of the foregoing approaches to codon selection can be combined with the minimal adenine codons shown above, e.g., by starting with the codons of Table 4, and then where more than one option is available, using the codon that corresponds to a more highly-expressed tRNA, either in the organism (e.g., human) in general, or in an organ or cell type of interest, such as the liver or hepatocytes (e.g., human liver or human hepatocytes).

[00379] In some embodiments, at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in an ORF are codons from a codon set shown in Table 5 (e.g., the low U, low A, or low A/U codon set). The codons in the low A and low A/U sets use codons that minimize the indicated nucleotides while also using codons corresponding to highly expressed tRNAs where more than one option is available. In some embodiments, at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in an ORF are codons from the low U codon set shown in Table 5. In some embodiments, at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in an ORF are codons from the low A codon set shown in Table 5. In some embodiments, at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in an ORF are codons from the low A/U codon set shown in Table 5.

Table 5. Exemplary Codon Sets.

Amino Acid	Low U	Low A	Low A/U
Gly	GGC	GGC	GGC
Glu	GAG	GAG	GAG

Asp	GAC	GAC	GAC
Val	GTG	GTG	GTG
Ala	GCC	GCC	GCC
Arg	AGA	CGG	CGG
Ser	AGC	TCC	AGC
Lys	AAG	AAG	AAG
Asn	AAC	AAC	AAC
Met	ATG	ATG	ATG
Ile	ATC	ATC	ATC
Thr	ACC	ACC	ACC
Trp	TGG	TGG	TGG
Cys	TGC	TGC	TGC
Tyr	TAC	TAC	TAC
Leu	CTG	CTG	CTG
Phe	TTC	TTC	TTC
Gln	CAG	CAG	CAG
His	CAC	CAC	CAC

3. Exemplary sequences

[00380] In some embodiments, the ORF encoding the RNA-guided DNA binding agent comprises a sequence with at least 93% identity to SEQ ID NO: 311; and/or the ORF has at least 93% identity to SEQ ID NO: 311 over at least its first 50, 200, 250, or 300 nucleotides, or at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides; and/or the ORF consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4 or 5; and/or the ORF has an adenine content ranging from its minimum adenine content to 123% of the minimum adenine content; and/or the ORF has an adenine dinucleotide content ranging

from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content.

[00381] In some embodiments, the ORF encoding the RNA-guided DNA binding agent comprises a sequence with at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 377.

[00382] In some embodiments, the ORF encoding the RNA-guided DNA binding agent comprises a sequence with at least 90% identity to any one of SEQ ID NOs: 311-313, 328, 329, 346-348, 355, 356, 363, or 364. In some embodiments, the mRNA comprises an ORF encoding an RNA-guided DNA binding agent, wherein the RNA-guided DNA binding agent comprises an amino acid sequence with at least 90% identity to any one of SEQ ID NOs: 203, 213, 268, or 386-396, wherein the ORF has an adenine content ranging from its minimum adenine content to 150% of the minimum adenine content, and/or has a adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content. In some embodiments, the encoded RNA-guided DNA binding agent comprises an amino acid sequence with at least 90% identity to any one of SEQ ID NOs: 203, 213, 268, or 386-396, wherein the ORF has a uridine content ranging from its minimum uridine content to 150% of the minimum uridine content, and/or has a uridine dinucleotide content ranging from its minimum uridine dinucleotide content to 150% of the minimum uridine dinucleotide content. In some such embodiments, both the adenine and uridine nucleotide contents are less than or equal to 150% of their respective minima. In some embodiments, both the adenine and uridine dinucleotide contents are less than or equal to 150% of their respective minima. In some embodiments, any of the foregoing levels of identity is at least 95%, at least 98%, at least 99%, or 100%.

[00383] In some embodiments, the ORF encoding an RNA-guided DNA binding agent has at least 90% identity to any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides. The first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides are measured from the first nucleotide of the start codon (typically ATG), such that the A is nucleotide 1, the T is nucleotide 2, etc. In some embodiments, the open reading frame has at least 90% identity to any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364 over at least its first 10%, 12%, 15%, 20%, 25%, 30%, or 35% of its sequence. The length of the sequence of the ORF is the number of nucleotides from the beginning of the start codon to the end of the stop codon, and the first 10%, 12%, 15%, 20%, 25%, 30%, or 35% of its sequence corresponds to the number

of nucleotides starting from the first nucleotide of the start codon that make up the indicated percentage of the length of the total sequence.

[00384] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 243 in which the ORF of SEQ ID NO: 243 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00385] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 244 in which the ORF of SEQ ID NO: 244 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00386] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 256 in which the ORF of SEQ ID NO: 256 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00387] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 257 in which the ORF of SEQ ID NO: 257 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00388] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 258 in which the ORF of SEQ ID NO: 258 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00389] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 259 in which the ORF of SEQ ID NO: 259 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00390] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 260 in which the ORF of SEQ ID NO: 260 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00391] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 261 in which the ORF of SEQ ID NO: 261 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00392] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 376, optionally wherein the ORF of SEQ ID NO: 376 is substituted with an alternative ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00393] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 377, optionally wherein the ORF of SEQ ID NO: 377 is substituted with an alternative ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00394] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 378, optionally wherein the ORF of SEQ ID NO: 378 is substituted with an alternative ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00395] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 379 in which the ORF of SEQ ID NO: 379 is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00396] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 380 in which the ORF of SEQ ID NO: 380 is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00397] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 381 in which the ORF of SEQ ID NO: 381 is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00398] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 382 in which the ORF of SEQ ID NO: 382 is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00399] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 383 in which the ORF of SEQ ID NO: 383 is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00400] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID

NO: 384 in which the ORF of SEQ ID NO: 384 is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00401] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 385 in which the ORF of SEQ ID NO: 385 is substituted with the ORF of any one of SEQ ID NO: 311-313, 328, 329, 346-348, 355, 356, 363, or 364.

[00402] In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, 256-261, or 376-385 is at least 95%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, 256-261, or 376-385 is at least 98%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, 256-261, or 176-385 is at least 99%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, 256-261, or 376-385 is 100%.

4. Additional Features of nucleic acids, mRNAs, and ORFs

[00403] Any of the additional features described herein may be combined to the extent feasible with any of the embodiments described above.

a) Low uridine content

[00404] In some embodiments, the ORF encoding the RNA-guided DNA-binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, has a uridine content ranging from its minimum uridine content to about 150% of its minimum uridine content. In some embodiments, the uridine content of the ORF is less than or equal to about 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of its minimum uridine content. In some embodiments, the ORF has a uridine content equal to its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 150% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 145% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 140% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 135% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 130% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 125% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to

about 120% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 115% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 110% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 105% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 104% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 103% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 102% of its minimum uridine content. In some embodiments, the ORF has a uridine content less than or equal to about 101% of its minimum uridine content.

[00405] In some embodiments, the ORF has a uridine dinucleotide content ranging from its minimum uridine dinucleotide content to 200% of its minimum uridine dinucleotide content. In some embodiments, the uridine dinucleotide content of the ORF is less than or equal to about 195%, 190%, 185%, 180%, 175%, 170%, 165%, 160%, 155%, 150%, 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content equal to its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 200% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 195% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 190% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 185% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 180% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 175% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 170% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 165% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 160% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 155% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content equal to its minimum uridine

dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 150% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 145% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 140% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 135% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 130% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 125% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 120% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 115% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 110% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 105% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 104% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 103% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 102% of its minimum uridine dinucleotide content. In some embodiments, the ORF has a uridine dinucleotide content less than or equal to about 101% of its minimum uridine dinucleotide content.

[00406] In some embodiments, the ORF has a uridine dinucleotide content ranging from its minimum uridine dinucleotide content to the uridine dinucleotide content that is 90% or lower of the maximum uridine dinucleotide content of a reference sequence that encodes the same protein as the mRNA in question. In some embodiments, the uridine dinucleotide content of the ORF is less than or equal to about 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% of the maximum uridine dinucleotide content of a reference sequence that encodes the same protein as the mRNA in question.

[00407] In some embodiments, the ORF has a uridine trinucleotide content ranging from 0 uridine trinucleotides to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, or 50 uridine trinucleotides (where a longer run of uridines counts as the number of unique three-uridine segments within

it, e.g., a uridine tetranucleotide contains two uridine trinucleotides, a uridine pentanucleotide contains three uridine trinucleotides, etc.). In some embodiments, the ORF has a uridine trinucleotide content ranging from 0% uridine trinucleotides to 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, or 2% uridine trinucleotides, where the percentage content of uridine trinucleotides is calculated as the percentage of positions in a sequence that are occupied by uridines that form part of a uridine trinucleotide (or longer run of uridines), such that the sequences UUUAAA and UUUUAAAA would each have a uridine trinucleotide content of 50%. For example, in some embodiments, the ORF has a uridine trinucleotide content less than or equal to 2%. For example, in some embodiments, the ORF has a uridine trinucleotide content less than or equal to 1.5%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 1%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.9%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.8%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.7%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.6%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.5%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.4%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.3%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.2%. In some embodiments, the ORF has a uridine trinucleotide content less than or equal to 0.1%. In some embodiments, the ORF has no uridine trinucleotides.

[00408] In some embodiments, the ORF has a uridine trinucleotide content ranging from its minimum uridine trinucleotide content to the uridine trinucleotide content that is 90% or lower of the maximum uridine trinucleotide content of a reference sequence that encodes the same protein as the mRNA in question. In some embodiments, the uridine trinucleotide content of the ORF is less than or equal to about 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% of the maximum uridine trinucleotide content of a reference sequence that encodes the same protein as the mRNA in question.

[00409] A given ORF can be reduced in uridine content or uridine dinucleotide content or uridine trinucleotide content, for example, by using minimal uridine codons in a sufficient fraction of the ORF. For example, an amino acid sequence for an RNA-guided DNA-binding agent can be back-translated into an ORF sequence by converting amino acids to codons, wherein some or all of the ORF uses the exemplary minimal uridine codons shown below. In

some embodiments, at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons in the ORF are codons listed in Table 6.

Table 6. Exemplary minimal uridine codons

	Amino Acid	Minimal uridine codon
A	Alanine	GCA or GCC or GCG
G	Glycine	GGA or GGC or GGG
V	Valine	GUC or GUA or GUG
D	Aspartic acid	GAC
E	Glutamic acid	GAA or GAG
I	Isoleucine	AUC or AUA
T	Threonine	ACA or ACC or ACG
N	Asparagine	AAC
K	Lysine	AAG or AAA
S	Serine	AGC
R	Arginine	AGA or AGG
L	Leucine	CUG or CUA or CUC
P	Proline	CCG or CCA or CCC
H	Histidine	CAC
Q	Glutamine	CAG or CAA
F	Phenylalanine	UUC
Y	Tyrosine	UAC
C	Cysteine	UGC
W	Tryptophan	UGG
M	Methionine	AUG

[00410] In some embodiments, the ORF consists of a set of codons of which at least about 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons are codons listed in Table 6.

b) Low adenine and uridine content

[00411] To the extent feasible, any of the features described herein with respect to low adenine content can be combined with any of the features described herein with respect to low uridine content. For example, a nucleic acid (e.g., mRNA) may be provided that encodes an RNA-guided DNA-binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, comprising an ORF having a uridine content ranging from its minimum uridine content to about 150% of its minimum uridine content (e.g., a uridine content of the ORF is less than or equal to about 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of its minimum uridine content) and an adenine content ranging from its minimum adenine content to about 150% of its minimum adenine content (e.g., less than or equal to about 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%,

102%, or 101% of its minimum adenine content). So too for uridine and adenine dinucleotides. Similarly, the content of uridine nucleotides and adenine dinucleotides in the ORF may be as set forth above. Similarly, the content of uridine dinucleotides and adenine nucleotides in the ORF may be as set forth above.

[00412] A given ORF can be reduced in uridine and adenine nucleotide and/or dinucleotide content, for example, by using minimal uridine and adenine codons in a sufficient fraction of the ORF. For example, an amino acid sequence for an RNA-guided DNA-binding agent can be back-translated into an ORF sequence by converting amino acids to codons, wherein some or all of the ORF uses the exemplary minimal uridine and adenine codons shown below. In some embodiments, at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons in the ORF are codons listed in Table 7.

Table 7. Exemplary minimal uridine and adenine codons

	Amino Acid	**Minimal uridine codon**
A	Alanine	GCC or GCG
G	Glycine	GGC or GGG
V	Valine	GUC or GUG
D	Aspartic acid	GAC
E	Glutamic acid	GAG
I	Isoleucine	AUC
T	Threonine	ACC or ACG
N	Asparagine	AAC
K	Lysine	AAG
S	Serine	AGC or UCC or UCG
R	Arginine	CGC or CGG
L	Leucine	CUG or CUC
P	Proline	CCG or CCC
H	Histidine	CAC
Q	Glutamine	CAG
F	Phenylalanine	UUC
Y	Tyrosine	UAC
C	Cysteine	UGC
W	Tryptophan	UGG
M	Methionine	AUG

[00413] In some embodiments, the ORF consists of a set of codons of which at least about 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons are codons listed in Table 7. As can be seen in Table 7, each of the three listed serine codons contains either one A or one

U. In some embodiments, uridine minimization is prioritized by using AGC codons for serine. In some embodiments, adenine minimization is prioritized by using UCC and/or UCG codons for serine.

c) Encoded RNA-guided DNA binding agent

[00414] In some embodiments, the RNA-guided DNA-binding agent is a Class 2 Cas nuclease. In some embodiments, the RNA-guided DNA-binding agent has cleavase activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease, such as a Class 2 Cas nuclease (which may be, e.g., a Cas nuclease of Type II, V, or VI). Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, and C2c3 proteins and modifications thereof. Examples of Cas9 nucleases include those of the type II CRISPR systems of *S. pyogenes*, *S. aureus*, and other prokaryotes (see, e.g., the list in the next paragraph), and modified (e.g., engineered or mutant) versions thereof. See, e.g., US2016/0312198 A1; US 2016/0312199 A1. Other examples of Cas nucleases include a Csm or Cmr complex of a type III CRISPR system or the Cas10, Csm1, or Cmr2 subunit thereof; and a Cascade complex of a type I CRISPR system, or the Cas3 subunit thereof. In some embodiments, the Cas nuclease may be from a Type-IIA, Type-IIB, or Type-IIC system. For discussion of various CRISPR systems and Cas nucleases see, e.g., Makarova et al., Nat. Rev. Microbiol. 9:467-477 (2011); Makarova et al., Nat. Rev. Microbiol, 13: 722-36 (2015); Shmakov et al., Molecular Cell, 60:385-397 (2015). In some embodiments, the RNA-guided DNA buiding agent is a Cas cleavase, e.g. a Cas9 cleavagse. In some embodiments, the RNA-guided DNA binding agent is a Cas nickase, e.g. a Cas9 nickase. In some embodiments, the RNA-guided DNA binding agent is an *S. pyogenes* Cas9 nuclease, e.g. a cleavase.

[00415] Non-limiting exemplary species that the Cas nuclease can be derived from include *Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Staphylococcus aureus, Listeria innocua, Lactobacillus gasseri, Francisella novicida, Wolinella succinogenes, Sutterella wadsworthensis, Gammaproteobacterium, Neisseria meningitidis, Campylobacter jejuni, Pasteurella multocida, Fibrobacter succinogene, Rhodospirillum rubrum, Nocardiopsis dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii,*

Lactobacillus salivarius, Lactobacillus buchneri, Treponema denticola, Microscilla marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas sp., Crocosphaera watsonii, Cyanothece sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, Ammonifex degensii, Caldicelulosiruptor becscii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthrospira maxima, Arthrospira platensis, Arthrospira sp., Lyngbya sp., Microcoleus chthonoplastes, Oscillatoria sp., Petrotoga mobilis, Thermosipho africanus, Streptococcus pasteurianus, Neisseria cinerea, Campylobacter lari, Parvibaculum lavamentivorans, Corynebacterium diphtheria, Acidaminococcus sp., Lachnospiraceae bacterium ND2006, and *Acaryochloris marina*.

[00416] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Neisseria meningitidis*. In some embodiments, the Cas nuclease is the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Acidaminococcus sp.* In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Lachnospiraceae* bacterium ND2006. In further embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella tularensis, Lachnospiraceae bacterium, Butyrivibrio proteoclasticus, Peregrinibacteria bacterium, Parcubacteria bacterium, Smithella, Acidaminococcus, Candidatus Methanoplasma termitum, Eubacterium eligens, Moraxella bovoculi, Leptospira inadai, Porphyromonas crevioricanis, Prevotella disiens,* or *Porphyromonas macacae*. In certain embodiments, the Cas nuclease is a Cpf1 nuclease from an *Acidaminococcus* or *Lachnospiraceae*.

[00417] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 nuclease comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 nuclease is a wild type Cas9. In some embodiments, the Cas9 is capable of inducing a double strand break in target DNA. In certain embodiments, the Cas nuclease may cleave dsDNA, it may cleave one strand of

dsDNA, or it may not have DNA cleavase or nickase activity. An exemplary Cas9 amino acid sequence is provided as SEQ ID NO: 203. An exemplary Cas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 311. An exemplary Cas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 346.

[00418] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as Fok1. In some embodiments, a Cas nuclease may be a modified nuclease.

[00419] In other embodiments, the Cas nuclease may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

d) Heterologous functional domains; nuclear localization signals

[00420] In some embodiments, the RNA-guided DNA-binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, comprises one or more heterologous functional domains (e.g., is or comprises a fusion polypeptide).

[00421] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, into the nucleus of a cell. For example, the heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be linked at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. In some embodiments, the RNA-guided DNA-binding agent may be fused C-terminally to at least one NLS. An NLS may also be inserted within the RNA-guided DNA binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (*e.g.*, two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two SV40

NLS sequences linked at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a monopartite sequence, such as, *e.g.*, the SV40 NLS, PKKKRKV (SEQ ID NO: 278) or PKKKRRV (SEQ ID NO: 290). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 291). In some embodiments, the NLS sequence may comprise LAAKRSRTT (SEQ ID NO: 279), QAAKRSRTT (SEQ ID NO: 280), PAPAKRERTT (SEQ ID NO: 281), QAAKRPRTT (SEQ ID NO: 282), RAAKRPRTT (SEQ ID NO: 283), AAAKRSWSMAA (SEQ ID NO: 284), AAAKRVWSMAF (SEQ ID NO: 285), AAAKRSWSMAF (SEQ ID NO: 286), AAAKRKYFAA (SEQ ID NO: 287), RAAKRKAFAA (SEQ ID NO: 288), or RAAKRKYFAV (SEQ ID NO: 289). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 278) NLS may be linked at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site. In some embodiments, one or more NLS(s) according to any of the foregoing embodiments are present in the RNA-guided DNA-binding agent in combination with one or more additional heterologous functional domains, such as any of the heterologous functional domains described below. Exemplary coding sequences for NLSs are provided as SEQ ID NOs: 292-304.

[00422] In some embodiments, the heterologous functional domain may be capable of modifying the intracellular half-life of the RNA-guided DNA binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9. In some embodiments, the half-life of the RNA-guided DNA binding agent may be increased. In some embodiments, the half-life of the RNA-guided DNA-binding agent may be reduced. In some embodiments, the heterologous functional domain may be capable of increasing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may be capable of reducing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may act as a signal peptide for protein degradation. In some embodiments, the protein degradation may be mediated by proteolytic enzymes, such as, for example, proteasomes, lysosomal proteases, or calpain proteases. In some embodiments, the heterologous functional domain may comprise a PEST sequence. In some embodiments, the RNA-guided DNA-binding agent may be modified by addition of ubiquitin or a polyubiquitin chain. In some embodiments, the ubiquitin may be a ubiquitin-like protein (UBL). Non-

limiting examples of ubiquitin-like proteins include small ubiquitin-like modifier (SUMO), ubiquitin cross-reactive protein (UCRP, also known as interferon-stimulated gene-15 (ISG15)), ubiquitin-related modifier-1 (URM1), neuronal-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8, also called Rub1 in *S. cerevisiae*), human leukocyte antigen F-associated (FAT10), autophagy-8 (ATG8) and -12 (ATG12), Fau ubiquitin-like protein (FUB1), membrane-anchored UBL (MUB), ubiquitin fold-modifier-1 (UFM1), and ubiquitin-like protein-5 (UBL5).

[00423] In some embodiments, the heterologous functional domain may be a marker domain. Non-limiting examples of marker domains include fluorescent proteins, purification tags, epitope tags, and reporter gene sequences. In some embodiments, the marker domain may be a fluorescent protein. Non-limiting examples of suitable fluorescent proteins include green fluorescent proteins (*e.g.*, GFP, GFP-2, tagGFP, turboGFP, sfGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreen1), yellow fluorescent proteins (*e.g.*, YFP, EYFP, Citrine, Venus, YPet, PhiYFP, ZsYellow1), blue fluorescent proteins (*e.g.*, EBFP, EBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire,), cyan fluorescent proteins (*e.g.*, ECFP, Cerulean, CyPet, AmCyan1, Midoriishi-Cyan), red fluorescent proteins (*e.g.*, mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRed1, AsRed2, eqFP611, mRasberry, mStrawberry, Jred), and orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato) or any other suitable fluorescent protein. In other embodiments, the marker domain may be a purification tag and/or an epitope tag. Non-limiting exemplary tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein (MBP), thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AU5, E, ECS, E2, FLAG, HA, nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, 6xHis, 8xHis, biotin carboxyl carrier protein (BCCP), poly-His, and calmodulin. Non-limiting exemplary reporter genes include glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, or fluorescent proteins.

[00424] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, to a specific organelle, cell type, tissue, or organ. In some embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to mitochondria.

[00425] In further embodiments, the heterologous functional domain may be an effector domain. When the RNA-guided DNA-binding agent is directed to its target sequence, *e.g.*, when a Cas nuclease is directed to a target sequence by a gRNA, the effector domain may modify or affect the target sequence. In some embodiments, the effector domain may be chosen from a nucleic acid binding domain, a nuclease domain (e.g., a non-Cas nuclease domain), an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. In some embodiments, the heterologous functional domain is a nuclease, such as a FokI nuclease. See, e.g., US Pat. No. 9,023,649. In some embodiments, the heterologous functional domain is a transcriptional activator or repressor. See, e.g., Qi et al., "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression," *Cell* 152:1173-83 (2013); Perez-Pinera et al., "RNA-guided gene activation by CRISPR-Cas9-based transcription factors," *Nat. Methods* 10:973-6 (2013); Mali et al., "CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," *Nat. Biotechnol.* 31:833-8 (2013); Gilbert et al., "CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes," *Cell* 154:442-51 (2013). As such, the RNA-guided DNA-binding agent essentially becomes a transcription factor that can be directed to bind a desired target sequence using a guide RNA. In certain embodiments, the DNA modification domain is a methylation domain, such as a demethylation or methyltransferase domain. In certain embodiments, the effector domain is a DNA modification domain, such as a base-editing domain. In particular embodiments, the DNA modification domain is a nucleic acid editing domain that introduces a specific modification into the DNA, such as a deaminase domain. *See, e.g.*, WO 2015/089406; US 2016/0304846. The nucleic acid editing domains, deaminase domains, and Cas9 variants described in WO 2015/089406 and US 2016/0304846 are hereby incorporated by reference.

e) UTRs; Kozak sequences

[00426] In some embodiments, the polynucleotide (e.g. mRNA) comprises a 5' UTR, a 3' UTR, or 5' and 3' UTRs. In some embodiments, the polynucleotide (e.g. mRNA) comprises at least one UTR from Hydroxysteroid 17-Beta Dehydrogenase 4 (HSD17B4 or HSD), e.g., a 5' UTR from HSD. In some embodiments, the polynucleotide (e.g. mRNA) comprises at least one UTR from a globin mRNA, for example, human alpha globin (HBA) mRNA, human beta globin (HBB) mRNA, or Xenopus laevis beta globin (XBG) mRNA. In some embodiments, the polynucleotide (e.g. mRNA) comprises a 5' UTR, 3' UTR, or 5' and 3' UTRs from a globin mRNA, such as HBA, HBB, or XBG. In some embodiments, the

polynucleotide (e.g. mRNA) comprises a 5' UTR from bovine growth hormone, cytomegalovirus (CMV), mouse Hba-a1, HSD, an albumin gene, HBA, HBB, or XBG. In some embodiments, the polynucleotide (e.g. mRNA)comprises a 3' UTR from bovine growth hormone, cytomegalovirus, mouse Hba-a1, HSD, an albumin gene, HBA, HBB, or XBG. In some embodiments, the polynucleotide (e.g. mRNA) comprises 5' and 3' UTRs from bovine growth hormone, cytomegalovirus, mouse Hba-a1, HSD, an albumin gene, HBA, HBB, XBG, heat shock protein 90 (Hsp90), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta-actin, alpha-tubulin, tumor protein (p53), or epidermal growth factor receptor (EGFR).

[00427] In some embodiments, the polynucleotide (e.g. mRNA) comprises 5' and 3' UTRs that are from the same source, e.g., a constitutively expressed mRNA such as actin, albumin, or a globin such as HBA, HBB, or XBG.

[00428] In some embodiments, a nucleic acid disclosed herein comprises a 5' UTR with at least 90% identity to any one of SEQ ID NOs: 232, 234, 236, 238, 241, or 275-277. In some embodiments, a nucleic acid disclosed herein comprises a 3' UTR with at least 90% identity to any one of SEQ ID NOs: 233, 235, 237, 239, or 240. In some embodiments, any of the foregoing levels of identity is at least 95%, at least 98%, at least 99%, or 100%. In some embodiments, a nucleic acid disclosed herein comprises a 5' UTR having the sequence of any one of SEQ ID NOs: 232, 234, 236, 238, or 241. In some embodiments, a nucleic acid disclosed herein comprises a 3' UTR having the sequence of any one of SEQ ID NOs: 233, 235, 237, 239, or 240.

[00429] In some embodiments, the polynucleotide (e.g. mRNA) does not comprise a 5' UTR, e.g., there are no additional nucleotides between the 5' cap and the start codon. In some embodiments, the polynucleotide (e.g. mRNA) comprises a Kozak sequence (described below) between the 5' cap and the start codon, but does not have any additional 5' UTR. In some embodiments, the polynucleotide (e.g. mRNA) does not comprise a 3' UTR, e.g., there are no additional nucleotides between the stop codon and the poly-A tail.

[00430] In some embodiments, the polynucleotide (e.g. mRNA) comprises a Kozak sequence. The Kozak sequence can affect translation initiation and the overall yield of a polypeptide translated from a nucleic acid. A Kozak sequence includes a methionine codon that can function as the start codon. A minimal Kozak sequence is NNNRUGN wherein at least one of the following is true: the first N is A or G and the second N is G. In the context of a nucleotide sequence, R means a purine (A or G). In some embodiments, the Kozak sequence is RNNRUGN, NNNRUGG, RNNRUGG, RNNAUGN, NNNAUGG, or RNNAUGG. In some embodiments, the Kozak sequence is rccRUGg with zero mismatches

or with up to one or two mismatches to positions in lowercase. In some embodiments, the Kozak sequence is rccAUGg with zero mismatches or with up to one or two mismatches to positions in lowercase. In some embodiments, the Kozak sequence is gccRccAUGG (nucleotides 4-13 of SEQ ID NO: 305) with zero mismatches or with up to one, two, or three mismatches to positions in lowercase. In some embodiments, the Kozak sequence is gccAccAUG with zero mismatches or with up to one, two, three, or four mismatches to positions in lowercase. In some embodiments, the Kozak sequence is GCCACCAUG. In some embodiments, the Kozak sequence is gccgccRccAUGG (SEQ ID NO: 305) with zero mismatches or with up to one, two, three, or four mismatches to positions in lowercase.

f) Poly-A tail

[00431] In some embodiments, the polynucleotide (e.g. mRNA) further comprises a poly-adenylated (poly-A) tail. In some instances, the poly-A tail is "interrupted" with one or more non-adenine nucleotide "anchors" at one or more locations within the poly-A tail. The poly-A tails may comprise at least 8 consecutive adenine nucleotides, but also comprise one or more non-adenine nucleotide. As used herein, "non-adenine nucleotides" refer to any natural or non-natural nucleotides that do not comprise adenine. Guanine, thymine, and cytosine nucleotides are exemplary non-adenine nucleotides. Thus, the poly-A tails on the polynucleotide (e.g. mRNA) described herein may comprise consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA-binding agent or a sequence of interest. In some instances, the poly-A tails on mRNA comprise non-consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA-binding agent or a sequence of interest, wherein non-adenine nucleotides interrupt the adenine nucleotides at regular or irregularly spaced intervals.

[00432] In some embodiments, the poly-A tail is encoded in the plasmid used for in vitro transcription of mRNA and becomes part of the transcript. The poly-A sequence encoded in the plasmid, i.e., the number of consecutive adenine nucleotides in the poly-A sequence, may not be exact, e.g., a 100 poly-A sequence in the plasmid may not result in a precisely 100 poly-A sequence in the transcribed mRNA. In some embodiments, the poly-A tail is not encoded in the plasmid, and is added by PCR tailing or enzymatic tailing, e.g., using *E. coli* poly(A) polymerase.

[00433] In some embodiments, the one or more non-adenine nucleotides are positioned to interrupt the consecutive adenine nucleotides so that a poly(A) binding protein can bind to a stretch of consecutive adenine nucleotides. In some embodiments, one or more non-adenine

nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-100 consecutive adenine nucleotides. In some embodiments, the non-adenine nucleotide is after one, two, three, four, five, six, or seven adenine nucleotides and is followed by at least 8 consecutive adenine nucleotides.

[00434] The poly-A tail of the present disclosure may comprise one sequence of consecutive adenine nucleotides followed by one or more non-adenine nucleotides, optionally followed by additional adenine nucleotides.

[00435] In some embodiments, the poly-A tail comprises or contains one non-adenine nucleotide or one consecutive stretch of 2-10 non-adenine nucleotides. In some embodiments, the non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some instances, the one or more non-adenine nucleotides are located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotides are located after at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 consecutive adenine nucleotides.

[00436] In some embodiments, the non-adenine nucleotide is guanine, cytosine, or thymine. In some instances, the non-adenine nucleotide is a guanine nucleotide. In some embodiments, the non-adenine nucleotide is a cytosine nucleotide. In some embodiments, the non-adenine nucleotide is a thymine nucleotide. In some instances, where more than one non-adenine nucleotide is present, the non-adenine nucleotide may be selected from: a) guanine and thymine nucleotides; b) guanine and cytosine nucleotides; c) thymine and cytosine nucleotides; or d) guanine, thymine and cytosine nucleotides. An exemplary poly-A tail comprising non-adenine nucleotides is provided as SEQ ID NO: 262.

g) Modified nucleotides

[00437] In some embodiments, the nucleic acid comprising an ORF encoding an RNA-guided DNA-binding agent comprises a modified uridine at some or all uridine positions. In some embodiments, the modified uridine is a uridine modified at the 5 position, e.g., with a halogen or C1-C3 alkoxy. In some embodiments, the modified uridine is a pseudouridine modified at the 1 position, e.g., with a C1-C3 alkyl. The modified uridine can be, for example, pseudouridine, N1-methyl-pseudouridine, 5-methoxyuridine, 5-iodouridine, or a combination thereof. In some embodiments the modified uridine is 5-methoxyuridine. In

some embodiments the modified uridine is 5-iodouridine. In some embodiments the modified uridine is pseudouridine. In some embodiments the modified uridine is N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of N1-methyl pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-iodouridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and 5-methoxyuridine.

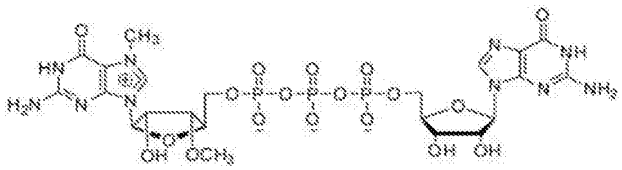
[00438] In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the uridine positions in the nucleic acid are modified uridines. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the nucleic acid are modified uridines, e.g., 5-methoxyuridine, 5-iodouridine, N1-methyl pseudouridine, pseudouridine, or a combination thereof. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the nucleic acid are 5-methoxyuridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the nucleic acid are pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the nucleic acid are N1-methyl pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the nucleic acid are 5-iodouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the nucleic acid are 5-methoxyuridine, and the remainder are N1-methyl pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the nucleic acid are 5-iodouridine, and the remainder are N1-methyl pseudouridine.

h) 5' Cap

[00439] In some embodiments, the nucleic acid (e.g., mRNA) comprising an ORF encoding an RNA-guided DNA-binding agent comprises a 5' cap, such as a Cap0, Cap1, or

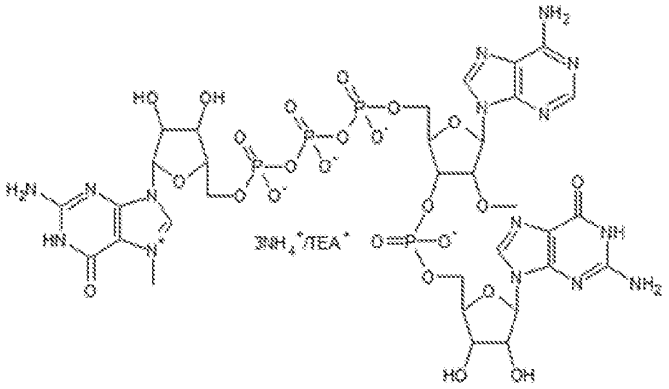
Cap2. A 5' cap is generally a 7-methylguanine ribonucleotide (which may be further modified, as discussed below e.g. with respect to ARCA) linked through a 5'-triphosphate to the 5' position of the first nucleotide of the 5'-to-3' chain of the nucleic acid, i.e., the first cap-proximal nucleotide. In Cap0, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-hydroxyl. In Cap1, the riboses of the first and second transcribed nucleotides of the mRNA comprise a 2'-methoxy and a 2'-hydroxyl, respectively. In Cap2, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-methoxy. See, e.g., Katibah et al. (2014) *Proc Natl Acad Sci USA* 111(33):12025-30; Abbas et al. (2017) *Proc Natl Acad Sci USA* 114(11):E2106-E2115. Most endogenous higher eukaryotic mRNAs, including mammalian nucleic acids such as human nucleic acids, comprise Cap1 or Cap2. Cap0 and other cap structures differing from Cap1 and Cap2 may be immunogenic in mammals, such as humans, due to recognition as "non-self" by components of the innate immune system such as IFIT-1 and IFIT-5, which can result in elevated cytokine levels including type I interferon. Components of the innate immune system such as IFIT-1 and IFIT-5 may also compete with eIF4E for binding of a nucleic acid with a cap other than Cap1 or Cap2, potentially inhibiting translation of the mRNA.

[00440] A cap can be included in an RNA co-transcriptionally. For example, ARCA (anti-reverse cap analog; Thermo Fisher Scientific Cat. No. AM8045) is a cap analog comprising a 7-methylguanine 3'-methoxy-5'-triphosphate linked to the 5' position of a guanine ribonucleotide which can be incorporated in vitro into a transcript at initiation. ARCA results in a Cap0 cap in which the 2' position of the first cap-proximal nucleotide is hydroxyl. See, e.g., Stepinski et al., (2001) "Synthesis and properties of mRNAs containing the novel 'anti-reverse' cap analogs 7-methyl(3'-O-methyl)GpppG and 7-methyl(3'deoxy)GpppG," *RNA* 7: 1486–1495. The ARCA structure is shown below.



[00441] CleanCap™ AG (m7G(5')ppp(5')(2'OMeA)pG; TriLink Biotechnologies Cat. No. N-7113) or CleanCap™ GG (m7G(5')ppp(5')(2'OMeG)pG; TriLink Biotechnologies Cat. No. N-7133) can be used to provide a Cap1 structure co-transcriptionally. 3'-O-methylated versions of CleanCap™ AG and CleanCap™ GG are also available from TriLink Biotechnologies as Cat. Nos. N-7413 and N-7433, respectively. The CleanCap™ AG structure is shown below. CleanCap™ structures are sometimes referred to herein using the

last three digits of the catalog numbers listed above (e.g., "CleanCap™ 113" for TriLink Biotechnologies Cat. No. N-7113).



[00442] Alternatively, a cap can be added to an RNA post-transcriptionally. For example, Vaccinia capping enzyme is commercially available (New England Biolabs Cat. No. M2080S) and has RNA triphosphatase and guanylyltransferase activities, provided by its D1 subunit, and guanine methyltransferase, provided by its D12 subunit. As such, it can add a 7-methylguanine to an RNA, so as to give Cap0, in the presence of S-adenosyl methionine and GTP. See, e.g., Guo, P. and Moss, B. (1990) *Proc. Natl. Acad. Sci. USA* 87, 4023-4027; Mao, X. and Shuman, S. (1994) *J. Biol. Chem.* 269, 24472-24479. For additional discussion of caps and capping approaches, see, e.g., WO2017/053297 and Ishikawa et al., *Nucl. Acids. Symp. Ser.* (2009) No. 53, 129-130.

D. Determination of efficacy of RNAs

[00443] In some embodiments, the efficacy of a gRNA is determined when delivered together with other components, e.g., a nucleic acid encoding an RNA-guided DNA binding agent such as any of those described herein. In some embodiments, the efficacy of a combination of a gRNA and a nucleic acid encoding an RNA-guided DNA binding agent is determined.

[00444] As described herein, use of an RNA-guided DNA nuclease and a guide RNA disclosed herein can lead to double-stranded breaks in the DNA which can produce errors in the form of insertion/deletion (indel) mutations upon repair by cellular machinery. Many mutations due to indels alter the reading frame or introduce premature stop codons and, therefore, produce a non-functional protein.

[00445] In some embodiments, the efficacy of particular gRNAs or combinations is determined based on *in vitro* models. In some embodiments, the *in vitro* model is HEK293

cells. In some embodiments, the *in vitro* model is HUH7 human hepatocarcinoma cells. In some embodiments, the *in vitro* model is HepG2 cells. In some embodiments, the *in vitro* model is primary human hepatocytes. In some embodiments, the *in vitro* model is primary cynomolgus hepatocytes. With respect to using primary human hepatocytes, commercially available primary human hepatocytes can be used to provide greater consistency between experiments. In some embodiments, the number of off-target sites at which a deletion or insertion occurs in an in vitro model (e.g., in primary human hepatocytes) is determined, e.g., by analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA and the guide RNA. In some embodiments, such a determination comprises analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide. Exemplary procedures for such determinations are provided in the working examples below.

[00446] In some embodiments, the efficacy of particular gRNAs or combinations is determined across multiple *in vitro* cell models for a gRNA selection process. In some embodiments, a cell line comparison of data with selected gRNAs is performed. In some embodiments, cross screening in multiple cell models is performed.

[00447] In some embodiments, the efficacy of particular gRNAs or combinations is determined based on *in vivo* models. In some embodiments, the *in vivo* model is a rodent model. In some embodiments, the rodent model is a mouse which expresses a human *TTR* gene, which may be a mutant human *TTR* gene. In some embodiments, the *in vivo* model is a non-human primate, for example cynomolgus monkey.

[00448] In some embodiments, the efficacy of a guide RNA or combination is measured by percent editing of *TTR*. In some embodiments, the percent editing of *TTR* is compared to the percent editing necessary to acheive knockdown of TTR protein, e.g., in the cell culture media in the case of an *in vitro* model or in serum or tissue in the case of an *in vivo* model.

[00449] In some embodiements, the efficacy of a guide RNA or combination is measured by the number and/or frequency of indels at off-target sequences within the genome of the target cell type. In some embodiments, efficacious guide RNAs and combinations are provided which produce indels at off target sites at very low frequencies (e.g., <5%) in a cell population and/or relative to the frequency of indel creation at the target site. Thus, the disclosure provides for guide RNAs which do not exhibit off-target indel formation in the target cell type (e.g., a hepatocyte), or which produce a frequency of off-target indel formation of <5% in a cell population and/or relative to the frequency of indel creation at the target site. In some embodiments, the disclosure provides guide RNAs and combinations

which do not exhibit any off target indel formation in the target cell type (e.g., hepatocyte). In some embodiments, guide RNAs and combinations are provided which produce indels at less than 5 off-target sites, e.g., as evaluated by one or more methods described herein. In some embodiments, guide RNAs and combinations are provided which produce indels at less than or equal to 4, 3, 2, or 1 off-target site(s) e.g., as evaluated by one or more methods described herein. In some embodiments, the off-target site(s) does not occur in a protein coding region in the target cell (e.g., hepatocyte) genome.

[00450] In some embodiments, detecting gene editing events, such as the formation of insertion/deletion ("indel") mutations and homology directed repair (HDR) events in target DNA utilize linear amplification with a tagged primer and isolating the tagged amplification products (herein after referred to as "LAM-PCR," or "Linear Amplification (LA)" method), as described in WO2018/067447 or Schmidt et al., Nature Methods 4:1051-1057 (2007).

[00451] In some embodiments, detecting gene editing events, such as the formation of insertion/deletion ("indel") mutations and homology directed repair (HDR) events in target DNA, further comprises sequencing the linear amplified products or the further amplified products. Sequencing may comprise any method known to those of skill in the art, including, next generation sequencing, and cloning the linear amplification products or further amplified products into a plasmid and sequencing the plasmid or a portion of the plasmid. Exemplary next generation sequencing methods are discussed, e.g., in Shendure et al., *Nature* 26:1135-1145 (2008). In other aspects, detecting gene editing events, such as the formation of insertion/deletion ("indel") mutations and homology directed repair (HDR) events in target DNA, further comprises performing digital PCR (dPCR) or droplet digital PCR (ddPCR) on the linear amplified products or the further amplified products, or contacting the linear amplified products or the further amplified products with a nucleic acid probe designed to identify DNA comprising HDR template sequence and detecting the probes that have bound to the linear amplified product(s) or further amplified product(s). In some embodiments, the method further comprises determining the location of the HDR template in the target DNA.

[00452] In certain embodiments, the method further comprises determining the sequence of an insertion site in the target DNA, wherein the insertion site is the location where the HDR template incorporates into the target DNA, and wherein the insertion site may include some target DNA sequence and some HDR template sequence.

[00453] In some embodiments, the efficacy of a guide RNA or combination is measured by secretion of TTR. In some embodiments, secretion of TTR is measured using an enzyme-linked immunosorbent assay (ELISA) assay with cell culture media or serum. In some

embodiments, secretion of TTR is measured in the same *in vitro* or *in vivo* systems or models used to measure editing. In some embodiments, secretion of TTR is measured in primary human hepatocytes. In some embodiments, secretion of TTR is measured in HUH7 cells. In some embodiments, secretion of TTR is measured in HepG2 cells.

[00454] ELISA assays are generally known to the skilled artisan and can be designed to determine serum TTR levels. In one exemplary embodiment, blood is collected and the serum is isolated. The total TTR serum levels may be determined using a Mouse Prealbumin (Transthyretin) ELISA Kit (Aviva Systems Biology, Cat. OKIA00111) or similar kit for measuring human TTR. If no kit is available, an ELISA can be developed using plates that are pre-coated with with capture antibody specific for the TTR one is measuring. The plate is next incubated at room temperature for a period of time before washing. Enzyme-anti-TTR antibody conjugate is added and inncubated. Unbound antibody conjugate is removed and the plate washed before the addition of the chromogenic substrate solution that reactes with the enzyme. The plate is read on an appropriate plate reader at an absorbance specific for the enzyme and substrate used.

[00455] In some embodiments, the amount of TTR in cells (including those from tissue) measures efficacy of a gRNA or combination. In some embodiments, the amount of TTR in cells is measured using western blot. In some embodiments, the cell used is HUH7 cells. In some embodiments, the cell used is a primary human hepatocyte. In some embodiments, the cell used is a primar cell obtained from an animal. In some embodiments, the amount of TTR is compared to the amount of glyceraldehyde 3-phosphate dehydrogenase GAPDH (a housekeeping gene) to control for changes in cell number.

III. LNP formulations and Treatment of ATTR

[00456] In some embodiments, a method of inducing a double-stranded break (DSB) within the *TTR* gene is provided comprising administering a composition comprising a guide RNA as described herein, e.g. comprising any one or more guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID Nos: 5-82 are administered to induce a DSB in the *TTR* gene. The guide RNA is administered together with a nucleic acid (e.g., mRNA) or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease

are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00457] In some embodiments, a method of inducing a double-stranded break (DSB) within the *TTR* gene is provided comprising administering a composition comprising a guide RNA, such as a chemically modified guide RNA, comprising any one or more guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124. In some embodiments, any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124 or gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82 are administered to induce a DSB in the *TTR* gene. The guide RNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00458] In some embodiments, a method of modifying the *TTR* gene is provided comprising administering a composition comprising a guide RNA as described herein, e.g. comprising any one or more of the guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124, are administered to modify the *TTR* gene. The guide RNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00459] In some embodiments, a method of modifying the *TTR* gene is provided comprising administering a composition comprising a guide RNA comprising any one or

more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124, are administered to modify the *TTR* gene. The guide RNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00460] In some embodiments, a method of treating ATTR is provided comprising administering a composition comprising a guide RNA as described herein, e.g. comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to treat ATTR. The guide RNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00461] In some embodiments, a method of treating ATTR is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124are administered to treat ATTR. The guide RNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the

guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00462] In some embodiments, a method of reducing TTR serum concentration is provided comprising administering a guide RNA as described herein, e.g. comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82 or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to reduce or prevent the accumulation of TTR in amyloids or amyloid fibrils. The gRNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00463] In some embodiments, a method of reducing TTR serum concentration is provided comprising administering a guide RNA as described herein, e.g., comprising any one or more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124are administered to reduce or prevent the accumulation of TTR in amyloids or amyloid fibrils. The gRNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00464] In some embodiments, a method of reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject is provided comprising administering a composition comprising a guide RNA as described herein, e.g. comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, a method of reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject is provided comprising administering a composition comprising any one or more of the sgRNAs of SEQ ID Nos: 87-113. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82 or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to reduce or prevent the accumulation of TTR in amyloids or amyloid fibrils. The gRNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00465] In some embodiments, a method of reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject is provided comprising administering a composition comprising a guide RNA as described herein, e.g. comprising any one or more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, a method of reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject is provided comprising administering a composition comprising any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82 or any one or more of the sgRNAs of SEQ ID Nos: 87-113, 115-120, and 122-124are administered to reduce or prevent the accumulation of TTR in amyloids or amyloid fibrils. The gRNA is administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). The RNA-guided DNA nuclease may be an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such

as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00466] In some embodiments, the gRNA comprising a guide sequence of Table 1 or one or more sgRNAs from Table 2 together with an RNA-guided DNA nuclease such as a Cas nuclease translated from the nucleic acid induce DSBs, and non-homologous ending joining (NHEJ) during repair leads to a mutation in the *TTR* gene. In some embodiments, NHEJ leads to a deletion or insertion of a nucleotide(s), which induces a frame shift or nonsense mutation in the *TTR* gene.

[00467] In some embodiments, administering the guide RNA and nucleic acid encoding an RNA-guided DNA binding agent (e.g., in a composition provided herein) reduces levels (e.g., serum levels) of TTR in the subject, and therefore prevents accumulation and aggregation of TTR in amyloids or amyloid fibrils.

[00468] In some embodiments, reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject comprises reducing or preventing TTR deposition in one or more tissues of the subject, such as stomach, colon, or nervous tissue. In some embodiments, the nervous tissue comprises sciatic nerve or dorsal root ganglion. In some embodiments, TTR deposition is reduced in two, three, or four of the stomach, colon, dorsal root ganglion, and sciatic nerve. The level of deposition in a given tissue can be determined using a biopsy sample, e.g., using immunostaining. In some embodiments, reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject and/or reducing or preventing TTR deposition is inferred based on reducing serum TTR levels for a period of time. As discussed in the examples, it has been found that reducing serum TTR levels in accordance with methods and uses provided herein can result in clearance of deposited TTR from tissues such as those discussed above and in the examples, e.g., as measured 8 weeks after administration of the composition.

[00469] In some embodiments, the subject is mammalian. In some embodiments, the subject is human. In some embodiments, the subject is cow, pig, monkey, sheep, dog, cat, fish, or poultry.

[00470] In some embodiments, the use of one or more guide RNAs as described herein, e.g. comprising any one or more of the guide sequences in Table 1 or one or more sgRNAs from Table 2 (e.g., in a composition provided herein) and of a nucleic acid (e.g. mRNA) described herein encoding an RNA-guided DNA-binding agent is provided for the preparation of a medicament for treating a human subject having ATTR. The RNA-guided

DNA-binding agent may be a Cas9, e.g. an *S. pyogenes* Cas9. In particular embodiments, the guide RNA is chemically modified.

[00471] In some embodiments, the composition comprising the guide RNA and nucleic acid is administered intravenously. In some embodiments, the composition comprising the guide RNA and nucleic acid is administered into the hepatic circulation.

[00472] In some embodiments, a single administration of a composition comprising a guide RNA and nucleic acid provided herein is sufficient to knock down expression of the mutant protein. In some embodiments, a single administration of a composition comprising a guide RNA and nucleic acid provided herein is sufficient to knock out expression of the mutant protein in a population of cells. In other embodiments, more than one administration of a composition comprising a guide RNA and nucleic acid provided herein may be beneficial to maximize editing via cumulative effects. For example, a composition provided herein can be administered 2, 3, 4, 5, or more times, such as 2 times. Administrations can be separated by a period of time ranging from, e.g., 1 day to 2 years, such as 1 to 7 days, 7 to 14 days, 14 days to 30 days, 30 days to 60 days, 60 days to 120 days, 120 days to 183 days, 183 days to 274 days, 274 days to 366 days, or 366 days to 2 years.

[00473] In some embodiments, a composition is administered in an effective amount in the range of 0.01 to 10 mg/kg (mpk), e.g., 0.01 to 0.1 mpk, 0.1 to 0.3 mpk, 0.3 to 0.5 mpk, 0.5 to 1 mpk, 1 to 2 mpk, 2 to 3 mpk, 3 to 5 mpk, 5 to 10 mpk, or 0.1, 0.2, 0.3, 0.5, 1, 2, 3, 5, or 10 mpk. In some embodiments, a composition is administered in the amount of 2-4 mg/kg, such as 2.5-3.5 mg/kg. In some embodiments, a composition is administered in the amount of about 3 mg/kg.

[00474] In some embodiments, the efficacy of treatment with the compositions of the invention is seen at 1 year, 2 years, 3 years, 4 years, 5 years, or 10 years after delivery. In some embodiments, efficacy of treatment with the compositions of the invention is assessed by measuring serum levels of TTR before and after treatment. In some embodiments, efficacy of treatment with the compositions assessed via a reduction of serum levels of TTR is seen at 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, or at 11 months.

[00475] In some embodiments, treatment slows or halts disease progression.

[00476] In some embodiments, treatment slows or halts progression of FAP. In some embodiments, treatment results in improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy or autonomic neuropathy.

[00477] In some embodiments, treatment results in improvement, stabilization, or slowing of change in symptoms of FAC. In some embodiments, treatment results in improvement, stabilization, or slowing of change symptoms of restrictive cardiomyopathy or congestive heart failure.

[00478] In some embodiments, efficacy of treatment is measured by increased survival time of the subject.

[00479] In some embodiments, efficacy of treatment is measured by improvement or slowing of progression in symptoms of sensorimotor or autonomic neuropathy. In some embodiments, efficacy of treatment is measured by an increase or a a slowing of decrease in ability to move an area of the body or to feel in any area of the body. In some embodiments, efficacy of treatment is measured by improvement or a slowing of decrease in the ability to swallow; breath; use arms, hands, legs, or feet; or walk. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of neuralgia. In some embodiments, the neuralgia is characterized by pain, burning, tingling, or abnormal feeling. In some embodiments, efficacy of treatment is measured by improvement or a slowing of increase in postural hypotension, dizziness, gastrointestinal dysmotility, bladder dysfunction, or sexual dysfunction. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of weakness. In some embodiments, efficacy of treatment is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

[00480] In some embodiments, efficacy of treatment is measured by improvement or slowing of progression of symptoms of congestive heart failure or CHF. In some embodiments, efficacy of treatment is measured by an decrease or a slowing of increase in shortness of breath, trouble breathing, fatigue, or swelling in the ankles, feet, legs, abdomen, or veins in the the neck. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of fluid buildup in the body, which may be assessed by measures such as weight gain, frequent urination, or nighttime cough. In some embodiments, efficacy of treatment is measured using cardiac biomarker tests (such as B-type natriuretic peptide [BNP] or N-terminal pro b-type natriuretic peptide [NT-proBNP]), lung function tests, chest x-rays, or electrocardiography.

A. Combination Therapy

[00481] In some embodiments, the invention comprises combination therapies comprising administering any one of the gRNAs as described herein, e.g., comprising any one or more of

the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 and a nucleic acid encoding an RNA-guided DNA-binding agent (e.g., in a composition provided herein) as described herein, such as a nucleic acid (e.g. mRNA) or vector described herein encoding an *S. pyogenes* Cas9, together with an additional therapy suitable for alleviating symptoms of ATTR. In particular embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA and the nucleic acid encoding an RNA-guided DNA nuclease are administered in an LNP described herein, such as an LNP comprising a CCD lipid (e.g., an amine lipid, such as lipid A), a helper lipid (e.g., cholesterol), a stealth lipid (e.g., a PEG lipid, such as PEG2k-DMG), and optionally a neutral lipid (e.g., DSPC).

[00482] In some embodiments, the additional therapy for ATTR is a treatment for sensorimotor or autonomic neuropathy. In some embodiments, the treatment for sensorimotor or autonomic neuropathy is a nonsteroidal anti-inflammatory drug, antidepressant, anticonvulsant medication, antiarrythmic medication, or narcotic agent. In some embodiments, the antidepressant is a tricylic agent or a serotonin-norepinephrine reuptake inhibitor. In some embodiments, the antidepressant is amitriptyline, duloxetine, or venlafaxine. In some embodiments, the anticonvulsant agent is gabapentin, pregabalin, topiramate, or carbamazepine. In some embodiments, the additional therapy for sensorimotor neuropathy is transcutaneous electrical nerve stimulation.

[00483] In some embodiments, the additional therapy for ATTR is a treatment for restrictive cardiomyopathy or congestive heart failure (CHF). In some embodiments, the treatment for CHF is a ACE inhibitor, aldosterone antagonist, angiotensin receptor blocker, beta blocker, digoxin, diuretic, or isosorbide dinitrate/hydralazine hydrochloride. In some embodiments, the ACE inhibitor is enalapril, captopril, ramipril, perindopril, imidapril, or quinapril. In some embodiments, the aldosterone antagonist is eplerenone or spironolactone. In some embodiments, the angiotensin receptor blocker is azilsartan, cadesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, or valsartan. In some embodiments, the beta blocker is acebutolol, atenolol, bisoprolol, metoprolol, nadolol, nebivolol, or propranolol. In some embodiments, the diuretic is chlorothiazide, chlorthalidone, hydrochlorothiazide, indapamide, metolazone, bumetanide, furosemide, torsemide, amiloride, or triameterene.

[00484] In some embodiments, the combination therapy comprises administering any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 and a nucleic acid encoding an RNA-guided DNA-binding agent (e.g., in a composition provided herein) together with a siRNA that targets TTR or mutant TTR. In some embodiments, the siRNA is any siRNA capable of further

reducing or eliminating the expression of wild type or mutant TTR. In some embodiments, the siRNA is the drug Patisiran (ALN-TTR02) or ALN-TTRsc02. In some embodiments, the siRNA is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein). In some embodiments, the siRNA is administered on a regular basis following treatment with any of the gRNA compositions provided herein.

[00485] In some embodiments, the combination therapy comprises administering any one of the gRNAs comprising any one or more of the guide sequences described herein, e.g., disclosed in Table 1 or any one or more of the sgRNAs in Table 2 and a nucleic acid encoding an RNA-guided DNA-binding agent described herein (e.g., in a composition provided herein) together with antisense nucleotide that targets TTR or mutant TTR. In some embodiments, the antisense nucleotide is any antisense nucleotide capable of further reducing or eliminating the expression of wild type or mutant TTR. In some embodiments, the antisense nucleotide is the drug Inotersen (IONS-TTR_{Rx}). In some embodiments, the antisense nucleotide is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 and a nucleic acid encoding an RNA-guided DNA-binding agent (e.g., in a composition provided herein). In some embodiments, the antisense nucleotide is administered on a regular basis following treatment with any of the gRNA compositions provided herein.

[00486] In some embodiments, the combination therapy comprises administering any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 and a nucleic acid encoding an RNA-guided DNA-binding agent (e.g., in a composition provided herein) together with a small molecule stabilizer that promotes kinetic stabilization of the correctly folded tetrameric form of TTR. In some embodiments, the small molecule stabilizer is the drug tafamidis (Vyndaqel®) or diflunisal. In some embodiments, the small molecule stabilizer is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein). In some embodiments, the small molecule stabilizer is administered on a regular basis following treatment with any of the compositions provided herein.

[00487] In any of the foregoing embodiments, the guide sequences disclosed in Table 1 may be selected from SEQ ID NOs: 5-72, 74-78, and 80-82, and/or the sgRNAs in Table 2 may be selected from SEQ ID Nos: 87-113, 115-120, and 122-124, and/or the guide RNA may be a chemically modified guide RNA.

[00488] In some embodiments, a method described herein comprises infusion prophylaxis. In some embodiments, an infusion prophylaxis is administered to a subject before the gene editing composition. In some embodiments, an infusion prophylaxis is administered to a subject 8-24 hours or 1-2 hours prior to the administration of the nucleic acid composition. In some embodiments, an infusion prophylaxis comprises corticosteroid. In some embodiments, the infusion prophylaxis comprises one or more, or all, of corticosteroid, an antipyretic (e.g. oral acetaminophen (also called paracetamol), which may reduce pain and fever and/or inhibit COX enzymes and/or prostaglandins), H1 blocker, or H2 blocker. In some embodiments, the infusion prophylaxis comprises an intravenous corticosteroid (e.g., dexamethasone 8-12 mg, such as 10 mg or equivalent) and an antipyretic (e.g. oral acetaminophen or paracetamol 500 mg). In some embodiments, the H1 blocker (e.g., diphenhydramine 50 mg or equivalent) and/or H2 blocker (e.g., ranitidine 50 mg or equivalent) are administered orally. In some embodiments, the H1 blocker (e.g., diphenhydramine 50 mg or equivalent) and/or H2 blocker (e.g., ranitidine 50 mg or equivalent) are administered intravenously. In some embodiments, an infusion prophylaxis is administered intravenously 1-2 hour before before infusion of the nucleic acid composition. In some embodiments an intravenous H1 blocker and/or an intravenous H2 blocker is substituted with an oral equivalent. The infusion prophylaxis may function to reduce adverse reactions associated with administering the nucleic acid composition. In some embodiments, the infusion prophylaxis is administered as a required premedication prior to administering the nucleic acid composition. The dosage, frequency and mode of administration of the corticosteroid, infusion prophylaxis, and the guide-RNA containing composition described herein can be controlled independently.

[00489] The corticosteroid used in the disclosed methods may be administered according to regimens known in the art, e.g., US FDA-approved regimens. In some embodiments, e.g., comprising administration to or for use in a human subject, the corticosteroid can be administered in an amount that ranges from about 0.75 mg to about 25 mg. In some embodiments, e.g., comprising administration to or for use in a human subject, the corticosteroid can be administered in an amount that ranges from about 0.01 – 0.5 mg/kg, such as 0.1 – 0.40 mg/kg or 0.25 – 0.40 mg/kg.

[00490] In some embodiments, the corticosteroid is administered before the guide RNA-containing composition described herein. In some embodiments, the corticosteroid is administered after the guide RNA-containing composition described herein. In some embodiments, the corticosteroid is administered simultaneously with the guide RNA-

containing composition described herein. In some embodiments, multiple doses of the corticosteroid are administered before or after the administration of the guide RNA-containing composition. In some embodiments, multiple doses of the guide RNA-containing composition are administered before or after the administration of the corticosteroid. In some embodiments, multiple doses of the corticosteroid and multiple doses of the the guide RNA-containing composition are administered.

[00491] If appropriate, a dose of corticosteroid may be administered as at least two sub-doses administered separately at appropriate intervals. In some embodiments, the corticosteroid is administered at least two times before the administration of the guide RNA-containing composition described herein. In some embodiments, a dose of corticosteroid is administered at least two times after the administration of the guide RNA-containing composition described herein. In some embodiments, the corticosteroid is administered (e.g., before, with, and/or after the administration of the guide RNA-containing composition described herein) at an interval of 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 18 hours; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks; or an amount of time in a range bounded by any two of the preceding values. In some embodiments, the corticosteroid is administered before the administration of the guide RNA-containing composition described herein at an interval of 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 18 hours; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks; or an amount of time in a range bounded by any two of the preceding values. In some embodiments, the corticosteroid is administered after the administration of the guide RNA-containing composition described herein at an interval of 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 18 hours; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks; or an amount of time in a range bounded by any two of the preceding values.

[00492] In some embodiments,the corticosteroid is administered at least two times. In some embodiments,the corticosteroid is administered is administered at least three times. In some embodiments,the corticosteroid is administered at least four times. In some embodiments,the corticosteroid is administered is up to five, six, seven, eight, nine, or ten times. A first dose may be oral and a second or subsequent dose may be by parenteral administration, e.g. infusion. Alternatively, a first dose may be perenteral and a second or subsequent dose may be by oral administration.

[00493] In some embodiments, the corticosteroid is administered orally before intravenous administration of a guide RNA-containing composition described herein. In some

embodiments, the corticosteroid is administered orally at or after intravenous administration of a guide RNA-containing composition described herein.

[00494] In some embodiments, corticosteroid is dexamethasone. In some embodiments, dexamethasone is administered intravenously 1-2 hour before before infusion of the nucleic acid composition. In some embodiments, dexamethasone is administered intravenously in the amount of 8-12 mg, such as 10 mg, 1-2 hour before before infusion of the nucleic acid composition. In some embodiments, dexamethasone is administered orally 8 to 24 hours before infusion of the nucleic acid composition. In some embodiments, dexamethasone is administered orally in the amount of 8-12 mg, such as 8 mg, 8 to 24 hours before infusion of the nucleic acid composition. In some embodiments, dexamethasone is administered orally in the amount of 8-12 mg, such as 8 mg, 8 to 24 hours before infusion of the nucleic acid composition and dexamethasone is administered intravenously in the amount of 8-12 mg, such as 10 mg, 1-2 hour before before infusion of the nucleic acid composition.

B. Delivery of Nucleic Acid Compositions

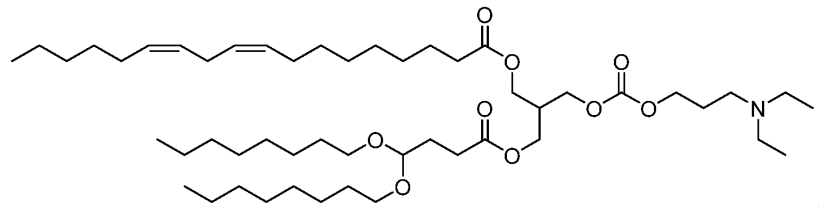
[00495] In some embodiments, the nucleic acid compositions described herein, comprising a gRNA and a nucleic acid described herein encoding an RNA-guided DNA-binding agent as RNA or encoded on one or more vectors, are formulated in or administered via a lipid nanoparticle; see e.g., WO2017173054A1 entitled "LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS," and WO2019067992A1 entiled "FORMULATIONS," the contents of which are hereby incorporated by reference in their entirety. Any lipid nanoparticle (LNP) known to those of skill in the art to be capable of delivering nucleotides to subjects may be utilized with the guide RNAs described herein and the nucleic acid encoding an RNA-guided DNA nuclease.

[00496] Disclosed herein are various embodiments of LNP formulations for RNAs, including CRISPR/Cas cargoes. Such LNP formulations may include (i) a CCD lipid, such as an amine lipid, (ii) a neutral lipid, (iii) a helper lipid, and (iv) a stealth lipid, such as a PEG lipid. Some embodiments of the LNP formulations include an "amine lipid", along with a helper lipid, a neutral lipid, and a stealth lipid such as a PEG lipid. In some embodiments, the LNP formulations include less than 1 percent neutral phospholipid. In some embodiments, the LNP formulations include less than 0.5 percent neutral phospholipid. By "lipid nanoparticle" is meant a particle that comprises a plurality of (*i.e.* more than one) lipid molecules physically associated with each other by intermolecular forces.

[00497] **CCD Lipids**

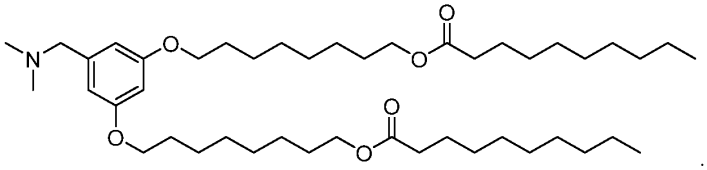
[00498] Lipid compositions for delivery of CRISPR/Cas mRNA and guide RNA components to a liver cell comprise a CCD Lipid.

[00499] In some embodiments, the CCD lipid is Lipid A, which is (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate. Lipid A can be depicted as:



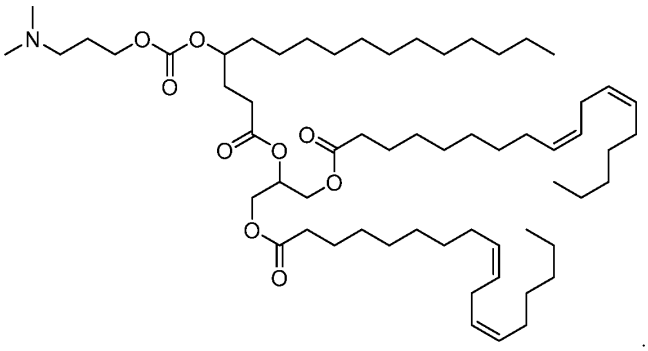
[00500] Lipid A may be synthesized according to WO2015/095340 (e.g., pp. 84-86).

[00501] In some embodiments, the CCD lipid is Lipid B, which is ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate), also called ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl) bis(decanoate). Lipid B can be depicted as:



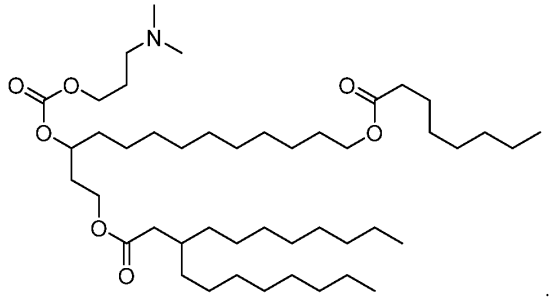
[00502] Lipid B may be synthesized according to WO2014/136086 (e.g., pp. 107-09).

[00503] In some embodiments, the CCD lipid is Lipid C, which is 2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1,3-diyl (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate). Lipid C can be depicted as:



[00504] In some embodiments, the CCD lipid is Lipid D, which is 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl 3-octylundecanoate.

[00505] Lipid D can be depicted as:



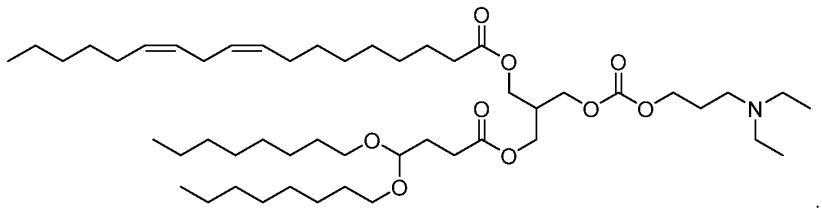
[00506] Lipid C and Lipid D may be synthesized according to WO2015/095340.

[00507] The CCD lipid can also be an equivalent to Lipid A, Lipid B, Lipid C, or Lipid D. In certain embodiments, the CCD lipid is an equivalent to Lipid A, an equivalent to Lipid B, an equivalent to Lipid C, or an equivalent to Lipid D.

[00508] **Amine Lipids**

[00509] In some embodiments, the LNP compositions for the delivery of biologically active agents comprise an "amine lipid", which is defined as Lipid A, Lipid B, Lipid C, Lipid D or equivalents of Lipid A (including acetal analogs of Lipid A), equivalents of Lipid B, equivalents of Lipid C, and equivalents of Lipid D.

[00510] In some embodiments, the amine lipid is Lipid A, which is (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate. Lipid A can be depicted as:



[00511] Lipid A may be synthesized according to WO2015/095340 (e.g., pp. 84-86). In certain embodiments, the amine lipid is an equivalent to Lipid A.

[00512] In certain embodiments, an amine lipid is an analog of Lipid A. In certain embodiments, a Lipid A analog is an acetal analog of Lipid A. In particular LNP compositions, the acetal analog is a C4-C12 acetal analog. In some embodiments, the acetal

analog is a C5-C12 acetal analog. In additional embodiments, the acetal analog is a C5-C10 acetal analog. In further embodiments, the acetal analog is chosen from a C4, C5, C6, C7, C9, C10, C11, and C12 acetal analog.

[00513] Amine lipids suitable for use in the LNPs described herein are biodegradable *in vivo* and suitable for delivering a biologically active agent, such as an RNA to a cell. The amine lipids have low toxicity (*e.g.*, are tolerated in an animal model without adverse effect in amounts of greater than or equal to 10 mg/kg of RNA cargo). In certain embodiments, LNPs comprising an amine lipid include those where at least 75% of the amine lipid is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days. In certain embodiments, LNPs comprising an amine lipid include those where at least 50% of the mRNA or gRNA is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days. In certain embodiments, LNPs comprising an amine lipid include those where at least 50% of the LNP is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days, for example by measuring a lipid (e.g., an amine lipid), RNA (e.g., mRNA), or another component. In certain embodiments, lipid-encapsulated versus free lipid, RNA, or nucleic acid component of the LNP is measured.

[00514] Lipid clearance may be measured as described in literature. *See Maier*, M.A., *et al.* "Biodegradable Lipids Enabling Rapidly Eliminated Lipid Nanoparticles for Systemic Delivery of RNAi Therapeutics," *Mol. Ther.* 2013, 21(8), 1570-78 ("Maier"). For example, in Maier, LNP-siRNA systems containing luciferases-targeting siRNA were administered to six- to eight-week old male C57Bl/6 mice at 0.3 mg/kg by intravenous bolus injection *via* the lateral tail vein. Blood, liver, and spleen samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, and 168 hours post-dose. Mice were perfused with saline before tissue collection and blood samples were processed to obtain plasma. All samples were processed and analyzed by LC-MS. Further, Maier describes a procedure for assessing toxicity after administration of LNP-siRNA formulations. For example, a luciferase-targeting siRNA was administered at 0, 1, 3, 5, and 10 mg/kg (5 animals/group) via single intravenous bolus injection at a dose volume of 5 mL/kg to male Sprague-Dawley rats. After 24 hours, about 1 mL of blood was obtained from the jugular vein of conscious animals and the serum was isolated. At 72 hours post-dose, all animals were euthanized for necropsy. Assessments of clinical signs, body weight, serum chemistry, organ weights and histopathology were performed. Although Maier describes methods for assessing siRNA-LNP formulations, these methods may be applied to assess clearance, pharmacokinetics, and toxicity of administration of LNP compositions of the present disclosure.

[00515] The amine lipids may lead to an increased clearance rate. In some embodiments, the clearance rate is a lipid clearance rate, for example the rate at which a lipid is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is an RNA clearance rate, for example the rate at which an mRNA or a gRNA is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is the rate at which LNP is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is the rate at which LNP is cleared from a tissue, such as liver tissue or spleen tissue. In certain embodiments, a high clearance rate leads to a safety profile with no substantial adverse effects. The amine lipids may reduce LNP accumulation in circulation and in tissues. In some embodiments, a reduction in LNP accumulation in circulation and in tissues leads to a safety profile with no substantial adverse effects.

[00516] The amine lipids of the present disclosure are ionizable (e.g., may form a salt) depending upon the pH of the medium they are in. For example, in a slightly acidic medium, the amine lipids may be protonated and thus bear a positive charge. Conversely, in a slightly basic medium, such as, for example, blood, where pH is approximately 7.35, the amine lipids may not be protonated and thus bear no charge. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 9. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 9. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 10.

[00517] The pH at which an amine lipid is predominantly protonated is related to its intrinsic pKa. In some embodiments, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.1 to about 7.4. In some embodiments, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.5 to about 6.6. In some embodiments, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.6 to about 6.4. In some embodiments, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.8 to about 6.2. For example, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.8 to about 6.5. The pKa of an amine lipid can be an important consideration in formulating LNPs as it has been found that cationic lipids with a pKa ranging from about 5.1 to about 7.4 are effective for delivery of cargo in vivo, e.g., to the liver. Furthermore, it has been found that cationic lipids with a pKa ranging from about 5.3 to about 6.4 are effective for delivery in vivo, e.g., to tumors. See, e.g., WO 2014/136086.

[00518] **Additional Lipids**

[00519] "Neutral lipids" suitable for use in a lipid composition of the disclosure include, for example, a variety of neutral, uncharged or zwitterionic lipids. Examples of neutral phospholipids suitable for use in the present disclosure include, but are not limited to, 5-heptadecylbenzene-1,3-diol (resorcinol), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), pohsphocholine (DOPC), dimyristoylphosphatidylcholine (DMPC), phosphatidylcholine (PLPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DAPC), phosphatidylethanolamine (PE), egg phosphatidylcholine (EPC), dilauryloylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), 1-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoyl-2-myristoyl phosphatidylcholine (PMPC), 1-palmitoyl-2-stearoyl phosphatidylcholine (PSPC), 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DBPC), 1-stearoyl-2-palmitoyl phosphatidylcholine (SPPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine (DEPC), palmitoyloleoyl phosphatidylcholine (POPC), lysophosphatidyl choline, dioleoyl phosphatidylethanolamine (DOPE), dilinoleoylphosphatidylcholine distearoylphosphatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE), lysophosphatidylethanolamine and combinations thereof. In one embodiment, the neutral phospholipid may be selected from the group consisting of distearoylphosphatidylcholine (DSPC) and dimyristoyl phosphatidyl ethanolamine (DMPE). In another embodiment, the neutral phospholipid may be distearoylphosphatidylcholine (DSPC). In another embodiment, the neutral phospholipid may be dipalmitoylphosphatidylcholine (DPPC).

[00520] "Helper lipids" include steroids, sterols, and alkyl resorcinols. Helper lipids suitable for use in the present disclosure include, but are not limited to, cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate. In one embodiment, the helper lipid may be cholesterol. In one embodiment, the helper lipid may be cholesterol hemisuccinate.

[00521] "Stealth lipids" are lipids that alter the length of time the nanoparticles can exist in vivo (e.g., in the blood). Stealth lipids may assist in the formulation process by, for example, reducing particle aggregation and controlling particle size. Stealth lipids used herein may modulate pharmacokinetic properties of the LNP. Stealth lipids suitable for use in a lipid composition of the disclosure include, but are not limited to, stealth lipids having a hydrophilic head group linked to a lipid moiety. Stealth lipids suitable for use in a lipid composition of the present disclosure and information about the biochemistry of such lipids

can be found in Romberg et al., Pharmaceutical Research, Vol. 25, No. 1, 2008, pg. 55-71 and Hoekstra et al., Biochimica et Biophysica Acta 1660 (2004) 41-52. Additional suitable PEG lipids are disclosed, e.g., in WO 2006/007712.

[00522] In one embodiment, the hydrophilic head group of stealth lipid comprises a polymer moiety selected from polymers based on PEG. Stealth lipids may comprise a lipid moiety. In some embodiments, the stealth lipid is a PEG lipid. PEG lipids may assist in the formulation process by, for example, reducing particle aggregation and controlling particle size. PEG lipids used herein may modulate pharmacokinetic properties of the LNPs. Typically, the PEG lipid comprises a lipid moiety and a polymer moiety based on PEG.

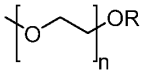
[00523] In one embodiment, a stealth lipid comprises a polymer moiety selected from polymers based on PEG (sometimes referred to as poly(ethylene oxide)), poly(oxazoline), poly(vinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), polyaminoacids and poly[N-(2-hydroxypropyl)methacrylamide].

[00524] In one embodiment, the PEG lipid comprises a polymer moiety based on PEG (sometimes referred to as poly(ethylene oxide)).

[00525] The PEG lipid further comprises a lipid moiety. In some embodiments, the lipid moiety may be derived from diacylglycerol or diacylglycamide, including those comprising a dialkylglycerol or dialkylglycamide group having alkyl chain length independently comprising from about C4 to about C40 saturated or unsaturated carbon atoms, wherein the chain may comprise one or more functional groups such as, for example, an amide or ester. In some embodiments, the alkyl chain length comprises about C10 to C20. The dialkylglycerol or dialkylglycamide group can further comprise one or more substituted alkyl groups. The chain lengths may be symmetrical or assymetric.

[00526] Unless otherwise indicated, the term "PEG" as used herein means any polyethylene glycol or other polyalkylene ether polymer. In one embodiment, PEG is an optionally substituted linear or branched polymer of ethylene glycol or ethylene oxide. In one embodiment, PEG is unsubstituted. In one embodiment, the PEG is substituted, e.g., by one or more alkyl, alkoxy, acyl, hydroxy, or aryl groups. In one embodiment, the term includes PEG copolymers such as PEG-polyurethane or PEG-polypropylene (see, e.g., J. Milton Harris, Poly(ethylene glycol) chemistry: biotechnical and biomedical applications (1992)); in another embodiment, the term does not include PEG copolymers. In one embodiment, the PEG has a molecular weight of from about 130 to about 50,000, in a sub-embodiment, about 150 to about 30,000, in a sub-embodiment, about 150 to about 20,000, in a sub-embodiment about 150 to about 15,000, in a sub-embodiment, about 150 to about

10,000, in a sub-embodiment, about 150 to about 6,000, in a sub-embodiment, about 150 to about 5,000, in a sub-embodiment, about 150 to about 4,000, in a sub-embodiment, about 150 to about 3,000, in a sub-embodiment, about 300 to about 3,000, in a sub-embodiment, about 1,000 to about 3,000, and in a sub-embodiment, about 1,500 to about 2,500.

[00527] In certain embodiments, the PEG (e.g., conjugated to a lipid moiety or lipid, such as a stealth lipid), is a "PEG-2K," also termed "PEG 2000," which has an average molecular weight of about 2,000 daltons. PEG-2K is represented herein by the following formula (I), wherein n is 45, meaning that the number averaged degree of polymerization comprises about 45 subunits 

(I). However, other PEG embodiments known in the art may be used, including, e.g., those where the number-averaged degree of polymerization comprises about 23 subunits (n=23), and/or 68 subunits (n=68). In some embodiments, n may range from about 30 to about 60. In some embodiments, n may range from about 35 to about 55. In some embodiments, n may range from about 40 to about 50. In some embodiments, n may range from about 42 to about 48. In some embodiments, n may be 45. In some embodiments, R may be selected from H, substituted alkyl, and unsubstituted alkyl. In some embodiments, R may be unsubstituted alkyl. In some embodiments, R may be methyl.

[00528] In any of the embodiments described herein, the PEG lipid may be selected from PEG-dilauroylglycerol, PEG-dimyristoylglycerol (PEG-DMG) (catalog # GM-020 from NOF, Tokyo, Japan), PEG-dipalmitoylglycerol, PEG-distearoylglycerol (PEG-DSPE) (catalog # DSPE-020CN, NOF, Tokyo, Japan), PEG-dilaurylglycamide, PEG-dimyristylglycamide, PEG-dipalmitoylglycamide, and PEG-distearoylglycamide, PEG-cholesterol (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl]carbamoyl-[omega]-methyl-poly(ethylene glycol), PEG-DMB (3,4-ditetradecoxylbenzyl-[omega]-methyl-poly(ethylene glycol)ether), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSPE) (cat. #880120C from Avanti Polar Lipids, Alabaster, Alabama, USA), 1,2-distearoyl-sn-glycerol, methoxypolyethylene glycol (PEG2k-DSG; GS-020, NOF Tokyo, Japan), poly(ethylene glycol)-2000-dimethacrylate (PEG2k-DMA), and 1,2-distearyloxypropyl-3-amine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSA). In one embodiment, the PEG lipid may be PEG2k-DMG. In some embodiments, the PEG lipid may be PEG2k-DSG. In one

embodiment, the PEG lipid may be PEG2k-DSPE. In one embodiment, the PEG lipid may be PEG2k-DMA. In one embodiment, the PEG lipid may be PEG2k-C-DMA. In one embodiment, the PEG lipid may be compound S027, disclosed in WO2016/010840 (paragraphs [00240] to [00244]). In one embodiment, the PEG lipid may be PEG2k-DSA. In one embodiment, the PEG lipid may be PEG2k-C11. In some embodiments, the PEG lipid may be PEG2k-C14. In some embodiments, the PEG lipid may be PEG2k-C16. In some embodiments, the PEG lipid may be PEG2k-C18.

[00529] LNP Formulations

[00530] The LNP may contain (i) an amine lipid for encapsulation and for endosomal escape, (ii) a neutral lipid for stabilization, (iii) a helper lipid, also for stabilization, and (iv) a stealth lipid, such as a PEG lipid. The neutral lipid may be omitted.

[00531] In some embodiments, an LNP composition may comprise an RNA component that includes one or more of an RNA-guided DNA-binding agent, a Cas nuclease mRNA, a Class 2 Cas nuclease mRNA, a Cas9 mRNA, and a gRNA. In some embodiments, an LNP composition includes an mRNA encoding a Class 2 Cas nuclease and a gRNA as the RNA component. In certain embodiments, an LNP composition may comprise the RNA component, an amine lipid, a helper lipid, a neutral lipid, and a stealth lipid. In certain LNP compositions, the helper lipid is cholesterol. In other compositions, the neutral lipid is DSPC. In additional embodiments, the stealth lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the LNP composition comprises Lipid A or an equivalent of Lipid A; a helper lipid; a neutral lipid; a stealth lipid; and a guide RNA. In certain compositions, the amine lipid is Lipid A. In certain compositions, the amine lipid is Lipid A or an acetal analog thereof; the helper lipid is cholesterol; the neutral lipid is DSPC; and the stealth lipid is PEG2k-DMG.

[00532] In certain embodiments, lipid compositions are described according to the respective molar ratios of the component lipids in the formulation. Embodiments of the present disclosure provide lipid compositions described according to the respective molar ratios of the component lipids in the formulation. In one embodiment, the mol-% of the amine lipid may be from about 30 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 40 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 45 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 50 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 55 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 50 mol-% to

about 55 mol-%. In one embodiment, the mol-% of the amine lipid may be about 50 mol-%. In one embodiment, the mol-% of the amine lipid may be about 55 mol-%. In some embodiments, the amine lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target mol-%. In some embodiments, the amine lipid mol-% of the LNP batch will be ±4 mol-%, ±3 mol-%, ±2 mol-%, ±1.5 mol-%, ±1 mol-%, ±0.5 mol-%, or ±0.25 mol-% of the target mol-%. All mol-% numbers are given as a fraction of the lipid component of the LNP compositions. In certain embodiments, LNP inter-lot variability of the amine lipid mol-% will be less than 15%, less than 10% or less than 5%.

[00533] In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be from about 5 mol-% to about 15 mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be from about 7 mol-% to about 12 mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be from about 0 mol-% to about 5 mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be from about 0 mol-% to about 10 mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be from about 5 mol-% to about 10 mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be from about 8 mol-% to about 10 mol-%.

[00534] In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be about 5 mol-%, about 6 mol-%, about 7 mol-%, about 8 mol-%, about 9 mol-%, about 10 mol-%, about 11 mol-%, about 12 mol-%, about 13 mol-%, about 14 mol-%, or about 15 mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be about 9 mol-%.

[00535] In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be from about 1 mol-% to about 5 mol-%. In one embodiment, the mol-% of the neutral lipid may be from about 0.1 mol-% to about 1 mol-%. In one embodiment, the mol-% of the neutral lipid such as neutral phospholipid may be about 0.1 mol-%, about 0.2 mol-%, about 0.5 mol-%, 1 mol-%, about 1.5 mol-%, about 2 mol-%, about 2.5 mol-%, about 3 mol-%, about 3.5 mol-%, about 4 mol-%, about 4.5 mol-%, or about 5 mol-%.

[00536] In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be less than about 1 mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be less than about 0.5 mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be about 0 mol-%, about 0.1 mol-%, about 0.2 mol-%, about 0.3 mol-%, about 0.4 mol-%, about 0.5 mol-%, about 0.6 mol-%, about 0.7 mol-%, about 0.8 mol-%, about 0.9 mol-%, or about 1 mol-%. In some embodiments, the

formulations disclosed herein are free of neutral lipid (i.e., 0 mol-% neutral lipid). In some embodiments, the formulations disclosed herein are essentially free of neutral lipid (i.e., about 0 mol-% neutral lipid). In some embodiments, the formulations disclosed herein are free of neutral phospholipid (i.e., 0 mol-% neutral phospholipid). In some embodiments, the formulations disclosed herein are essentially free of neutral phospholipid (i.e., about 0 mol-% neutral phospholipid).

[00537] In some embodiments, the neutral lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target neutral lipid mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[00538] In one embodiment, the mol-% of the helper lipid may be from about 20 mol-% to about 60 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 55 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 50 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 40 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 30 mol-% to about 50 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 30 mol-% to about 40 mol-%. In one embodiment, the mol-% of the helper lipid is adjusted based on amine lipid, neutral lipid, and PEG lipid concentrations to bring the lipid component to 100 mol-%. In one embodiment, the mol-% of the helper lipid is adjusted based on amine lipid and PEG lipid concentrations to bring the lipid component to 100 mol-%. In one embodiment, the mol-% of the helper lipid is adjusted based on amine lipid and PEG lipid concentrations to bring the lipid component to at least 99 mol-%. In some embodiments, the helper mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[00539] In one embodiment, the mol-% of the PEG lipid may be from about 1 mol-% to about 10 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 10 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 8 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 4 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2.5 mol-% to about 4 mol-%. In one embodiment, the mol-% of the PEG lipid may be about 3 mol-%. In one embodiment, the mol-% of the PEG lipid may be about 2.5 mol-%. In some embodiments, the PEG lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target PEG lipid mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[00540] In certain embodiments, the cargo includes a nucleic acid encoding an RNA-guided DNA-binding agent (e.g. a Cas nuclease, a Class 2 Cas nuclease, or Cas9), and a gRNA or a nucleic acid encoding a gRNA, or a combination of mRNA and gRNA. In one embodiment, an LNP composition may comprise a Lipid A or its equivalents. In some aspects, the amine lipid is Lipid A. In some aspects, the amine lipid is a Lipid A equivalent, e.g. an analog of Lipid A. In certain aspects, the amine lipid is an acetal analog of Lipid A. In various embodiments, an LNP composition comprises an amine lipid, a neutral lipid, a helper lipid, and a PEG lipid. In certain embodiments, the helper lipid is cholesterol. In certain embodiments, the neutral lipid is DSPC. In specific embodiments, PEG lipid is PEG2k-DMG. In some embodiments, an LNP composition may comprise a Lipid A, a helper lipid, a neutral lipid, and a PEG lipid. In some embodiments, an LNP composition comprises an amine lipid, DSPC, cholesterol, and a PEG lipid. In some embodiments, the LNP composition comprises a PEG lipid comprising DMG. In certain embodiments, the amine lipid is selected from Lipid A, and an equivalent of Lipid A, including an acetal analog of Lipid A. In additional embodiments, an LNP composition comprises Lipid A, cholesterol, DSPC, and PEG2k-DMG.

[00541] In various embodiments, an LNP composition comprises an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In various embodiments, an LNP composition comprises an amine lipid, a helper lipid, a neutral phospholipid, and a PEG lipid. In various embodiments, an LNP composition comprises a lipid component that consists of an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In various embodiments, an LNP composition comprises an amine lipid, a helper lipid, and a PEG lipid. In certain embodiments, an LNP composition does not comprise a neutral lipid, such as a neutral phospholipid. In various embodiments, an LNP composition comprises a lipid component that consists of an amine lipid, a helper lipid, and a PEG lipid. In certain embodiments, the neutral lipid is chosen from one or more of DSPC, DPPC, DAPC, DMPC, DOPC, DOPE, and DSPE. In certain embodiments, the neutral lipid is DSPC. In certain embodiments, the neutral lipid is DPPC. In certain embodiments, the neutral lipid is DAPC. In certain embodiments, the neutral lipid is DMPC. In certain embodiments, the neutral lipid is DOPC. In certain embodiments, the neutral lipid is DOPE. In certain embodiments, the neutral lipid is DSPE. In certain embodiments, the helper lipid is cholesterol. In specific embodiments, the PEG lipid is PEG2k-DMG. In some embodiments, an LNP composition may comprise a Lipid A, a helper lipid, and a PEG lipid. In some embodiments, an LNP composition may comprise a lipid component that consists of Lipid A, a helper lipid, and a PEG lipid. In some

embodiments, an LNP composition comprises an amine lipid, cholesterol, and a PEG lipid. In some embodiments, an LNP composition comprises a lipid component that consists of an amine lipid, cholesterol, and a PEG lipid. In some embodiments, the LNP composition comprises a PEG lipid comprising DMG. In certain embodiments, the amine lipid is selected from Lipid A and an equivalent of Lipid A, including an acetal analog of Lipid A. In certain embodiments, the amine lipid is a C5-C12 or a C4-C12 acetal analog of Lipid A. In additional embodiments, an LNP composition comprises Lipid A, cholesterol, and PEG2k-DMG.

[00542] Embodiments of the present disclosure also provide lipid compositions described according to the molar ratio between the positively charged amine groups of the amine lipid (N) and the negatively charged phosphate groups (P) of the nucleic acid to be encapsulated. This may be mathematically represented by the equation N/P. In some embodiments, an LNP composition may comprise a lipid component that comprises an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid; and a nucleic acid component, wherein the N/P ratio is about 3 to 10. In some embodiments, an LNP composition may comprise a lipid component that comprises an amine lipid, a helper lipid, and a PEG lipid; and a nucleic acid component, wherein the N/P ratio is about 3 to 10. In some embodiments, an LNP composition may comprise a lipid component that comprises an amine lipid, a helper lipid, a neutral lipid, and a helper lipid; and an RNA component, wherein the N/P ratio is about 3 to 10. In some embodiments, an LNP composition may comprise a lipid component that comprises an amine lipid, a helper lipid, and a PEG lipid; and an RNA component, wherein the N/P ratio is about 3 to 10. In one embodiment, the N/P ratio may be about 5 to 7. In one embodiment, the N/P ration may be about 3 to 7. In one embodiment, the N/P ratio may be about 4.5 to 8. In one embodiment, the N/P ratio may be about 6. In one embodiment, the N/P ratio may be 6 ± 1. In one embodiment, the N/P ratio may be 6 ± 0.5. In some embodiments, the N/P ratio will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target N/P ratio. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[00543] In some embodiments, the RNA component may comprise a nucleic acid, such as a nucleic acid disclosed herein, e.g., encoding a Cas nuclease described herein (such as a Cas9 mRNA described herein), and a gRNA described herein. In some embodiments, the RNA component comprises a Cas nuclease mRNA described herein and a gRNA described herein. In some embodiments, the RNA component comprises a Class 2 Cas nuclease mRNA described herein and a gRNA described herein. In any of the foregoing embodiments, the

gRNA may be an sgRNA described herein, such as a chemically modified sgRNA described herein.

[00544] In certain embodiments, an LNP composition may comprise an RNA component as discussed above, an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In certain LNP compositions, the helper lipid is cholesterol; the neutral lipid is DSPC; and/or the PEG lipid is PEG2k-DMG or PEG2k-C11. In specific compositions, the amine lipid is selected from Lipid A and its equivalents, such as an acetal analog of Lipid A. In one embodiment, the lipid component of the LNP composition consists of an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In one embodiment, the lipid component of the LNP composition consists of an amine lipid, a helper lipid, and a PEG lipid. In certain compositions comprising an mRNA encoding a Cas nuclease and a gRNA, the helper lipid is cholesterol. In some compositions comprising an mRNA encoding a Cas nuclease and a gRNA, the neutral lipid is DSPC. Certain compositions comprising an mRNA encoding a Cas nuclease and a gRNA comprise less than about 1 mol-% neutral lipid, e.g. neutral phospholipid. Certain compositions comprising an mRNA encoding a Cas nuclease and a gRNA comprise less than about 0.5 mol-% neutral lipid, e.g. neutral phospholipid. In certain compositions, the LNP does not comprise a neutral lipid, e.g., neutral phospholipid. In additional embodiments comprising an mRNA encoding a Cas nuclease and a gRNA, the PEG lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the amine lipid is selected from Lipid A and its equivalents, such as acetal analogs of Lipid A.

[00545] In certain embodiments, the LNP compositions include a Cas nuclease mRNA (such as a Class 2 Cas mRNA) described herein and at least one gRNA described herein. In certain embodiments, the LNP composition includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 25:1 to about 1:25. In certain embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 10:1 to about 1:10. In certain embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 8:1 to about 1:8. As measured herein, the ratios are by weight. In some embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas mRNA from about 5:1 to about 1:5. In some embodiments, ratio range is about 3:1 to 1:3, about 2:1 to 1:2, about 5:1 to 1:2, about 5:1 to 1:1, about 3:1 to 1:2, about 3:1 to 1:1, about 3:1, about 2:1 to 1:1. In some embodiments, the gRNA to mRNA ratio is about 3:1 or about 2:1 In some embodiments the ratio of gRNA to Cas nuclease mRNA, such as

Class 2 Cas nuclease is about 1:1. The ratio may be about 25:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10, or 1:25.

[00546] In some embodiments, LNPs are formed by mixing an aqueous RNA solution with an organic solvent-based lipid solution, e.g., 100% ethanol. Suitable solutions or solvents include or may contain: water, PBS, Tris buffer, NaCl, citrate buffer, ethanol, chloroform, diethylether, cyclohexane, tetrahydrofuran, methanol, isopropanol. A pharmaceutically acceptable buffer, e.g., for in vivo administration of LNPs, may be used. In certain embodiments, a buffer is used to maintain the pH of the composition comprising LNPs at or above pH 6.5. In certain embodiments, a buffer is used to maintain the pH of the composition comprising LNPs at or above pH 7.0. In certain embodiments, the composition has a pH ranging from about 7.2 to about 7.7. In additional embodiments, the composition has a pH ranging from about 7.3 to about 7.7 or ranging from about 7.4 to about 7.6. In further embodiments, the composition has a pH of about 7.2, 7.3, 7.4, 7.5, 7.6, or 7.7. The pH of a composition may be measured with a micro pH probe. In certain embodiments, a cryoprotectant is included in the composition. Non-limiting examples of cryoprotectants include sucrose, trehalose, glycerol, DMSO, and ethylene glycol. Exemplary compositions may include up to 10% cryoprotectant, such as, for example, sucrose. In certain embodiments, the LNP composition may include about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10% cryoprotectant. In certain embodiments, the LNP composition may include about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10% sucrose. In some embodiments, the LNP composition may include a buffer. In some embodiments, the buffer may comprise a phosphate buffer (PBS), a Tris buffer, a citrate buffer, and mixtures thereof. In certain exemplary embodiments, the buffer comprises NaCl. In certain emboidments, NaCl is omitted. Exemplary amounts of NaCl may range from about 20 mM to about 45 mM. Exemplary amounts of NaCl may range from about 40 mM to about 50 mM. In some embodiments, the amount of NaCl is about 45 mM. In some embodiments, the buffer is a Tris buffer. Exemplary amounts of Tris may range from about 20 mM to about 60 mM. Exemplary amounts of Tris may range from about 40 mM to about 60 mM. In some embodiments, the amount of Tris is about 50 mM. In some embodiments, the buffer comprises NaCl and Tris. Certain exemplary embodiments of the LNP compositions contain 5% sucrose and 45 mM NaCl in Tris buffer. In other exemplary embodiments, compositions contain sucrose in an amount of about 5% w/v, about 45 mM NaCl, and about 50 mM Tris at pH 7.5. The salt, buffer, and cryoprotectant amounts may be varied such that the osmolality of the overall formulation is maintained. For example, the final osmolality may be maintained at less than 450 mOsm/L. In further embodiments, the

osmolality is between 350 and 250 mOsm/L. Certain embodiments have a final osmolality of 300 +/- 20 mOsm/L.

[00547] In some embodiments, microfluidic mixing, T-mixing, or cross-mixing is used. In certain aspects, flow rates, junction size, junction geometry, junction shape, tube diameter, solutions, and/or RNA and lipid concentrations may be varied. LNPs or LNP compositions may be concentrated or purified, *e.g.*, via dialysis, tangential flow filtration, or chromatography. The LNPs may be stored as a suspension, an emulsion, or a lyophilized powder, for example. In some embodiments, an LNP composition is stored at 2-8° C, in certain aspects, the LNP compositions are stored at room temperature. In additional embodiments, an LNP composition is stored frozen, for example at -20° C or -80° C. In other embodiments, an LNP composition is stored at a temperature ranging from about 0° C to about -80° C. Frozen LNP compositions may be thawed before use, for example on ice, at room temperature, or at 25° C.

[00548] The LNPs may be, *e.g.*, microspheres (including unilamellar and multilamellar vesicles, *e.g.*, "liposomes"—lamellar phase lipid bilayers that, in some embodiments, are substantially spherical—and, in more particular embodiments, can comprise an aqueous core, *e.g.*, comprising a substantial portion of RNA molecules), a dispersed phase in an emulsion, micelles, or an internal phase in a suspension.

[00549] Moreover, the LNP compositions are biodegradable, in that they do not accumulate to cytotoxic levels *in vivo* at a therapeutically effective dose. In some embodiments, the LNP compositions do not cause an innate immune response that leads to substantial adverse effects at a therapeutic dose level. In some embodiments, the LNP compositions provided herein do not cause toxicity at a therapeutic dose level.

[00550] In some embodiments, the pdi may range from about 0.005 to about 0.75. In some embodiments, the pdi may range from about 0.01 to about 0.5. In some embodiments, the pdi may range from about zero to about 0.4. In some embodiments, the pdi may range from about zero to about 0.35. In some embodiments, the pdi may range from about zero to about 0.35. In some embodiments, the pdi may range from about zero to about 0.3. In some embodiments, the pdi may range from about zero to about 0.25. In some embodiments, the pdi may range from about zero to about 0.2. In some embodiments, the pdi may be less than about 0.08, 0.1, 0.15, 0.2, or 0.4.

[00551] The LNPs disclosed herein have a size (e.g., Z-average diameter) of about 1 to about 250 nm. In some embodiments, the LNPs have a size of about 10 to about 200 nm. In further embodiments, the LNPs have a size of about 20 to about 150 nm. In some

embodiments, the LNPs have a size of about 50 to about 150 nm. In some embodiments, the LNPs have a size of about 50 to about 100 nm. In some embodiments, the LNPs have a size of about 50 to about 120 nm. In some embodiments, the LNPs have a size of about 60 to about 100 nm. In some embodiments, the LNPs have a size of about 75 to about 150 nm. In some embodiments, the LNPs have a size of about 75 to about 120 nm. In some embodiments, the LNPs have a size of about 75 to about 100 nm. Unless indicated otherwise, all sizes referred to herein are the average sizes (diameters) of the fully formed nanoparticles, as measured by dynamic light scattering on a Malvern Zetasizer. The nanoparticle sample is diluted in phosphate buffered saline (PBS) so that the count rate is approximately 200-400 kcps. The data is presented as a weighted-average of the intensity measure (Z-average diameter).

[00552] In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from about 50% to about 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from about 50% to about 70%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from about 70% to about 90%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from about 90% to about 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from about 75% to about 95%.

[00553] In some embodiments, the LNPs are formed with an average molecular weight ranging from about 1.00E+05 g/mol to about 1.00E+10 g/mol. In some embodiments, the LNPs are formed with an average molecular weight ranging from about 5.00E+05 g/mol to about 7.00E+07g/mol. In some embodiments, the LNPs are formed with an average molecular weight ranging from about 1.00E+06 g/mol to about 1.00E+10 g/mol. In some embodiments, the LNPs are formed with an average molecular weight ranging from about 1.00E+07 g/mol to about 1.00E+09 g/mol. In some embodiments, the LNPs are formed with an average molecular weight ranging from about 5.00E+06 g/mol to about 5.00E+09 g/mol.

[00554] In some embodiments, the polydispersity (Mw/Mn; the ratio of the weight averaged molar mass (Mw) to the number averaged molar mass (Mn)) may range from about 1.000 to about 2.000. In some embodiments, the Mw/Mn may range from about 1.00 to about 1.500. In some embodiments, the Mw/Mn may range from about 1.020 to about 1.400. In some embodiments, the Mw/Mn may range from about 1.010 to about 1.100. In some embodiments, the Mw/Mn may range from about 1.100 to about 1.350.

[00555] Dynamic Light Scattering ("DLS") can be used to characterize the polydispersity index ("pdi") and size of the LNPs of the present disclosure. DLS measures the scattering of light that results from subjecting a sample to a light source. PDI, as determined from DLS measurements, represents the distribution of particle size (around the mean particle size) in a population, with a perfectly uniform population having a PDI of zero. In some embodiments, the pdi may range from 0.005 to 0.75. In some embodiments, the pdi may range from 0.01 to 0.5. In some embodiments, the pdi may range from 0.02 to 0.4. In some embodiments, the pdi may range from 0.03 to 0.35. In some embodiments, the pdi may range from 0.1 to 0.35.

[00556] In some embodiments, LNPs disclosed herein have a size of 1 to 250 nm. In some embodiments, the LNPs have a size of 10 to 200 nm. In further embodiments, the LNPs have a size of 20 to 150 nm. In some embodiments, the LNPs have a size of 50 to 150 nm. In some embodiments, the LNPs have a size of 50 to 100 nm. In some embodiments, the LNPs have a size of 50 to 120 nm. In some embodiments, the LNPs have a size of 75 to 150 nm. In some embodiments, the LNPs have a size of 30 to 200 nm. Unless indicated otherwise, all sizes referred to herein are the average sizes (diameters) of the fully formed nanoparticles, as measured by dynamic light scattering on a Malvern Zetasizer. The nanoparticle sample is diluted in phosphate buffered saline (PBS) so that the count rate is approximately 200-400 kcts. The data is presented as a weighted-average of the intensity measure. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 50% to 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 50% to 70%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 70% to 90%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 90% to 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 75% to 95%.

[00557] In some embodiments, LNPs associated with the gRNAs disclosed herein and nucleic acids (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein are for use in preparing a medicament for treating ATTR. In some embodiments, LNPs associated with the gRNAs disclosed herein and nucleic acids (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein are for use in preparing a medicament for reducing or preventing accumulation and aggregation of TTR in amyloids or amyloid fibrils in subjects having ATTR. In some embodiments, LNPs associated with the gRNAs disclosed herein and nucleic acids (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein

are for use in preparing a medicament for reducing serum TTR concentration. In some embodiments, LNPs associated with the gRNAs disclosed herein and nucleic acids (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein are for use in treating ATTR in a subject, such as a mammal, e.g., a primate such as a human. In some embodiments, LNPs associated with the gRNAs disclosed herein and nucleic acids (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein are for use in reducing or preventing accumulation and aggregation of TTR in amyloids or amyloid fibrils in subjects having ATTR, such as a mammal, e.g., a primate such as a human. In some embodiments, LNPs associated with the gRNAs disclosed herein and nucleic acids (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein are for use in reducing serum TTR concentration in a subject, such as a mammal, e.g., a primate such as a human.

[00558] Electroporation is also a well-known means for delivery of cargo, and any electroporation methodology may be used for delivery of any one of the gRNAs disclosed herein. In some embodiments, electroporation may be used to deliver any one of the gRNAs disclosed herein and an RNA-guided DNA nuclease such as Cas9 or a nucleic acid encoding an RNA-guided DNA nuclease such as Cas9.

[00559] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein and nucleic acids (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein to an ex vivo cell, wherein the gRNA and nucleic acid are associated with an LNP.

[00560] In certain embodiments, the invention comprises DNA or RNA vectors encoding any of the guide RNAs comprising any one or more of the guide sequences described herein. In some embodiments, in addition to guide RNA sequences, the vectors further comprise nucleic acids that do not encode guide RNAs. Nucleic acids that do not encode guide RNA include, but are not limited to, promoters, enhancers, regulatory sequences, and nucleic acids encoding an RNA-guided DNA nuclease, which can be a nuclease such as Cas9. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, or a crRNA and trRNA. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a sgRNA and a nucleic acid encoding an RNA-guided DNA nuclease, which can be a Cas nuclease, such as Cas9 or Cpf1. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, and a nucleic acid encoding an RNA-guided DNA nuclease, which can be a Cas protein, such as, Cas9. In one embodiment, the Cas9 is from *Streptococcus pyogenes* (i.e., Spy Cas9). In some

embodiments, the nucleotide sequence encoding the crRNA, trRNA, or crRNA and trRNA (which may be a sgRNA) comprises or consists of a guide sequence flanked by all or a portion of a repeat sequence from a naturally-occurring CRISPR/Cas system. The nucleic acid comprising or consisting of the crRNA, trRNA, or crRNA and trRNA may further comprise a vector sequence wherein the vector sequence comprises or consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

[00561] In some embodiments, the crRNA and the trRNA are encoded by non-contiguous nucleic acids within one vector. In other embodiments, the crRNA and the trRNA may be encoded by a contiguous nucleic acid. In some embodiments, the crRNA and the trRNA are encoded by opposite strands of a single nucleic acid. In other embodiments, the crRNA and the trRNA are encoded by the same strand of a single nucleic acid.

[00562] In some embodiments, the vector may be circular. In other embodiments, the vector may be linear. In some embodiments, the vector may be enclosed in a lipid nanoparticle, liposome, non-lipid nanoparticle, or viral capsid. Non-limiting exemplary vectors include plasmids, phagemids, cosmids, artificial chromosomes, minichromosomes, transposons, viral vectors, and expression vectors.

[00563] In some embodiments, the vector may be a viral vector. In some embodiments, the viral vector may be genetically modified from its wild type counterpart. For example, the viral vector may comprise an insertion, deletion, or substitution of one or more nucleotides to facilitate cloning or such that one or more properties of the vector is changed. Such properties may include packaging capacity, transduction efficiency, immunogenicity, genome integration, replication, transcription, and translation. In some embodiments, a portion of the viral genome may be deleted such that the virus is capable of packaging exogenous sequences having a larger size. In some embodiments, the viral vector may have an enhanced transduction efficiency. In some embodiments, the immune response induced by the virus in a host may be reduced. In some embodiments, viral genes (such as, e.g., integrase) that promote integration of the viral sequence into a host genome may be mutated such that the virus becomes non-integrating. In some embodiments, the viral vector may be replication defective. In some embodiments, the viral vector may comprise exogenous transcriptional or translational control sequences to drive expression of coding sequences on the vector. In some embodiments, the virus may be helper-dependent. For example, the virus may need one or more helper virus to supply viral components (such as, e.g., viral proteins) required to amplify and package the vectors into viral particles. In such a case, one or more helper components, including one or more vectors encoding the viral components, may be

introduced into a host cell along with the vector system described herein. In other embodiments, the virus may be helper-free. For example, the virus may be capable of amplifying and packaging the vectors without any helper virus. In some embodiments, the vector system described herein may also encode the viral components required for virus amplification and packaging.

[00564] Non-limiting exemplary viral vectors include adeno-associated virus (AAV) vector, lentivirus vectors, adenovirus vectors, helper dependent adenoviral vectors (HDAd), herpes simplex virus (HSV-1) vectors, bacteriophage T4, baculovirus vectors, and retrovirus vectors. In some embodiments, the viral vector may be an AAV vector. In some embodiments, the viral vector is AAV2, AAV3, AAV3B, AAV5, AAV6, AAV6.2, AAV7, AAVrh.64R1, AAVhu.37, AAVrh.8, AAVrh.32.33, AAV8, AAV9, AAVrh10, or AAVLK03. In other embodiments, the viral vector may a lentivirus vector.

[00565] In some embodiments, the lentivirus may be non-integrating. In some embodiments, the viral vector may be an adenovirus vector. In some embodiments, the adenovirus may be a high-cloning capacity or "gutless" adenovirus, where all coding viral regions apart from the 5' and 3' inverted terminal repeats (ITRs) and the packaging signal ('I') are deleted from the virus to increase its packaging capacity. In yet other embodiments, the viral vector may be an HSV-1 vector. In some embodiments, the HSV-1-based vector is helper dependent, and in other embodiments it is helper independent. For example, an amplicon vector that retains only the packaging sequence requires a helper virus with structural components for packaging, while a 30kb-deleted HSV-1 vector that removes non-essential viral functions does not require helper virus. In additional embodiments, the viral vector may be bacteriophage T4. In some embodiments, the bacteriophage T4 may be able to package any linear or circular DNA or RNA molecules when the head of the virus is emptied. In further embodiments, the viral vector may be a baculovirus vector. In yet further embodiments, the viral vector may be a retrovirus vector. In embodiments using AAV or lentiviral vectors, which have smaller cloning capacity, it may be necessary to use more than one vector to deliver all the components of a vector system as disclosed herein. For example, one AAV vector may contain sequences encoding an RNA-guided DNA nuclease such as a Cas nuclease, while a second AAV vector may contain one or more guide sequences.

[00566] In some embodiments, the vector may be capable of driving expression of one or more coding sequences in a cell. In some embodiments, the cell may be a prokaryotic cell, such as, e.g., a bacterial cell. In some embodiments, the cell may be a eukaryotic cell, such as, e.g., a yeast, plant, insect, or mammalian cell. In some embodiments, the eukaryotic cell

may be a mammalian cell. In some embodiments, the eukaryotic cell may be a rodent cell. In some embodiments, the eukaryotic cell may be a human cell. Suitable promoters to drive expression in different types of cells are known in the art. In some embodiments, the promoter may be wild type. In other embodiments, the promoter may be modified for more efficient or efficacious expression. In yet other embodiments, the promoter may be truncated yet retain its function. For example, the promoter may have a normal size or a reduced size that is suitable for proper packaging of the vector into a virus.

[00567] In some embodiments, the vector may comprise a nucleotide sequence encoding an RNA-guided DNA nuclease such as a nuclease described herein. In some embodiments, the nuclease encoded by the vector may be a Cas protein. In some embodiments, the vector system may comprise one copy of the nucleotide sequence encoding the nuclease. In other embodiments, the vector system may comprise more than one copy of the nucleotide sequence encoding the nuclease. In some embodiments, the nucleotide sequence encoding the nuclease may be operably linked to at least one transcriptional or translational control sequence. In some embodiments, the nucleotide sequence encoding the nuclease may be operably linked to at least one promoter.

[00568] In some embodiments, the promoter may be constitutive, inducible, or tissue-specific. In some embodiments, the promoter may be a constitutive promoter. Non-limiting exemplary constitutive promoters include cytomegalovirus immediate early promoter (CMV), simian virus (SV40) promoter, adenovirus major late (MLP) promoter, Rous sarcoma virus (RSV) promoter, mouse mammary tumor virus (MMTV) promoter, phosphoglycerate kinase (PGK) promoter, elongation factor-alpha (EF1a) promoter, ubiquitin promoters, actin promoters, tubulin promoters, immunoglobulin promoters, a functional fragment thereof, or a combination of any of the foregoing. In some embodiments, the promoter may be a CMV promoter. In some embodiments, the promoter may be a truncated CMV promoter. In other embodiments, the promoter may be an EF1a promoter. In some embodiments, the promoter may be an inducible promoter. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech).

[00569] In some embodiments, the promoter may be a tissue-specific promoter, e.g., a promoter specific for expression in the liver.

[00570] The vector may further comprise a nucleotide sequence encoding the guide RNA described herein. In some embodiments, the vector comprises one copy of the guide RNA. In

other embodiments, the vector comprises more than one copy of the guide RNA. In embodiments with more than one guide RNA, the guide RNAs may be non-identical such that they target different target sequences, or may be identical in that they target the same target sequence. In some embodiments where the vectors comprise more than one guide RNA, each guide RNA may have other different properties, such as activity or stability within a complex with an RNA-guided DNA nuclease, such as a Cas RNP complex. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to at least one transcriptional or translational control sequence, such as a promoter, a 3' UTR, or a 5' UTR. In one embodiment, the promoter may be a tRNA promoter, *e.g.*, tRNALys3, or a tRNA chimera. See Mefferd et al., RNA. 2015 21:1683-9; Scherer et al., Nucleic Acids Res. 2007 35: 2620–2628. In some embodiments, the promoter may be recognized by RNA polymerase III (Pol III). Non-limiting examples of Pol III promoters include U6 and H1 promoters. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human U6 promoter. In other embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human H1 promoter. In embodiments with more than one guide RNA, the promoters used to drive expression may be the same or different. In some embodiments, the nucleotide encoding the crRNA of the guide RNA and the nucleotide encoding the trRNA of the guide RNA may be provided on the same vector. In some embodiments, the nucleotide encoding the crRNA and the nucleotide encoding the trRNA may be driven by the same promoter. In some embodiments, the crRNA and trRNA may be transcribed into a single transcript. For example, the crRNA and trRNA may be processed from the single transcript to form a double-molecule guide RNA. Alternatively, the crRNA and trRNA may be transcribed into a single-molecule guide RNA (sgRNA). In other embodiments, the crRNA and the trRNA may be driven by their corresponding promoters on the same vector. In yet other embodiments, the crRNA and the trRNA may be encoded by different vectors.

[00571] In some embodiments, the nucleotide sequence encoding the guide RNA may be located on the same vector comprising the nucleotide sequence encoding an RNA-guided DNA nuclease such as a Cas nuclease. In some embodiments, expression of the guide RNA and of the RNA-guided DNA nuclease such as a Cas protein may be driven by their own corresponding promoters. In some embodiments, expression of the guide RNA may be driven by the same promoter that drives expression of the RNA-guided DNA nuclease such as a Cas protein. In some embodiments, the guide RNA and the RNA-guided DNA nuclease such as a Cas protein transcript may be contained within a single transcript. For example, the guide

RNA may be within an untranslated region (UTR) of the RNA-guided DNA nuclease such as a Cas protein transcript. In some embodiments, the guide RNA may be within the 5' UTR of the transcript. In other embodiments, the guide RNA may be within the 3' UTR of the transcript. In some embodiments, the intracellular half-life of the transcript may be reduced by containing the guide RNA within its 3' UTR and thereby shortening the length of its 3' UTR. In additional embodiments, the guide RNA may be within an intron of the transcript. In some embodiments, suitable splice sites may be added at the intron within which the guide RNA is located such that the guide RNA is properly spliced out of the transcript. In some embodiments, expression of the RNA-guided DNA nuclease such as a Cas protein and the guide RNA from the same vector in close temporal proximity may facilitate more efficient formation of the CRISPR RNP complex.

[00572] In some embodiments, the compositions comprise a vector system. In some embodiments, the vector system may comprise one single vector. In other embodiments, the vector system may comprise two vectors. In additional embodiments, the vector system may comprise three vectors. When different guide RNAs are used for multiplexing, or when multiple copies of the guide RNA are used, the vector system may comprise more than three vectors.

[00573] In some embodiments, the vector system may comprise inducible promoters to start expression only after it is delivered to a target cell. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech). In additional embodiments, the vector system may comprise tissue-specific promoters to start expression only after it is delivered into a specific tissue.

The vector may be delivered by liposome, a nanoparticle, an exosome, or a microvesicle. The vector may also be delivered by a lipid nanoparticle (LNP); see e.g., WO2017/173054, published October 5, 2017, and entitled "LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS," and WO2019067992A1 published April 4, 2019, entitled "FORMULATIONS," the contents of each of which are hereby incorporated by reference in their entirety. Any of the LNPs and LNP formulations described herein are suitable for delivery of the guides alone or together a cas nuclease or a nucleic acid encoding a cas nuclease. In some embodiments, an LNP composition is encompassed comprising: an RNA component and a lipid component, wherein the lipid component comprises an amine lipid, a neutral lipid, a helper lipid, and a stealth lipid; and wherein the N/P ratio is about 1-10.

In some instances, the the lipid component comprises Lipid A or its acetal analog, cholesterol, DSPC, and PEG-DMG; and wherein the N/P ratio is about 1-10. In some embodiments, the lipid component comprises: about 40-60 mol-% amine lipid; about 5-15 mol-% neutral lipid; and about 1.5-10 mol-% PEG lipid, wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-10. In some embodiments, the lipid component comprises about 50-60 mol-% amine lipid; about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% PEG lipid, wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-8. In some instances, the lipid component comprises: about 50-60 mol-% amine lipid; about 5-15 mol-% DSPC; and about 2.5-4 mol-% PEG lipid, wherein the remainder of the lipid component is cholesterol, and wherein the N/P ratio of the LNP composition is about 3-8. In some instances, the lipid component comprises: 48-53 mol-% Lipid A; about 8-10 mol-% DSPC; and 1.5-10 mol-% PEG lipid, wherein the remainder of the lipid component is cholesterol, and wherein the N/P ratio of the LNP composition is 3-8 ±0.2.

In some embodiments, the LNP comprises a lipid component and the lipid component comprises, consists essentially of, or consists of: about 50 mol-% amine lipid such as Lipid A; about 9 mol-% neutral lipid such as DSPC; about 3 mol-% of a stealth lipid such as a PEG lipid, such as PEG2k-DMG, and the remainder of the lipid component is helper lipid such as cholesterol, wherein the N/P ratio of the LNP composition is about 6. In some embodiments, the amine lipid is Lipid A. In some embodiemnts, the neutral lipid is DSPC. In some embodiments, the stealth lipid is a PEG lipid. In some embodiments, the stealth lipid is a PEG2k-DMG. In some embodiments, the helper lipid is cholesterol. In some embodiments, the LNP comprises a lipid component and the lipid component comprises: about 50 mol-% Lipid A; about 9 mol-% DSPC; about 3 mol-% of PEG2k-DMG, and the remainder of the lipid component is cholesterol wherein the N/P ratio of the LNP composition is about 6.

[00574] In some embodiments, the vector may be delivered systemically. In some embodiments, the vector may be delivered into the hepatic circulation.

[00575] This description and exemplary embodiments should not be taken as limiting. For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities, percentages, or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term "about," to the extent they are not already so modified. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be

obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[00576] It is noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the," and any singular use of any word, include plural referents unless expressly and unequivocally limited to one referent. As used herein, the term "include" and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

EXAMPLES

[00577] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

General Reagents and Methods

[00578] Unless otherwise indicated, mRNA was synthesized by *in vitro* transcription (IVT) using a linearized plasmid DNA template and T7 RNA polymerase. Transcription was generally performed from constructs comprising a T7 Promoter, a transcript sequence disclosed herein such as SEQ ID NO: 243 (which encodes the RNA ORF of SEQ ID NO: 204) and a poly-A tail (SEQ ID NO: 263) encoded in the plasmid.

[00579] For all methods, the transcript concentration was determined by measuring the light absorbance at 260 nm (Nanodrop), and the transcript was analyzed by capillary electrophoresis by Bioanlayzer (Agilent).

[00580] **LNP Formulation**

[00581] The lipid components were dissolved in 100% ethanol with the lipid component molar ratios described below. The chemically modified sgRNA and Cas9 mRNA were combined and dissolved in 25 mM citrate, 100 mM NaCl, pH 5.0, resulting in a concentration of total RNA cargo of approximately 0.45 mg/mL. The LNPs were formulated with an N/P ratio of about 6, with the ratio of chemically modified sgRNA: Cas9 mRNA at a 1:2 w/w ratio as described below. Unless otherwise indicated, LNPs were formulated with 50% Lipid A, 9% DSPC, 38% cholesterol, and 3% PEG2k-DMG.

[00582] The LNPs were formed by an impinging jet mixing of the lipid in ethanol with two volumes of RNA solution and one volume of water. The lipid in ethanol is mixed through a mixing cross with the two volumes of RNA solution. A fourth stream of water is mixed with the outlet stream of the cross through an inline tee. (See, e.g., WO2016010840,

Fig. 2.) A 2:1 ratio of aqueous to organic solvent was maintained during mixing using differential flow rates. The LNPs were held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v). Diluted LNPs were concentrated using tangential flow filtration on a flat sheet cartridge (Sartorius, 100kD MWCO) and then buffer exchanged by diafiltration into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS). The resulting mixture was then filtered using a 0.2 μm sterile filter. The final LNP was stored at 4°C or -80°C until further use.

[00583] **LNP Composition Analytics**

[00584] Dynamic Light Scattering ("DLS") is used to characterize the polydispersity index ("pdi") and size of the LNPs of the present disclosure. DLS measures the scattering of light that results from subjecting a sample to a light source. PDI, as determined from DLS measurements, represents the distribution of particle size (around the mean particle size) in a population, with a perfectly uniform population having a PDI of zero.

[00585] Electropheretic light scattering is used to characterize the surface charge of the LNP at a specified pH. The surface charge, or the zeta potential, is a measure of the magnitude of electrostatic repulsion/attraction between particles in the LNP suspension.

[00586] Asymetric-Flow Field Flow Fractionation – Multi-Angle Light Scattering (AF4-MALS) is used to separate particles in the composition by hydrodynamic radius and then measure the molecular weights, hydrodynamic radii and root mean square radii of the fractionated particles. This allows the ability to assess molecular weight and size distributions as well as secondary characteristics such as the Burchard-Stockmeyer Plot (ratio of root mean square ("rms") radius to hydrodynamic radius over time suggesting the internal core density of a particle) and the rms conformation plot (log of rms radius vs log of molecular weight where the slope of the resulting linear fit gives a degree of compactness vs elongation).

[00587] Nanoparticle tracking analysis (NTA, Malvern Nanosight) can be used to determine particle size distribution as well as particle concentration. LNP samples are diluted appropriately and injected onto a microscope slide. A camera records the scattered light as the particles are slowly infused through field of view. After the movie is captured, the Nanoparticle Tracking Analysis processes the movie by tracking pixels and calculating a diffusion coefficient. This diffusion coefficient can be translated into the hydrodynamic radius of the particle. The instrument also counts the number of individual particles counted in the analysis to give particle concentration.

[00588] Cryo-electron microscopy ("cryo-EM") can be used to determine the particle size, morphology, and structural characteristics of an LNP.

[00589] Lipid compositional analysis of the LNPs can be determined from liquid chromotography followed by charged aerosol detection (LC-CAD). This analysis can provide a comparison of the actual lipid content versus the theoretical lipid content.

[00590] LNP compositions are analyzed for average particle size, polydispersity index (pdi), total RNA content, encapsulation efficiency of RNA, and zeta potential. LNP compositions may be further characterized by lipid analysis, AF4-MALS, NTA, and/or cryo-EM. Average particle size and polydispersity are measured by dynamic light scattering (DLS) using a Malvern Zetasizer DLS instrument. LNP samples were diluted with PBS buffer prior to being measured by DLS. Z-average diameter which is an intensity-based measurement of average particle size is reported along with number average diameter and pdi. A Malvern Zetasizer instrument is also used to measure the zeta potential of the LNP. Samples are diluted 1:17 (50 µL into 800 µL) in 0.1X PBS, pH 7.4 prior to measurement.

[00591] A fluorescence-based assay (Ribogreen®, ThermoFisher Scientific) is used to determine total RNA concentration and free RNA. Encapsulation efficiency is calculated as (Total RNA - Free RNA)/Total RNA. LNP samples are diluted appropriately with 1x TE buffer containing 0.2% Triton-X 100 to determine total RNA or 1x TE buffer to determine free RNA. Standard curves are prepared by utilizing the starting RNA solution used to make the compositions and diluted in 1x TE buffer +/- 0.2% Triton-X 100. Diluted RiboGreen® dye (according to the manufacturer's instructions) is then added to each of the standards and samples and allowed to incubate for approximately 10 minutes at room temperature, in the absence of light. A SpectraMax M5 Microplate Reader (Molecular Devices) is used to read the samples with excitation, auto cutoff and emission wavelengths set to 488 nm, 515 nm, and 525 nm respectively. Total RNA and free RNA are determined from the appropriate standard curves.

[00592] Encapsulation efficiency is calculated as (Total RNA - Free RNA)/Total RNA. The same procedure may be used for determining the encapsulation efficiency of a DNA-based cargo component. In a fluorescence-based assay, for single-strand DNA Oligreen Dye may be used, and for double-strand DNA, Picogreen Dye. Alternatively, the total RNA concentration can be determined by a reverse-phase ion-pairing (RP-IP) HPLC method. Triton X-100 is used to disrupt the LNPs, releasing the RNA. The RNA is then separated from the lipid components chromatographically by RP-IP HPLC and quantified against a standard curve using UV absorbance at 260 nm.

[00593] AF4-MALS is used to look at molecular weight and size distributions as well as secondary statistics from those calculations. LNPs are diluted as appropriate and injected into

a AF4 separation channel using an HPLC autosampler where they are focused and then eluted with an exponential gradient in cross flow across the channel. All fluid is driven by an HPLC pump and Wyatt Eclipse Instrument. Particles eluting from the AF4 channel flow through a UV detector, multi-angle light scattering detector, quasi-elastic light scattering detector and differential refractive index detector. Raw data is processed by using a Debeye model to determine molecular weight and rms radius from the detector signals.

[00594] Lipid components in LNPs are analyzed quantitatively by HPLC coupled to a charged aerosol detector (CAD). Chromatographic separation of 4 lipid components is achieved by reverse phase HPLC. CAD is a destructive mass-based detector which detects all non-volatile compounds and the signal is consistent regardless of analyte structure.

[00595] **Cas9 mRNA and gRNA Cargos**

[00596] The Cas9 mRNA cargo was prepared by *in vitro* transcription. Capped and polyadenylated Cas9 mRNA was generated by *in vitro* transcription using a linearized plasmid DNA template and T7 RNA polymerase using a method as follows. Plasmid DNA containing a T7 promoter and a 90-100 nt poly(A/T) region is linearized by incubating at 37 °C for 2 hours with XbaI with the following conditions: 200 ng/μL plasmid, 2 U/μL XbaI (NEB), and 1x reaction buffer. The XbaI is inactivated by heating the reaction at 65 °C for 20 min. The linearized plasmid is purified from enzyme and buffer. The IVT reaction to generate Cas9 modified mRNA is performed by incubating at 37 °C for 1.5-4 hours in the following conditions: 50 ng/μL linearized plasmid; 2-5 mM each of GTP, ATP, CTP, and N1-methyl pseudo-UTP (Trilink); 10-25 mM ARCA (Trilink); 5 U/μL T7 RNA polymerase (NEB); 1 U/μL Murine RNase inhibitor (NEB); 0.004 U/μL Inorganic *E. coli* pyrophosphatase (NEB); and 1x reaction buffer. TURBO DNase (ThermoFisher) is added to a final concentration of 0.01 U/μL, and the reaction is incubated for an additional 30 minutes to remove the DNA template. The Cas9 mRNA was purified with TFF and/or an LiCl precipitation-containing method.

[00597] The sgRNAs in the following examples were chemically synthesized by known methods using phosphoramidites.

[00598] **Cas9 mRNA and guide RNA delivery *in vitro* by LNP**

[00599] Primary human liver hepatocytes (PHH) (Gibco) and primary cynomolgus liver hepatocytes (PCH) (InVitro Admet Laborotories) were thawed and resuspended in hepatocyte thawing medium with supplements (Gibco, Cat. CM7500) followed by centrifugation. The supernatant was discarded and the pelleted cells resuspended in hepatocyte plating medium plus supplement pack (Invitrogen, Cat. A1217601 and CM3000). Cells were counted and

plated on Bio-coat collagen I coated 96-well plates (ThermoFisher, Cat. 877272) at a density of 33,000 cells/well for PHH and 50,000 cells/well for PCH. Plated cells were allowed to settle and adhere for 5 hours in a tissue culture incubator at 37°C and 5% CO_2 atmosphere. After incubation cells were checked for monolayer formation and were washed once with hepatocyte culture medium (Invitrogen, Cat. A1217601 and CM4000).

[00600] PHH and PCH were treated with LNPs as further described below. Cells were incubated at 37°C, 5% CO_2 for 24 hours prior to treatment with LNPs. LNPs were incubated in media containing 3% cynomolgus serum or 3% fetal bovine serum at 37°C for 5 minutes and administered to cells in amounts as further provided herein.

[00601] **Genomic DNA isolation**

[00602] Treated cells were harvested post-treatment at 72 hours. The cells were lysed from each well of a 96-well plate using 50 µL/well Quick Extract DNA Extraction solution (Epicentre, Cat. QE09050) according to manufacturer's protocol. All DNA samples were subjected to PCR and subsequent NGS analysis, as described herein.

[00603] **NGS Sequencing**

[00604] In brief, to quantitatively determine the efficiency of editing at the target location in the genome, genomic DNA was isolated and deep sequencing was utilized to identify the presence of insertions and deletions introduced by gene editing.

[00605] PCR primers were designed around the target site (e.g., TTR), and the genomic area of interest was amplified. Additional PCR was performed according to the manufacturer's protocols (Illumina) to add the necessary chemistry for sequencing. The amplicons were sequenced on an Illumina MiSeq instrument. The reads were aligned to the appropriate reference genome (e.g., hg38, macFas5) after eliminating those having low quality scores. The resulting files containing the reads were mapped to the reference genome (BAM files), where reads that overlapped the target region of interest were selected and the number of wild-type reads versus the number of reads which contain an insertion, substitution, or deletion was calculated.

[00606] The editing percentage (e.g., the “editing efficiency” or “percent editing”) is defined as the total number of sequence reads with insertions or deletions over the total number of sequence reads, including wild type.

Example 1 – Dose response with LNP delivery of Cas9 mRNAs in primary hepatocytes

[00607] The efficacy of various guide and mRNA combinations was evaluated *in vitro* in primary cyno hepatocytes (PCH) and primary human hepatocytes (PHH). PHH and PCH

cells were plated as described above and then treated with various doses of LNP as described in Tables 8 and 9, respectively. The LNPs contained G000502 (SEQ ID No: 114) and either mRNA000001 (SEQ ID No: 1) or mRNA000042 (SEQ ID No: 377) as mRNA. Cells were lysed after 72 hrs and % editing was determined by NGS. Each condition was assayed in triplicate and Tables 8 and 9 show the mean and standard deviation for % Edit.

Table 8. *In vitro* editing using LNP delivery in PHHs

Guide ID	mRNA ID	Dose Cas9 mRNA (ng)	Mean % Edit	SD
G000502	mRNA01	50	46.1	3.97
G000502	mRNA01	10	32.37	2.64
G000502	mRNA01	5	24.87	0.06
G000502	mRNA01	1	5.2	0.75
G000502	mRNA01	0.01	0.43	0.12
G000502	mRNA01	0	0.23	0.15
G000502	mRNA42	50	42.27	1.8
G000502	mRNA42	10	41.67	0.81
G000502	mRNA42	5	32.7	2.93
G000502	mRNA42	1	10.13	1.62
G000502	mRNA42	0.01	0.8	0
G000502	mRNA42	0	0.13	0.06

Table 9. *In vitro* editing using LNP delivery in PCHs

Guide ID	mRNA ID	Dose Cas9 mRNA (ng)	Mean %Indel	SD
G000502	mRNA01	50	71.8	1.99
G000502	mRNA01	10	33.23	2.32
G000502	mRNA01	5	15.23	1.36
G000502	mRNA01	1	1.27	0.12
G000502	mRNA01	0.01	0.1	0
G000502	mRNA01	0	0.1	0
G000502	mRNA42	50	82.8	1.77
G000502	mRNA42	10	59.5	1.1
G000502	mRNA42	5	38.37	1
G000502	mRNA42	1	5.6	0.4
G000502	mRNA42	0.01	0.3	0.1
G000502	mRNA42	0	0.1	0

Example 2 – Dose response with LNP delivery of Cas9 mRNAs in primary hepatocytes

[00608] Various guide and mRNA combinations were evaluated *in vitro* in PCH and PHH cells. PHH cells from two donors and PCH cells were plated as described above and treated

with doses of LNP as described in Tables 10 and 11, respectively. The LNPs contained Cas9 mRNA000042 (SEQ ID No: 377) and one of 4 single guide RNAs. Guides G000480 and G000486 (SEQ ID Nos: 87 and 93) perfectly target to the human TTR gene. Guide G000502 (SEQ ID No: 114) targets the human TTR gene with a single mismatch in the targeting region. G000739 (SEQ ID No: 2) is a negative control. Cells were lysed after 72 hrs and % editing was determined by NGS. Each condition was assayed in triplicate. Table 10 and FIG. 1 show % Editing results in PHH cells. Table 11 and FIG. 2 show % Editing results in PCH cells.

Table 10 – *In vitro* editing in PHH cells

Guide	Guide concentration (nM)	PHH – Donor 1		PHH – Donor 2	
		Mean % Editing	Std. Dev	Mean % Editing	Std. Dev
G000480	15.51724	90.97	0.91	90.33	1.71
G000480	5.172414	92.23	0.85	87.57	3.63
G000480	1.724138	90.83	0.68	86.37	1.50
G000480	0.574713	85.37	1.86	80.53	1.97
G000480	0.191571	73.53	0.91	62.37	5.98
G000480	0.063857	51.57	0.40	32.80	3.10
G000480	0.021286	25.37	1.48	9.07	0.67
G000480	0.007095	7.23	0.40	1.97	0.57
G000486	15.51724	79.07	0.74	86.73	1.63
G000486	5.172414	82.83	1.69	88.03	2.69
G000486	1.724138	71.87	0.67	81.43	1.68
G000486	0.574713	54.33	0.32	66.90	2.10
G000486	0.191571	33.70	1.06	41.70	2.52
G000486	0.063857	16.67	0.67	17.33	1.29
G000486	0.021286	5.10	0.26	4.27	0.76
G000486	0.007095	1.33	0.06	1.37	0.15
G000502	15.51724	68.93	0.90	78.20	0.17
G000502	5.172414	72.90	0.53	80.73	1.70
G000502	1.724138	53.83	0.71	65.13	0.71
G000502	0.574713	31.23	1.33	37.53	0.84
G000502	0.191571	14.17	0.98	17.70	1.71
G000502	0.063857	5.03	0.32	5.27	0.12
G000502	0.021286	1.57	0.31	1.13	0.35
G000502	0.007095	0.33	0.06	0.33	0.06
G000739	15.51724	0.47	0.06	0.20	0.10
G000739	5.172414	0.40	0.10	0.20	0.10

G000739	1.724138	0.13	0.06	0.07	0.06
G000739	0.574713	0.10	0.00	0.07	0.06
G000739	0.191571	0.07	0.06	0.10	0.00
G000739	0.063857	0.07	0.06	0.07	0.06
G000739	0.021286	0.03	0.06	0.07	0.06
G000739	0.007095	0.10	0.00	0.03	0.06

Table 11 – *In vitro* editing in PCH cells

Guide	Guide concentration (nM)	Mean % Editing	Std. Dev
G000480	15.51724	8.87	1.29
G000480	5.172414	4.43	0.71
G000480	1.724138	0.80	0.20
G000480	0.574713	0.23	0.12
G000480	0.191571	0.23	0.06
G000480	0.063857	0.10	0.00
G000480	0.021286	0.10	0.00
G000480	0.007095	0.10	0.00
G000486	15.51724	73.10	2.00
G000486	5.172414	58.27	0.47
G000486	1.724138	23.23	1.40
G000486	0.574713	5.93	0.25
G000486	0.191571	1.43	0.21
G000486	0.063857	0.53	0.15
G000486	0.021286	0.40	0.17
G000486	0.007095	0.47	0.12
G000502	15.51724	88.10	0.53
G000502	5.172414	83.53	0.55
G000502	1.724138	73.07	2.68
G000502	0.574713	47.87	1.38
G000502	0.191571	18.37	0.86
G000502	0.063857	4.80	0.26
G000502	0.021286	1.27	0.50
G000502	0.007095	0.57	0.12
G000739	15.51724	0.10	0.00
G000739	5.172414	0.13	0.06
G000739	1.724138	0.13	0.06
G000739	0.574713	0.10	0.00
G000739	0.191571	0.10	0.00

G000739	0.063857	0.10	0.00
G000739	0.021286	0.10	0.00
G000739	0.007095	0.10	0.00

Example 3 - Characterization of additional mRNAs

[00609] Materials and methods for this example were as described in International Patent Application No. PCT/US2018/053439, filed September 28, 2018. Cas9 sequences using different codon schemes were designed to test for improved protein expression. Each sequence was designed to encode the Cas9 amino acid of SEQ ID No: 203 using a distinct set of codons. In each open reading frame sequence, a single codon was used to encode each amino acid. Sequences vary based on the frequency with which codons occur in complete protein coding genes in *Homo sapiens* based on the NCBI-GenBank Flat File Release 160.0 (Nakamura et al. (2000) *Nucl. Acids Res.* 28, 292; Benson et al. (2006) *Nucleic Acids Res.* 34(Database issue), D16-20) and the abundance of a particular nucleotide among the codons. Based on the codon schemes shown in Table 5, several different open reading frames for Cas9 (SEQ ID No: 252, 311, and 312) were constructed that encode Cas9 protein of SEQ ID NO: 203. These were incorporated into constructs also containing the HSD 5' UTR (SEQ ID NO: 241), an albumin 3' UTR, a T7 promoter and a polyA tail. An exemplary sequence containing the albumin 3' UTR and polyA tail is SEQ ID NO: 253, in which the 3' UTR and polyA tail follow the HSD 5' UTR and the ORF of SEQ ID NO: 252.

[00610] Messenger RNA was produced for each construct by IVT using 100% N1-methyl pseudouridine in place of uridine. HepG2 cells were transfected with 800 ng of each Cas9 mRNA using Lipofectamine™ MessengerMAX™ Transfection Reagent (ThermoFisher). Six hours post transfection, cells were lysed by freeze thaw and cleared by centrifugation. Cas9 protein levels were determined by ELISA assay. Briefly, total protein concentration was determined by bicinchoninic acid assay. An MSD GOLD 96-well Streptavidin SECTOR Plate (Meso Scale Diagnostics, Cat. L15SA-1) was prepared according to manufacturer's protocol using Cas9 mouse antibody (Origene, Cat. CF811179) as the capture antibody and Cas9 (7A9-3A3) Mouse mAb (Cell Signaling Technology, Cat. 14697) as the detection antibody. Recombinant Cas9 protein was used as a calibration standard in Diluent 39 (Meso Scale Diagnostics) with 1X Halt™ Protease Inhibitor Cocktail, EDTA-Free (ThermoFisher, Cat. 78437). ELISA plates were read using the Meso Quickplex SQ120 instrument (Meso Scale Discovery) and data was analyzed with Discovery Workbench 4.0 software package (Meso Scale Discovery).

[00611] Editing efficiency was assessed *in vitro* by transfecting mRNA together with a guide (G502; SEQ ID NO: 114) targeting transthyretin (*TTR*) into HepG2 cells and measuring percentage editing. Cas9 mRNAs comprising SEQ ID Nos indicated in Table 12 were assessed at concentrations of mRNA from 3 ng-100 ng. Untreated cells did not show measurable editing. Table 12 shows the effects of the different codon sets on Cas9 protein expression and editing *in vitro*.

Table 12. In vitro editing and expression of ORFs with different codon sets

ORF (codon set)	ng Cas9/mg total protein	ng Cas9/mg total protein Standard Deviation	% Editing (30 ng mRNA transfected)	Editing Standard Deviation
SEQ ID No: 252 (Table 4 low U 1)	31.23	4.47	22.2	2.83
SEQ ID No: 311 (Table 4 low A)	74.62	15.53	41.3	3.56
SEQ ID No: 312 (Table 4 low A/U)	77.32	10.60	34.8	7.32

[00612] To determine the effectiveness of the codon schemes *in vivo*, Cas9 protein expression was measured when expressed *in vivo* from mRNAs encoding Cas9 using codon schemes described in Table 4. Messenger RNAs as indicated in Table 26 were formulated as LNPs with a guide RNA targeting TTR (G282; SEQ ID NO: 242). The LNPs were assembled using the cross flow procedure and contained 50% Lipid A, 9% DSPC, 38% cholesterol, and 3% PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had a N:P ratio of 6.0. LNPs were purified using Amicon PD-10 filters (GE Healthcare) and used at a concentration of 0.32 mg/ml (LNP concentration). CD-1 female mice (n=5 per group) were dosed i.v. at 1 mpk. At 3 hours post-dose, animals were sacrificed, the liver was collected and Cas9 expression in liver were measured. Cas9 protein expression was measured in the liver using the Meso Scale Discovery ELISA assay described above. Approximately 40-50 mg liver tissue was homogenized by bead mill in RIPA Buffer (Boston Bioproducts BP-115) with 1x Complete Protease Inhibitor Tablet (Roche, Cat.11836170001). Table 13 shows Cas9 expression results in liver. mRNAs for the low A and low A/U codon schemes (ORFs of SEQ ID NOs: 311 and 312) showed the highest Cas9 expression of the tested ORFs. Cas9 protein expression of the negative control was below the lower limit of quantitation (LLOQ).

Table 13

ORF	Average Cas9 (ng/g liver)	Standard Deviation
TSS	< LLOQ	0.0
SEQ ID No: 204	1644	1172
SEQ ID NO: 252	1562	951
SEQ ID NO: 311	2630	730
SEQ ID NO: 312	2134	362

[00613] To determine the effectiveness of the codon schemes in vivo, genome editing was measured in vivo from mRNAs encoding Cas9 using different codon schemes. Messenger RNAs as indicated in Table 27 were formulated as LNPs with a guide RNA targeting TTR (G282; SEQ ID NO: 242). The LNPs were assembled using the cross flow procedure and contained 50% Lipid A, 9% DSPC, 38% cholesterol, and 3% PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had a N:P ratio of 6.0. LNPs were purified using Amicon PD-10 filters (GE Healthcare), and used at a concentration of 0.05 mg/ml (LNP concentration). CD-1 female mice (n=5 per group, except n=4 for the group treated with SEQ ID NO: 252) were dosed i.v. at 0.1 mpk. At 6 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured. Table 14 shows in vivo editing results and serum TTR levels.

Table 14

ORF	Avg % Editing	Editing Standard Deviation	Serum TTR (µg/ml)	Serum TTR Standard Deviation	n
TSS	0.06	0.05	856	68	5
SEQ ID No: 204	40.96	8.41	329	143	5
SEQ ID No: 252	60.10	8.07	143	78	4
SEQ ID No: 311	57.26	4.15	216	62	5
SEQ ID No: 312	61.44	4.50	100	79	5

[00614] To determine the efficacy of the codon schemes at different mRNA concentrations, an *in vivo* dose response experiment was performed. Messenger RNAs as indicated in Table 15 were formulated as LNPs with a guide RNA targeting TTR (G282;

SEQ ID NO: 242). The LNPs were assembled using the cross flow method and contained 50% Lipid A, 9% DSPC, 38% cholesterol, and 3% PEG2k-DMG. LNPs were purified using Amicon PD-10 filters (GE Healthcare and used at a concentration of 0.7 mg/ml (LNP concentration). CD-1 female mice (n=5 per group) were dosed i.v. at 0.03, 0.1, or 0.3 mpk. At 7 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured. Table 14 shows in vivo editing results and serum TTR levels.

Table 15

ORF	Dose (mpk)	Liver editing (%)	Serum TTR (ug/mL)	Serum TTR (%KD)
TSS	n/a	0.1	576.8	0.0
SEQ ID No: 204	0.3	51.3	165.6	71.3
	0.1	17.3	540.7	6.3
	0.03	1.9	761.4	-32.0
SEQ ID No: 252	0.3	57.0	100.8	82.5
	0.1	29.6	336.1	41.7
	0.03	5.0	636.4	-10.3
SEQ ID NO: 311	0.3	59.4	93.8	83.7
	0.1	30.6	373.5	35.2
	0.03	5.9	559.6	3.0
SEQ ID NO: 312	0.3	60.6	92.0	87.2
	0.1	25.5	397.5	31.1
	0.03	7.8	555.3	3.7

[00615] To determine the effectiveness of the codon schemes with different UTRs, genome editing was measured *in vivo* following administration of mRNAs encoding Cas9. Messenger RNAs as indicated in Table 15 were formulated as LNPs with a guide RNA targeting TTR (G282; SEQ ID NO: 242). The LNPs were assembled using the cross flow procedure and contained 50% Lipid A, 9% DSPC, 38% cholesterol, and 3% PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had a N:P ratio of 6.0. LNPs were purified using Amicon PD-10 filters (GE Healthcare) and used at a concentration of 0.05 mg/ml (LNP concentration). CD-1 female mice (n=5 per group; n=4 for SEQ ID No: 243 editing) were dosed i.v. at 0.1 mpk. At 6 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured. Table 16 shows *in vivo* editing and serum TTR results.

Table 16

mRNA construct	% Editing	Standard Deviation	Serum TTR (µg/ml)	Standard Deviation
TSS	0	0	1274	214
SEQ ID No: 243	28	4	630	152
SEQ ID No: 376	35	8	482	138
SEQ ID No: 377	37	9	316	143
SEQ ID No: 378	42	6	524	192

Example 4 - Multiple Dose LNP Study Administered via 30 Minute and 2 Hour IV Infusion in Cynomolgus Monkeys

[00616] Male cynomolgus monkeys in cohorts of n=3 were administered dexamethasone (Dex) via IV bolus injection at 2 mg/kg a minimum of 1 hour prior to LNP or vehicle control administration. Each cohort received varying doses of LNP to provide 3 mg/kg, or 6 mg/kg (RNA) per NHP. Dosing groups are shown in Table 17. Two cohorts received an LNP dose of 3 mg/kg in order to compare infusion time. Formulations contained a Cas9 mRNA comprising SEQ ID No. 377) and guide RNA (gRNA) G000502 (SEQ ID No. 114) in a gRNA:mRNA ratio of 1:2 by weight. The cohorts receiving an LNP dose of 3mg/kg (total RNA content), were administered by 30-minute or 120-minute IV infusion. All other cohorts with various doses of LNP (in mg/kg, total RNA content), were administered by 120-minute IV infusion.

Table 17: Infusion Study Dosing Groups

Group Number	Test Material	Dose Level (mg/kg)	Infusion Time(min)	# of Animals
1	TSS	0	120	3
2	Cyn-	3.0	120	3
3	Cyn-	3.0	30	3
4	Cyn-	6.0	120	3

Table 18: % Editing and Serum TTR

Group Number	Liver Editing (%)	TTR % Reduction

1	0.0 (0.0, 0.0, 0.0)	-28 (-34, -23, -
2	63.3 (50.8, 69.0, 69.9)	85 (66, 95, 94)
3	63.3 (65.0, 66.0, 58.8)	88 (90, 89,86)
4	74.5 (75.3, 74.6, 73.6)	96 (97, 96, 95)

[00617] At day 29 post-dose, liver specimens were collected through single ultrasound-guided percutaneous biopsy targeting the right lobe/side of the liver, using a 16-gauge SuperCore biopsy needle under an intramuscular injection of ketamine/xylazine. A sample between 1.0 cm^3 and 1.5 cm^3 of total liver biopsy were collected per animal. Each biopsy specimen was flash frozen in liquid nitrogen and stored at -80 °C. Editing analysis of the liver specimens was performed through NGS sequencing as previously described. Results for the liver editing demonstrated up to about 70% editing. Corticosteroid pre-treatment with the described LNP treatment was well tolerated.

Table 19: Alanine Transaminase (ALT) Levels

	Pre- Bleed		6 Hour		24 Hour		48 Hour		Day 7		Day 29	
	Avg	St Dev	Avg	St Dev	Avg	St Dev	Avg	St Dev	Avg	St Dev	Avg	St Dev
Grp1-TSS	49.0	11.1	173.6	30.2	175.3	29.1	155.6	21.7	76.0	4.5	49.0	8.8
Grp2-3 mpk, 2 hr infusion	40.3	9.0	77.6	18.4	74.0	19.3	56.0	16.3	44.0	7.5	37.3	7.0
Grp3-3 mpk, 30 min infusion	50.3	7.5	149.0	130.0	285.3	352.2	236.3	294.1	88.3	88.1	35.6	6.3
Grp4-6 mpk, 2 hr infusion	30.6	12.5	108.3	48.4	162.0	87.1	209.0	174.6	65.0	32.0	27.0	7.5

[00618] Liver samples were biopsied and analyzed for percent editing data, serum TTR data, and Alanine Transaminase (ALT) levels as seen in Table 18 and Figures 3A-B and Table 19 and Figure 3C, respectively. Results for the liver editing and Serum TTR data demonstrate that there is no significant difference in efficacy between the 3 mg/kg dose with a 30 minute infusion time and a 3 mg/kg dose with a 120 minute infusion time. The greater than 30' infusion time administrations, however, demonstrate lower levels of ALT, a liver injury biomarker. ALT levels were observed to be higher in the 3 mg/kg dose with a 30 minute infusion time which indicated potential liver stress.

[00619] **Materials and Methods for Example 4**. mRNA was synthesized by *in vitro* transcription (IVT) using a linearized plasmid DNA template and T7 RNA polymerase. Transcription was generally performed from constructs comprising a T7 Promoter (SEQ ID NO: 231), a transcript sequence disclosed herein such as SEQ ID NO: 377 (which encodes the RNA ORF of SEQ ID NO: 311), and a poly-A tail (SEQ ID NO: 263) encoded in the plasmid.

[00620] For all methods, the transcript concentration was determined by measuring the light absorbance at 260 nm (Nanodrop), and the transcript was analyzed by capillary electrophoresis by Bioanalyzer (Agilent).

[00621] **LNP Formulation**

[00622] The lipid components were dissolved in 100% ethanol with the lipid component molar ratios described below. The chemically modified sgRNA and Cas9 mRNA were combined and dissolved in 25 mM citrate, 100 mM NaCl, pH 5.0, resulting in a concentration of total RNA cargo of approximately 1.5 mg/mL. The LNPs were formulated with an N/P ratio of about 6, with the ratio of chemically modified sgRNA: Cas9 mRNA at a 1:2 w/w ratio as described below. LNPs were formulated with 50% Lipid A, 9% DSPC, 38% cholesterol, and 3% PEG2k-DMG, and LNPs were formed by cross-flow technique as described in Example 1. During mixing, a 2:1 ratio of aqueous to organic solvent was maintained using differential flow rates. Diluted LNPs were concentrated using tangential flow filtration and then buffer exchanged by diafiltration prior to filtering and storage.

[00623] **Cas9 mRNA and gRNA Cargos**

[00624] Capped and polyadenylated Cas9 mRNA was generated by *in vitro* transcription using a linearized plasmid DNA template and T7 RNA polymerase using the method described in Example 1.

[00625] **Genomic DNA isolation**

[00626] Genomic DNA was extracted from liver samples using 50 µL/well BuccalAmp DNA Extraction solution (Epicentre, Cat. QE09050) according to manufacturer's protocol. All DNA samples were subjected to PCR and subsequent NGS analysis, as described herein.

[00627] **NGS Sequencing**

[00628] In brief, to quantitatively determine the efficiency of editing at the target location in the genome, genomic DNA was isolated and deep sequencing was utilized to identify the presence of insertions and deletions introduced by gene editing.

[00629] PCR primers were designed around the target site (e.g., TTR), and the genomic area of interest was amplified. Primer sequences are provided below. Additional PCR was performed according to the manufacturer's protocols (Illumina) to add the necessary chemistry for sequencing. The amplicons were sequenced on an Illumina MiSeq instrument. The reads were aligned to a cyno reference genome (e.g., macFas5) after eliminating those having low quality scores. The resulting files containing the reads were mapped to the reference genome (BAM files), where reads that overlapped the target region of interest were selected and the number of wild type reads versus the number of reads which contain an insertion, substitution, or deletion was calculated.

[00630] The editing percentage (e.g., the “editing efficiency” or “percent editing”) is defined as the total number of sequence reads with insertions or deletions over the total number of sequence reads, including wild type.

Sequence Table

[00631] The following sequence table provides a listing of sequences disclosed herein. It is understood that if a DNA sequence (comprising Ts) is referenced with respect to an RNA, then Ts should be replaced with Us (which may be modified or unmodified depending on the context), and vice versa.

Description	Sequence	SEQ ID No.
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGAA GTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAA GGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAAC AAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGG AAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGC AGACCTGAGACTGATCTACCTGGCACTGGCCCACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAA CAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGA CGCAAAGGCAATCCTGAGCGCCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGGGAGAAAAGAAGAACGG ACTGTTCGGAAACCTGATCGCCCTGGGCCTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCA GCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGAGACCAGTACGCCGACCTGTTCCTGGCAGCAAA GAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAA GAGATACGACGAACACCACCAGGACCTGACACTGCTGGTCAAGGCACTGGTCAGACACGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTT CGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGATTCTACAAGTTCATCAAGCCGATCCTGGA AAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTGGACAACGGAAGCAT CCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAA GATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACACAGAGGCTTCATCGAAAGAATGACAAA CTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGAC AAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGA AGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGA AGACATCCTGGAAGACATCGTCCTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAACATACGCACACCT GTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAG AGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACGA CAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGG AAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAA CATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGG AATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCT GCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACATCGACAACAAGGTCCTGACAAGAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGA AGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGC AGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGC	1

	ACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAA CGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAG AAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGAC AGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGG AAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAG CAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGA CAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCT GGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGA CCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAA GGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGT CATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACAT CATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAG CACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGG AGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAG AAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTC TTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAG	
G000739 – sgRNA negative control	mG*mA*mU*CACGUCGGCCGUUGGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	2
modified sgRNA sequence ("N" may be any natural or non-natural nucleotide)	mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	3
30/30/39 poly-A sequence	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAA	4
CR003335 gRNA targeting	CUGCUCCUCCUCUGCCUUGC	5

Human TTR (Exon 1)		6
CR003336 gRNA targeting Human TTR (Exon 1)	CCUCCUCUGCCUUGCUGGAC	
CR003337 gRNA targeting Human TTR (Exon 1)	CCAGUCCAGCAAGGCAGAGG	7
CR003338 gRNA targeting Human TTR (Exon 1)	AUACCAGUCCAGCAAGGCAG	8
CR003339 gRNA targeting Human TTR (Exon 1)	ACACAAAUACCAGUCCAGCA	9
CR003340 gRNA targeting Human TTR (Exon 1)	UGGACUGGUAUUUGUGUCUG	10
CR003341 gRNA targeting Human TTR (Exon 1)	CUGGUAUUUGUGUCUGAGGC	11
CR003342 gRNA targeting Human TTR (Exon 2)	CUUCUCUACACCCAGGGCAC	12
CR003343 gRNA	CAGAGGACACUUGGAUUCAC	13

targeting Human TTR (Exon 2)		14
CR003344 gRNA targeting Human TTR (Exon 2)	UUUGACCAUCAGAGGACACU	15
CR003345 gRNA targeting Human TTR (Exon 2)	UCUAGAACUUUGACCAUCAG	16
CR003346 gRNA targeting Human TTR (Exon 2)	AAAGUUCUAGAUGCUGUCCG	17
CR003347 gRNA targeting Human TTR (Exon 2)	CAUUGAUGGCAGGACUGCCU	18
CR003348 gRNA targeting Human TTR (Exon 2)	AGGCAGUCCUGCCAUCAAUG	19
CR003349 gRNA targeting Human TTR (Exon 2)	UGCACGGCCACAUUGAUGGC	20
CR003350 gRNA targeting Human TTR (Exon 2)	CACAUGCACGGCCACAUUGA	21
CR003351	AGCCUUUCUGAACACAUGCA	

gRNA targeting Human TTR (Exon 2)		22
CR003352 gRNA targeting Human TTR (Exon 2)	GAAAGGCUGCUGAUGACACC	23
CR003353 gRNA targeting Human TTR (Exon 2)	AAAGGCUGCUGAUGACACCU	24
CR003354 gRNA targeting Human TTR (Exon 2)	ACCUGGGAGCCAUUUGCCUC	25
CR003355 gRNA targeting Human TTR (Exon 2)	CCCAGAGGCAAAUGGCUCCC	26
CR003356 gRNA targeting Human TTR (Exon 2)	GCAACUUACCCAGAGGCAAA	27
CR003357 gRNA targeting Human TTR (Exon 2)	UUCUUUGGCAACUUACCCAG	28
CR003358 gRNA targeting Human TTR (Exon 3)	AUGCAGCUCUCCCAGACUCAC	

CR003359 gRNA targeting Human TTR (Exon 3)	AGUGAGUCUGGAGAGCUGCA	29
CR003360 gRNA targeting Human TTR (Exon 3)	GUGAGUCUGGAGAGCUGCAU	30
CR003361 gRNA targeting Human TTR (Exon 3)	GCUGCAUGGGCUCACAACUG	31
CR003362 gRNA targeting Human TTR (Exon 3)	GCAUGGGCUCACAACUGAGG	32
CR003363 gRNA targeting Human TTR (Exon 3)	ACUGAGGAGGAAUUUGUAGA	33
CR003364 gRNA targeting Human TTR (Exon 3)	CUGAGGAGGAAUUUGUAGAA	34
CR003365 gRNA targeting Human TTR (Exon 3)	UGUAGAAGGGAUAUACAAAG	35
CR003366 gRNA targeting Human TTR	AAAUAGACACCAAAUCUUAC	36

(Exon 3)		
CR003367 gRNA targeting Human TTR (Exon 3)	AGACACCAAAUCUUACUGGA	37
CR003368 gRNA targeting Human TTR (Exon 3)	AAGUGCCUUCCAGUAAGAUU	38
CR003369 gRNA targeting Human TTR (Exon 3)	CUCUGCAUGCUCAUGGAAUG	39
CR003370 gRNA targeting Human TTR (Exon 3)	CCUCUGCAUGCUCAUGGAAU	40
CR003371 gRNA targeting Human TTR (Exon 3)	ACCUCUGCAUGCUCAUGGAA	41
CR003372 gRNA targeting Human TTR (Exon 3)	UACUCACCUCUGCAUGCUCA	42
CR003373 gRNA targeting Human TTR (Exon 4)	GUAUUCACAGCCAACGACUC	43
CR003374 gRNA targeting	GCGGCGGGGGCCGGAGUCGU	44

Human TTR (Exon 4)		45
CR003375 gRNA targeting Human TTR (Exon 4)	AAUGGUGUAGCGGCGGGGGC	46
CR003376 gRNA targeting Human TTR (Exon 4)	CGGCAAUGGUGUAGCGGCGG	47
CR003377 gRNA targeting Human TTR (Exon 4)	GCGGCAAUGGUGUAGCGGCG	48
CR003378 gRNA targeting Human TTR (Exon 4)	GGCGGCAAUGGUGUAGCGGC	49
CR003379 gRNA targeting Human TTR (Exon 4)	GGGCGGCAAUGGUGUAGCGG	50
CR003380 gRNA targeting Human TTR (Exon 4)	GCAGGGCGGCAAUGGUGUAG	51
CR003381 gRNA targeting Human TTR (Exon 4)	GGGGCUCAGCAGGGCGGCAA	52
CR003382 gRNA	GGAGUAGGGGCUCAGCAGGG	

targeting Human TTR (Exon 4)		53
CR003383 gRNA targeting Human TTR (Exon 4)	AUAGGAGUAGGGGCUCAGCA	54
CR003384 gRNA targeting Human TTR (Exon 4)	AAUAGGAGUAGGGGCUCAGC	55
CR003385 gRNA targeting Human TTR (Exon 4)	CCCCUACUCCUAUUCCACCA	56
CR003386 gRNA targeting Human TTR (Exon 4)	CCGUGGUGGAAUAGGAGUAG	57
CR003387 gRNA targeting Human TTR (Exon 4)	GCCGUGGUGGAAUAGGAGUA	58
CR003388 gRNA targeting Human TTR (Exon 4)	GACGACAGCCGUGGUGGAAU	59
CR003389 gRNA targeting Human TTR (Exon 4)	AUUGGUGACGACAGCCGUGG	60
CR003390	GGGAUUGGUGACGACAGCCG	

gRNA targeting Human TTR (Exon 4)		61
CR003391 gRNA targeting Human TTR (Exon 4)	GGCUGUCGUCACCAAUCCCA	
CR003392 gRNA targeting Human TTR (Exon 4)	AGUCCCUCAUUCCUUGGGAU	62
CR005298 gRNA targeting Human TTR (Exon 1)	UCCACUCAUUCUUGGCAGGA	63
CR005299 gRNA targeting Human TTR (Exon 4)	AGCCGUGGUGGAAUAGGAGU	64
CR005300 gRNA targeting Human TTR (Exon 1)	UCACAGAAACACUCACCGUA	65
CR005301 gRNA targeting Human TTR (Exon 1)	GUCACAGAAACACUCACCGU	66
CR005302 gRNA targeting Human TTR (Exon 2)	ACGUGUCUUCUCUACACCCA	67

CR005303 gRNA targeting Human TTR (Exon 2)	UGAAUCCAAGUGUCCUCUGA	68
CR005304 gRNA targeting Human TTR (Exon 2)	GGCCGUGCAUGUGUUCAGAA	69
CR005305 gRNA targeting Human TTR (Exon 3)	UAUAGGAAAACCAGUGAGUC	70
CR005306 gRNA targeting Human TTR (Exon 3)	AAAUCUUACUGGAAGGCACU	71
CR005307 gRNA targeting Human TTR (Exon 4)	UGUCUGUCUUCUCUCAUAGG	72
CR000689 gRNA targeting Cyno TTR	ACACAAAUACCAGUCCAGCG	73
CR005364 gRNA targeting Cyno TTR	AAAGGCUGCUGAUGAGACCU	74
CR005365 gRNA targeting Cyno TTR	CAUUGACAGCAGGACUGCCU	75
CR005366 gRNA	AUACCAGUCCAGCGAGGCAG	76

targeting Cyno TTR		
CR005367 gRNA targeting Cyno TTR	CCAGUCCAGCGAGGCAGAGG	77
CR005368 gRNA targeting Cyno TTR	CCUCCUCUGCCUCGCUGGAC	78
CR005369 gRNA targeting Cyno TTR	AAAGUUCUAGAUGCCGUCCG	79
CR005370 gRNA targeting Cyno TTR	ACUUGUCUUCUCUAUACCCA	80
CR005371 gRNA targeting Cyno TTR	AAGUGACUUCCAGUAAGAUU	81
CR005372 gRNA targeting Cyno TTR	AAAAGGCUGCUGAUGAGACC	82
	Not Used	83
	Not Used	84
	Not Used	85

	Not Used		86
G000480 sgRNA modified sequence targeting Human TTR	mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	87	
G000481 sgRNA modified sequence targeting Human TTR	mU*mC*mU*AGAACUUUGACCAUCAGGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	88	
G000482 sgRNA modified sequence targeting Human TTR	mU*mG*mU*AGAAGGGAUAUACAAAGGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	89	
G000483 sgRNA modified sequence targeting Human TTR	mU*mC*mC*ACUCAUUCUUGGCAGGAGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	90	
G000484 sgRNA modified sequence targeting	mA*mG*mA*CACCAAAUCUUACUGGAGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	91	

Human TTR		
G000485 sgRNA modified sequence targeting Human TTR	mC*mC*mU*CCUCUGCCUUGCUGGACGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	92
G000486 sgRNA modified sequence targeting Human TTR	mA*mC*mA*CAAAUACCAGUCCAGCAGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	93
G000487 sgRNA modified sequence targeting Human TTR	mU*mU*mC*UUUGGCAACUUACCCAGGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	94
G000488 sgRNA modified sequence targeting Human TTR	mA*mA*mA*GUUCUAGAUGCUGUCCGGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	95
G000489 sgRNA modified sequence targeting Human TTR	mU*mU*mU*GACCAUCAGAGGACACUGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	96
G000490 sgRNA modified sequence targeting Human TTR	mA*mA*mA*UAGACACCAAAUCUUACGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	97
G000491 sgRNA	mA*mU*mA*CCAGUCCAGCAAGGCAGGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	98

modified sequence targeting Human TTR		
G000492 sgRNA modified sequence targeting Human TTR	mC*mU*mU*CUCUACACCCAGGGCACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	99
G000493 sgRNA modified sequence targeting Human TTR	mA*mA*mG*UGCCUUCCCAGUAAGAUUGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	100
G000494 sgRNA modified sequence targeting Human TTR	mG*mU*mG*AGUCUGGAGAGCUGCAUGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	101
G000495 sgRNA modified sequence targeting Human TTR	mC*mA*mG*AGGACACUUGGAUUCACGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	102
G000496 sgRNA modified sequence targeting Human TTR	mG*mG*mC*CGUGCAUGUGUUCAGAAGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	103
G000497 sgRNA modified sequence targeting	mC*mU*mG*CUCCUCCUGCCUUGCGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	104

Human TTR		
G000498 sgRNA modified sequence targeting Human TTR	mA*mG*mU*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	105
G000499 sgRNA modified sequence targeting Human TTR	mU*mG*mA*AUCCAAGUGUCCUCUGAGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	106
G000500 sgRNA modified sequence targeting Human TTR	mC*mC*mA*GUCCAGCAAGGCAGAGGGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	107
G000501 sgRNA modified sequence targeting Human TTR	mU*mC*mA*CAGAAACACUCACCGUAGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	108
G000567 sgRNA modified sequence targeting Human TTR	mG*mA*mA*AGGCUGCUGAUGACACCGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCmAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	109
G000568 sgRNA modified sequence targeting Human TTR	mG*mG*mC*UGUCGUCACCAAUCCCAGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCmAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	110
G000570 sgRNA	mC*mA*mU*UGAUGGCAGGACUGCCUGUUUUAGAmGmCmCmUmAmGmAmAmAmUmAmGmCmAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	111

modified sequence targeting Human TTR		
G000571 sgRNA modified sequence targeting Human TTR	mG*mU*mC*ACAGAAACACUCACCGUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	112
G000572 sgRNA modified sequence targeting Human TTR	mC*mC*mC*CUACUCCUAUUCCACCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	113
G000502 sgRNA modified sequence targeting Cyno TTR	mA*mC*mA*CAAAUACCAGUCCAGCGGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	114
G000503 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*AGGCUGCUGAUGAGACCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	115
G000504 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*GGCUGCUGAUGAGACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	116
G000505 sgRNA modified sequence targeting	mC*mA*mU*UGACAGCAGGACUGCCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	117

Cyno TTR		
G000506 sgRNA modified sequence targeting Cyno TTR	mA*mU*mA*CCAGUCCAGCGAGGCAGGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	118
G000507 sgRNA modified sequence targeting Cyno TTR	mC*mC*mA*GUCCAGCGAGGCAGAGGGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	119
G000508 sgRNA modified sequence targeting Cyno TTR	mC*mC*mU*CCUCUGCCUCGCUGGACGUUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	120
G000509 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*GUUCUAGAUGCCGUCCGGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	121
G000510 sgRNA modified sequence targeting Cyno TTR	mA*mC*mU*UGUCUUCUCUAUACCCAGUUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	122
G000511 sgRNA modified sequence targeting Cyno TTR	mA*mA*mG*UGACUUCCCAGUAAGAUUGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	123
G000282 sgRNA	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmCmUmAmGmGmAmAmAmUmAmGmCmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	124

modified sequence targeting Mouse TTR		125
exemplary nucleotide sequence following the 3′ end of the Guide Sequence to form a sgRNA	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	126
exemplary nucleotide sequence following the 3′ end of the Guide Sequence to form a crRNA	Not used	127 to 202
Cas9 amino acid sequence	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV	203

	PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKRKV	204
Cas9 mRNA open reading frame (ORF) 2	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGC AAGAAGUUCAAGGUCCUGGGAAACACAGACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACA GCAGAAGCAACAAGACUGAAGAGAACACAGAAGAAGAAACACAAGAAGAAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGC AACGAAAUGGCAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACAC CCGAUCUUCGGAAACAUCGUCGACGAAGUACCCGACACAAUCUACCACCUGAGAAAGAAGCUGGUCGACAGC ACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGACCUG AACCCGGACAACAGCGUCGACAAGCUGUUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCA AGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCCAGCUGCCGGGAGAA AAGAAGAACGGACUGUUCGGAAACCUGAUCGCCUGAGCCUGGGAGCUGACACCGAACUUCAAGAGCAACUUCGACCUGGCAGAAGAC GCAAAGCUGCAGCUGAGCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGUCGGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCA AGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACGAGCUGCCGAAAAGUACAAG GAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAUAUCUACAAGUUCAUCAAG CCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGAC AACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACUUCUACCCGUUCCUGAAGGAC AACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGCAGAUUCGCAUGG AUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUACUUCACAGUCUACAAC GAACUGACAAAGGUCAAGUACGUCACAGAAGGGAAUGAGAAAGCCGGCGAAGAACAGAAGAAUACAUCGUCGACCUG CUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAAAUC AGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGATCAUCAAGGACAAGGACUUCCUGGACAAC GAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACGUUGUUCGAAGACAGAGAAAUGAUCGAAGAACUGAAAGACA UACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAUACACAGGAUGGGGAAGACUGAGCAGAAAGGCUGAUC AACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCAACAGAAACUUCAUGCAGCUG AUCCACGACGACAGCCUGACAAUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACACAUCGCA AACCUGGCAGGAAGCCCGCCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAGGUCAUGGGAAGACAC AAGCCGGAAAACAUCGUCAUCGAAUGGCAGAGAAAACCAGACAACACGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGCGACUACGACGUCGACCACAUC GUCCCGCAGAGCUUCCUGAAGGACGACAUCGACAACAAGGUCCUGACAAGAACGAGGAAAGAGCGACAACGUC CCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCUAAAGUCACACAGAGAAAGUUCGACAAC CUGACAAAGGCAGAGAGGAGGACUGAGCGACAAGGCAGGAUUCAUCAAGAGACAGCUGGUCGAAACAAGACAGAUCACA	

	AAGCACGUCGCACAGAUCCUGGACAGCAGGAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUCAAGGUCACUCACA	
	CUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACGCCCACGACGAC	
	GCAUACCUGAACGCAGUCGUCGGAACACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUC	
	UACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAAC	
	UUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUCGUC	
	UGGGACAAGGGAAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAACAUCGUCAAGAAAGACAGAAGUCCAGACA	
	GGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAGGACUGGGACCCGAAGAAGUAC	
	GGAGGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAAGCUGAAGAGCGUC	
	AAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAA	
	GUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCUUCGAACUGGAAAACGGAAGAAGAGAUGAAUGCUGGCAAGCGCAGGA	
	GAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGA	
	AGCCCGGAAGACAACGAAGCAGCUGUUCGUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC	
	AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAG	
	GCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGGAGCACCCGGCAGCAUUCAAGUACUUCGACACACAACAAUCGACAGAAAG	
	AGAUACACAAGCACAAAGGAAGUCCUGGACGCCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGC	
	CAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGUCUAG	
		205
Cas9 mRNA ORF 1	AUGGAUAAGAAGUACUCAAUCGGGCUGGAUAUCGGAACUAAUUCCGUGGGUUGGGCAGUGAUCACGGAUGAAUACAAAGUGCCGUCC	
	AAGAAGUUCAAGGUCCUGGGGAACACCGAUAGACACAGCAUCAAGAAAAAUCUCAUCGGAGCCCUGCUGUUUGACUCCGGCGAAACC	
	GCAGAAGCGACCCGGCUCAAACGUACCGCGAGGCGACGCUACACCGGCGGAAGAAUCGCAUCUGCUAUCUGCAAGAGAUCUUUUCG	
	AACGAAAUGGCAAGGUCGACGACAGCUUCUUCCACCGCCUGGAAGAAUCUUUCCUGGUGGAGGAGGACAAGAAGCAUGAACGGCAU	
	CCUAUCUUUGGAAACAUCGUCGACGAAGUGGCGUACCACGAAAAGUACCCGACCAUCUACCACCUGCGGAAGAAGUUGGUUGACUCA	
	ACUGACAAGGCCGACCUCAGAUUGAUCUACUUGGCCCUCGCCCACAUGAUCAAGUUCCGCGGACACUUCCUGAUCGAAGGCGAUCUG	
	AACCCUGAUAACUCCGACGUGGAUAAGCUUUUCAUCCAACUGGUGCAGACCUACAACCAGCUGUUCGAAGAAAACCCAAUCAACGCU	
	AGCGGCGUCGAUGCCAAGGCCAUCCUGUCCGCCCGGCUGUCGAAGUCGCGGCGCCUCGAAAACCUGAUCGCCAGCUGCCCGGGAGAG	
	AAAAAGAACGGACUUUUCGGCAACUUGAUCGCUCUCUCACUGGGACUCACUCCCCAAUUUCAAGUCCAAUUUGACCUGGCCGAGGAC	
	GCGAAGCUGCAACUCUCAAAGGACACCUACGACGACGACCUGGACAACUUGCUGGCACAAAUUGGCGAUCAGUACGCGGAUCUGUUC	
	CUUGCCGCUAAGAACCUUUCGGACGCAAUCUUGCUGUCCGAUAUCCUGCGCGUGAACACCGAAAUAACCAAAGGCGCCGCUUAGCGCC	
	UCGAUGAUUAAGCGGUACGACGAGCAUCACCAGGAUCUCACGCUGCUCAAAGCGCUCGUGAGACGCAACUGCCUGAAAAGUACAAG	
	GAGAUCUUCUUCGACCAGUCCAAGAAUGGGUACGCAGGUACAUCGAUGGAGGCGCUAGCCAGGAAGAGUUCUAUAAGUUCAUCAAG	
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	AACGGAUCCAUUCCCCACCAGAUCCAUCUGGGUGAGCUGCACGCCAUCUUGCGGCGCCAGGAGGACUUUUACCCAUUCCUCAAGGAC	
	AACCGGGAAAAGAUCGAGAAAAUUCUGACGUUCCGCAUCCCGUAUUACGUGGGCCCACUGGCGCGCGGCAAUUCGCGCUUCGCGUGG	
	AUGACUAGAAAAUCAGAGGAAACCAUCACUCCUUGGAAUUUCGAGGAAGUUGUGGACAAGGGAGCUUCGGCACAAAGCUUCAUCGAA	
	CGAAUGACCAACUUCGACAAGAAUCUCCCAAACGAGAAGGUGCUUCCUAAGCACAGCCUCCUUUACGAAUACUUCACGUGUCUACAAC	
	GAACUGACGAAAGUGAAAUACGUUACUGAAGGAAUGAGAAGCCGGCCUUUCUGUCCGGAGAACAGAAGAAAGCAAUUGUCGAUCUG	
	CUGUUCAAGACCAACCGCAAGGUGACCGUCAAGCAGCUUAAAGAGGACUACUUCAAGAAGAUCGAGUGUUUCGACUCAGUGGAAAUC	
	AGCGGGGUGGAGGACAGAUUCAACGCUUCGCUGGGAACCUACAUCAUGAUCUCCUGAAGAUCAUCAAGGACAAGGACUUCCUUGACAAC	
	GAGGAGAACGAGGACAUCCUGGAAGAUAUCGUCCUGACCUUGACCCUUUUCGAGGAUCGCGAGAUGAUCGAGGAGAGGCUUAAGACC	

UACGCUCAUCUCUUCGACGAUAAGGUCAUGAAACAACUCAAGCGCCGCCGGUACACUGGUUGGGGCCGCCUCUCCCGCAAGCUGAUC AACGGUAUUCGCGAUAAACAGAGCGGUAAAACUAUCCUGGAUUUCCUCAAAUCGGAUGGCUUCGCUAAUCGUAACUUCAUGCAAUUG AUCCACGACGACAGCCUGACCUUUAAGGAGGACAUCCAAAAAGCACAAGUGUCCGGACAGGGAGACUCACUCCAUGAACACAUCGCG AAUCUGGCCGGUUCGCCGCGAUAAGAAGGGAAUUCUGCAAACUGUGAAGGUGGUCGACGAGCUGGUGAAGGUCAUGGGACGGCAC AAACCGGAGAAUAUCGUGAUUGAAAUGGCCCGAGAAAACCAGACUACCCAGAAGGGCCAGAAAAACUCCCGCGAAAGGAUGAAGCGG AUCGAAGAAGGAAUCAAGGAGCUGGGCAGCCAGAUCCUGAAAGAGCACCCGGUGGAAAACACGCAGCUGCAGAACGAGAAGCUCUAC CUGUACUAUUUGCAAAAUGGACGGGACAUGUACGUGGACCAAGAGCUGGACAUCAATCGGUUGUCUGAUUACGACGUGGACCACAUC GUUCCACAGUCCUUUCUGAAGGAUGACUCGAUCGAUAACAAGGUGUUGACUCGCAGCGACAAGAACAGAGGGAAGUCAGAUAAUGUG CCAUCGGAGGAGGUCGUGAAGAAGAUGAAGAAUUACUGGCGGCAGCUCCUGAAUGCGAAGCUGAUUACCCAGAGAAAGUUUGACAAU CUCACUAAAGCCGAGCGCGGCGGACUCUCAGAGCUGGAUUCAUCAAACGGCAGCUGGUCGAGACUCGGCAGAUUACC AAGCACGUGGCGCAGAUCUUGGACUCCCGCAUGAACACUAAAUACGACGAGAACGUAAAGCUCAUCCUGGUCGAAGUGAAGGUGAUUACC CUGAAAAGCAAACUUGUGUCGGAAGGACUUUUCAGUUUUACAAAGUGAGAGAAAUCAACAUACCAUCACGCGCAUGAC GCAUACCUCAACGCUGUGGUCGGUAACCGCCCUGAUCAAAAAGUACCCUAAACUUGAAUCGGAGUUUGUGUACGGAGACUACAAGGUC UACGACGUGAGGAGAUGAUAGCCAAGUCCGAACAGGAAAUCGGGAAAGCAACUGCGAAAUACUUCUUUUACUCAAACAUCAUGAAC UUUUUCAAGACUGAAAUUACGCUGGCCAAUGGAGAAAUCAGGAAGAGGCCACUGAUCGAAACUAACGAGACGGCGAAAUCGUG UGGGACAAGGGCAGGGACUUCGCCAAAGUGCUCUCUAUGCCGCAAGUCAAUAUUGUGAAGAAAACCGAAGUGCAAACC GGCGGAUUUUCAAAGGAAUCGAUCCUCCCAAAGAGAAAUAGCGACACAAGCUCAUUGCACGCAAGAAAGACUGGGACCCGAAGAAGUAC GGAGGAUUCGAUUCGCCGACUGUCGCCAUACUCCGUCCUCGGUGGUGGCCAAGGUGGAGAAGGGAAAGAGCAAAAAGCUCAAAUCCGUC AAAGAGCUGCUGGGGAUUACCAUCAUGGAACGAUCCUCGUUCGAGAAGAACCCGAUUGAUUCCUCGAGGCGAAGGUUACAAGGAG GUGAAGAAGGAUCUGAUCAUCAAACUCCCCAAGUACUCACUGUUCGAACUGGAAAUGGUCGGAAGCGCAUGCUGGCUUCGGCCGGA GAACUCCAAAAGGAAAUGAGCUGGCCUUGCCUAGCAAGUACGUCAACUUCCUCUAUCUUGCUUCGCACUACGAAAAACUCAAAGGG UCACCGGAAGAUAACGAACAGAAGCAGCUUUUCGUGGAGCAGCACAAGCAUUAUCUGGAUGAAAUCAUCGAACAAAUCUCCGAGUUU UCAAAGCGCGUGAUCCUCGCCGACGCCAACCUCGACAAAGUCCUGUCGGCCUACAAUAAGCGAGAUAAGCCGAUCAGAGAACAG GCCGAGAACAUUAUCCACUUGUUCACCCUGACUAACCUGGGAGCCCCCAGCCGCCUUCAAGUACUUCGAUACUACUAUCGAUCGCAAA AGAUACACGUCCACCACGAAGUUCUGGACGCGACCCUGAUCCACCAAAGCAUCACACUGGACUCUACGAAACUACGGAUCGAUCUGUCG CAGCUGGGUGGCGAUGGCGGUGGAUCUCCGAAAAAGAAGAGAAAGGUGUAAUGA	Not Used	206-212
Amino acid sequence of Cas9 (without NLS)	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN	213

EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	214-221	
Not Used		
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD GSGSPKKKRKVDGSPKKKRKVDSG	222	
Not Used		223-230

T7 promoter	TAATACGACTCACTATA	231
Human beta-globin 5′ UTR	ACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACC	232
Human beta-globin 3′ UTR	GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCT TGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGC	233
Human alpha-globin 5′ UTR	CATAAACCCTGGCGCGCTCGCGGCCCGGCACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACC	234
Human alpha-globin 3′ UTR	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTT TGAATAAAGTCTGAGTGGGCGGC	235
Xenopus laevis beta-globin 5′ UTR	AAGCTCAGAATAAACGCTCAACTTTGGCC	236
Xenopus laevis beta-globin 3′ UTR	ACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAATACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGCC ATTCGTATCTGCTCCTAATAAAAAGAAAGTTTCTTCACATTCT	237
Bovine Growth Hormone 5′ UTR	CAGGGTCCTGTGGACAGCTCACCAGCT	238
Bovine Growth Hormone 3′ UTR	TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGA GGAAATTGCATCGCA	239
Mus musculus hemoglobin alpha, adult chain 1 (Hba-a1), 3′UTR	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGT AGGAAG	240

HSD17B4 5′ UTR	TCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTTCGTGTGTCGTTGCAGGCCTTATTC	241
G282 guide RNA targeting TTR	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmGmCmU*mU*mU*mU	242
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTTCGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGAA GTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAA GGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAAC AAGACTGAAGAGAACAGCAAGAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGG AAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGC AGACCTGAGATCGATCTACCTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAA CAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGA CGCAAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGGGAGAAAAGAAGAACGG ACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCA GCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAA GAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAA GAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAGTACAAGGAAATCTTCTT CGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGA AAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCAT CCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAA GATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGGAGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACACAGAGCTTCATCGAAAGAATGACAAA CTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGAC AAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGA AGACAGATTCAACGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGA AGACATCCTGGAAGACATCGTCCTGACACTGACGTTGTTCGAAGACAGAGAAATGATCGAAGAGACTGAAGACATACGCACACACCT GTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAG AGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAACTTCATGCAGCTGATCCACGACGA CAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGG AAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAA CATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAGAATGAAGAGAATCGAAGAAGG AATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCT GCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACCAGAGCGACAAGAACAAGGGAAAGAGCGACAACGTCCCGAGCGAAGA AGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGC AGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGC	243

	ACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAA CGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAG AAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGAC AGAAATCACACTGGCAAACGGAGAAATCAGAAAGACGCCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGG AAGAGACTTCGCCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAG CAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGA CAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCT GGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGA CCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAGAAGAATGCTGGCAAGCCGCAGGAGAACTGCAGAA GGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAGCAAATCAGCGAATTCAGCAAGAGAGT CATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACAT CATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAG CACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGG AGACGGAGGAGGAAGAGAAGAGGAAAGGTCTAGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAG AAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTC TTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAATGGAAAGAACCTCGAG	244
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 204, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCATGGACAAGAAGTACAG CATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCT GGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAACAAGACT GAAGAGAACAGCAAGAAGAAGATACACAAGAAGAACAGATCTTCCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGT CGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACAT CGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCT GAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGA CGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAA GGCAATCCTGAGCGCAAGAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTT CGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAG CAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCT GAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATA CGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCA GAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGAT GGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCA CCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGA AAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAGAGCGA AGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGA CAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAA GTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAG	

AAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAG ATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACAT CCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGA CGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACGGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAA GCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACAGCCTT GACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCC GGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGT CATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAA GGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAA CGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGTCCACCACATCGTCCCGCAGAGCTTCCT GAAGGACGACAGCATCGACAACAAGGTCCTGACCAGATCCGCAAGAACAGAGGAAAGACGACAACGTCCCGAGCGAAGAAGTCGT CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAG AGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGAT CCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGT CAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCCAGT CGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGAT GATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAAT CACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGA CTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACACAGAAGTCCAGACAGGAGGATTCAGCAAGGA AAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCC GACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAAT CACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGAT CATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAA CGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGAAGACAACGA ACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCT GGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAAGAATACATCCA CCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACCGTAAGCGATACACCAGCACAAA GGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGTGACGG AGGAGGAAGCCCGAAGAAGAGAAAGGTCTAGCTACCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAA AATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAAT CATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAG	245	
Not Used		
Cas9 transcript	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCTGCCACCATGGATAAGAA GTACTCGATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAA	246

with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 245, Kozak sequence, and 3′ UTR of ALB	GGTCCTGGGGAACACCGATAGACACAGCATCAAGAAGAATCTCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGCAGAAGCGAC CCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGCCGAAGAATCGCATCTGCTATCTGCAAGAAATCTTTTCGAACGAAATGGC AAAGGTGGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTTGG AAACATCGTGGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGC CGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGATAA CTCCGACGTGGATAAGCTGTTCATTCAACTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCCAGCGGCGTCGA TGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAAACCTGATCGCCAATTTTGACCTGGCCGAGGACGCGAAGCTGCA ACTCTCAAAGGACACCTACGACGACGACTTGGACAACAATTTGCTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAA GAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAA GCGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGAGATTTCTT CGACCAGTCCAAGAATGGGTACGCAGGGTACATCGATGGAGGCGCCAGCCAGGAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGA AAAGATGGACGGAACCGAAGAACTGCTGGTCAAGCTGAACAGGGAGGATCTGCTCCGCAAACAGAGAACCTTTGACAACGGAAGCAT TCCACACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGGAAAA GATCGAGAAAATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCCGGCAATTCGCGCTTCGCGTGGATGACTAGAAA ATCAGAGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAATCCTTCATCGAACGAATGACCAA CTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAA AGTGAAATACGTTACTGAAGGAATGAGGAAGCCGGCTTTCTGAGCGGAGAACAGAAGAAAGCGATTGTCGATCTGCTGTTCAAGAC CAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCGGAGTGGA GGACAGATTCAACGCTTCGCTGGGAACCTATCATGATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAACGA GGACATCCTGGAAGATATCGTCCTGACCTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCT CTTCGACGATAAGGTCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAACGGTATTCG CGATAAACAGAGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGGCTTCGCTAATCGTGATTACTTCATGCAGTTGATCCACGACGA CAGCCTGACCTTTAAGGAGGACATCCAGAAAGCACAAGGTGGACGACAAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGG TTCGCCGGCGATTAAGAAGGGAATCCTGCAAACTGTGAAGGTGGTGGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGAGAA TATCGTGATTGAAATGGCCCGAGAAAACCAGACTACCCAAGAAGGGCCAGAAGAACTCCCGCGAAAGGATGAAGCGGATCGAAGAAGG AATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCACCCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTT GCAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTGTCTGATTACGACGTGGACCACATCGTTCCACAGTC CTTTCTGAAGGATGACTCCATCGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGA GGTCGTGAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGC CGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGC GCAGATCCTGGACTCCCGCATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAA ACTTGTGTCGGACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCACGATGACGCATACCTCAA CGCTGTGGTCGGCACCGCCCTGATCAAGAAGTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAG GAAGATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTTTACTCAAACATCATGAACTTCTTCAAGAC TGAAATTACGCTGGCCAATGGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGG CAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTC AAAGGAATCGATCCTCCCAAAGAGAAATAGCGACACAAGCTCATTGCACGCAAGATAAGACTGGGACCCGAAGAAGTACGGAGGATTCGA TTCGCCGACTGTCGCATACTCCGTCCTCGTGGTGGCCAAGGTGGAAGGGAAAGAGCAAGAAGCTCAAATCCGTCAAAGAGCTGCT

	GGGGATTACCATCATGGAAGAACGATCCTCGTTCGAGAAGAACCCGATTGATTTCCTGGAGGCGAAGGGTTACAAGGAGGTGAAGAAGGA TCTGATCATCAAACTGCCCAAGTACTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAGAA AGGAAATGAGCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGAGAAACTCAAAGGGTCACCGGAAGA TAACGAACAGAAGCAGCTTTTCGTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGT GATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAAGCATAGATAAGCCGATCAGAGAACAGGCCGAGAACAT TATCCACTTGTTCACCCTGACTAACCTGGGAGCTCCCAGCCGCCTTCAAGTACTTCGATACTACATCGACCGCAAAAGATACACGTC CACCAAGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGTGG CGATGGTGGCGGTGGATCCTACCCATACGACGTGCCTGACTACGCCTCCGGAGGTGGTGGCCCCAAGAAGAAACGGAAGGTGTGATA GCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAAGAAATGAAGATCAATAGCTTATTCATCTCTTTTTC TTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAAT AAAAAATGGAAGAACCTCGAG	247
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 245, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCTATGGATAAGAAGTACTC GATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTCCT GGGGAACACCGATAGACACAGCATCAAGAAGAATCTCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCT CAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAAATCTTTCGAACGAAATGGCAAAGGT GGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTTGGAAACAT CGTGGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCT CAGATTGATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGATAACTCCGA CGTGGATAAGCTGTTCATTCAACTGGTGCAGACCTACAACCAGCTGTTCGAAGAAAACCCAATCAATGCCAGCGGCGTCGATGCCAA GGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAAACCTGATCGCCAGCTGCCGGGAGAGAAGAACGGACTTTT CGGCAACTTGATCGCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTC AAAGGACACCTACGACGACGACTTGGACAACTTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCT TTCGGACGCAATCTTGTCGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAAGCGGTA CGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGACAGCCAACTGCCTGAAAAGTACAAGGAGATTTTCTTCGACCA GTCCAAGAATGGGTACGCAGGGTACATCGATGGAGGCGCCAGCCAGGAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGAT GGACGGAACCGAAGAACTGCTGGTCAAGCTGAACAGGGAGGATCTGCTCCGCAAACAGAGGAACCTTTGACAACGGAAGCATTCCACA CCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGA GAAAATTCTGACGTTCCGCATATCCTGCGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAAGCGGTA GGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAATCCTTCATCGAACGAATGACCAACTTCGA CAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGTGAA ATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGAGCGGAGAACAGAAGAAAGCGATTGTCGATCTGCTGTTCAAGACCAACCG CAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCGGAGTGGAGGACAG ATTCAACGCTTCGCTGGGAACCTATCATGATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAACGAGGACAT CCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGA CGATAAGGTCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAACGGTATTCGCGATAA ACAGAGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAGTTGATCCACGACGACAGCCT GACCTTTAAGGAGGACATCCAGAAAGCACAAGTGAGCGGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCC GGCGATTAAGAAGGGAATCCTGCAAACTGTGAAGGTGGTGGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGAGAATATCGT	

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	Not Used	248-251
Cas9 ORF with minimal uridine codons frequently used in humans in general;	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGC	252
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12.75% U content	GCCAAGCTGCAGCTGAGCAAGGACGACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTC
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	CAGCTGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAGAGAAAGGTGTGA

Cas9 transcript with 5' UTR of HSD, ORF correspondi ng to SEQ ID NO: 252, Kozak sequence, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGAA GTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTCAA GGTGCTGGGCAACACCGACAGACACAGCATCAAGAAGAACCTGATCGGCGCCCTGTTGTTCGACAGCGGCGAGACCGCCGAGGCCAC CAGACTGAAGAGAACCGCCAGAAGAAGATACACCAGAAGAAACAGAATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATGGC CAAGGTGGACGACAGCTTCTTCCACAGACTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGAGACACCCCATCTTCGG CAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAGCTGGTGGACAGCACCGACAAGGC CGACCTGAGACTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCAGAGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAA CAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGA CGCCAAGGCCATCCTGAGCGCCAGACTGAGCAAGAGCAGAAGACTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAACGG CCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGCA GCTGAGCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTGGCCGCCAA GAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCATGATCAA GAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGAGACAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTT CGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTGGA GAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACAGAGAGGACCTGCTGAGAAAGCAGAGAACCTTCGACAACGGCAGCAT CCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGAGAAGACAGGAGGACTTCTACCCCTTCCTGAAGGACAACAGAGAAG GATCGAGAAGATCCTGACCTTCAGAATCCCCTACTACGTGGGCCCCCTGGCCAGAGGCAACAGCAGATTCGCCTGGATGACCAGAAA GAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAGAGAATGACCAA CTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAA GGTGAAGTACGTGACCGAGGGCATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAAGAAGTCGTGGACCTGCTGTTCAAGAC CAACAGAAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGA GGACAGATTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAACGA GGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACAGAGAGATGATCGAGGAGAGACTGAAGACCTACGCCCACCT GTTCGACGACAAGGTGATGAAGCAGCTGAAGAGAAGATACACCGGCTGGGGCAGACTGAGCAGAAGCTGATCAACGGCATCAG AGACAAGCAGAGCGGCAAGACCATCCTGGATCTTCCTGAAGAGCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGA CAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGG CAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCAGACACAAGCCCGAGAA CATCGTGATCGAGATGGCCAGAGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCAGAGAGAGAATGAAGCGAATCGAGGAGGG CATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCT GCAGAACGGCAGAGACATGTACGTGGACCAGGAGCTGGACATCAACAGACTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAG CTTCCTGAAGGACGACATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACAGAGGCAAGAGCGACAACGTGCCCAGCGAGGA GGTGGTGAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAGCTGATCACCCAGAGAAAGTTCGACAACCTGACCAAGGC CGAGAGAGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAGACCAGACAGATCACCAAGCACGTGGC CCAGATCCTGGACAGCAGAATGAACACCAAGTACGACGAGAACGACAAGCTGATCAGAGAGGTGAAGGTGATCACCCTGAAGAGCAA GCTGGTGAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTGAGAGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAA CGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGAG AAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGAC CGAGATCACCCTGGCCAACGGCGAGATCAGAAAGAGACCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGG	253

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Cas9 transcript with AGG as first three nucleotides for use with CleanCap™, 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of ALB	Not Used	AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGAA GTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAA GGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAAC AAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGG AAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGC AGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAA CAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGA CGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGG ACTGTTCGGAAACCTGATCGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCA GCTGAGCAAGGACACATACGACGACGACCTGGACAACATGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAA GAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAA GAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTT CGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGA AAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCAT CCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAA GATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACACAGAGCTTCATCGAAAGAATGACAAA CTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGAC AAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGA AGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGA	

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Cas9 transcript with 5′ UTR from CMV, ORF corresponding to SEQ ID NO: 204, Kozak	GGGCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACGG	257
	TGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACTCACCGTCCTTGACACGGCCACCATGGACAAGAAGTACAGCATCGGACTGG	
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sequence, and 3' UTR of ALB	GCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGA
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Cas9 transcript with 5′ UTR from HBB, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of HBB	GGGacatttgcttctgacaacaactgtgttcactagcaacctcaaacagacaccggatcctgccaccATGGACAAGAAGTACAGCATCG GACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAA ACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGA GAACAGCAAGAAGAAGATACACAAGAAGAACACGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACG ACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACCCGATCTTCGGAAACATCGTCG ACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGAC TGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCG ACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAA TCCTGAGCGCCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAA ACCTGATCGCCCTGGGCCTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCAGCTGAGCAAGG ACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCG ACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCCGTGAGCGCAAGCATGATCAAGAGATACGACG AACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCA AGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACG GAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGA TCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGA TCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAA CAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGA ACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACG TCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGG TCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAGATCAGCGGAGTCGAAGACAGATTCA ACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGG AAGACATCGTCCTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACA AGGTCATGAAGCAGCTGAAGAGAAGAATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGA GCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAACTTCATGCAGCTGATCCACGACGCACTGCCTGACAT TCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAACCTGGCAGGAAGCCCGGCAA TCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGACACAAGCCGGAAACATCGTCATCG AAATGGCAAGAGAAACCAGACAACACAGAAGGACAGAAGAACAGCAGAGAAAGAATGAAGAGGAATCGAAGAAGGAATCAAGGAAC TGGGAAGCCAGATCCTGAAGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAA GAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGG ACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGA AGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAG GACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGG ACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCG	

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	TGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGA	
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	GAAGCCCGAAGAGAAGAGAAAGGTCTAGctagcgctcgcttttcttgctgtccaatttctattaaaggttcctttgttccctaagtc	
	caactactaaactgggggatattatgaagggccttgagcatctggattctgcctaataaaaaacatttatttttcattgcctcgag	
Cas9 transcript with 5′ UTR from XBG, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of XBG	GGGaagctcagaataaacgctcaacttttggccggatctgccacCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACA	259
	GCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCA	
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	ACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGG	
	TCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCA	
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	ACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCC	
	TGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAG	
	CACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCC	
	ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAAGCCCGAAGAAGAAGAAA	
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	ccaaaatgtagccattcgtatctgctcctaataaaaagaaagttcttcacattcctctcgag	
		260
Cas9 transcript with AGG as first three nucleotides for use with CleanCap™, 5′ UTR from XBG, ORF	AGGaagctcagaataaacgctcaacttttggccggatcttgccacCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACA	
	GCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGCACACAGCATCA	
	AGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGATGGCAAAGTCAGCTGTTCATCCAGACTGG	
	CAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGG	
	AAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAA	
	AGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCAC	
	ACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGG	
	TCCAGACATACAACCAGTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCA	
	AGAGCAGAAGACTGGAAAACCTGATCGCCACAGCTGCCGGGAGAAAAGAAGCTGGACTGTTCGGAAACCTGATCGCCACTGAGCCTGG	
	GACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGG	

corresponding to SEQ ID NO: 204, Kozak sequence, and 3' UTR of XBG	ACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACA TCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACAC TGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACA TCGACGGAGGAGGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCA AGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACG CAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGT ACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAGAGCGAGAAGAACAATCACACCGTGGAACTTCG AAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCC TGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGC CGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGG AAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAGCCTGGGAACATACC ACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGA CACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGA GAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACT TCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGG CACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGA CAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGA CAACACAGAAGGGACAGAAGAACAGCAGAAGAAGAATGAAGAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGG AACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGG AACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGG TCCTGACAAGAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGAC AGCTGCTGAACGCCAAAGCTGATCAAGTTCCGCAAGCGCCACGACAACCTGACAAAGGCAGAGAGGAGGACTGGAGCGAACTGGAG CAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACACAAAGT ACGACGAAAACGACAAGCTGATCAAGGTCAAGTCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGT TCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGT ACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCG GAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAA AGAGACCGCTGATCGAAACAAACGGAGAAACAGGGAGAAGAGACTTCGCAACAGTCAGTCAGAAAGGTCCTGA GCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCG ACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACATCGCATACAGCGTCCTGGTCG TCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACACAATCATGGAAAGAAGCAGCTTCG AAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGT TCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAACTGCAGAAGGAAACGAACTGGCACTGCCGAGCAAGTACG TCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGC ACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCC TGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAG CACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACGTCCACCAAGGAAGTCCTGGACGCAACACTGATCC ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGACGGAGGAGGGAAGCCCGAAGAAGAGAAGA AGGTCTAGctagcaccagcctcaagaacacccgaatggagtcctaagctacataataccaacttacacttacaaaatgttgtccc

	ccaaaatgtagccattcgtatctgctccctaataaaaagaaagtttcttcacattcctcgag	
Cas9 transcript with AGG as first three nucleotides for use with CleanCap™, 5′ UTR from HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3′ UTR of ALB	AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGAA GTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGCGAATACAAGGTCCCGAGCAAGAAGTTCAA GGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAAC AAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGC AAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGG AAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGC AGACCTGAGACTGATCTACCTGGCACTGGCCCACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAA CAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGA CGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCACAGCTGCCGGGAGAAAAGAAGACGG ACTGTTCGGAAACCTGATCGCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCA GCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGAGACCAGTACGCCGACCTGTTCCTGGCAGCAAA GAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAA GAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCTGCCGGAAAAGTACAAGGAAATCTTCTT CGACCAGAGCAAGAACGGATACGCGGAGGATACATCGACGGAGGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGA AAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCAT CCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAA GATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGGGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAACTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAA CTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAA GGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGAC AAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGA AGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGA AGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCT GTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAG AGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAACTTCATGCAGCTGATCCACGACGA CAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGG AAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAA CATCGTCATCGAAATGGCAAGAGAAACCAGACACACAGAGGACAGAAGAAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGG AATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCT GCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAG CTTCCTGAAGGACGACTCCATCGACAACAAGGTCCTGACCAGAGCGACAAGAACAAGGGCAAGAGCGACAACGTCCCGAGCGAAGA AGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGC AGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGC ACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAA CGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAG AAAGATGATCGCCAAAGAGCAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGAC	261

	AGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGG AAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAG CAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGA CAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCT GGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGA CCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAA GGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGA CAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGT CATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACAT CATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAG CACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGG AGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAG AAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTC TTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAG	
30/30/39 poly-A sequence	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAA	262
poly-A 100 sequence	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAA	263
G209 guide RNA	mC*mC*mA*GUCCAGCGAGGCAAAGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCmU*mU*mU*U	264
	Not Used	265-267
Amino acid sequence of Neisseria meningitidis Cas9	MAAFKPNSINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRVFERAEVPKTGDSLAMARRLARSVRRLTRRRAHRLLRTRRLLK REGVLQAANFDENGLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVAGNAHALQTGDF RTPAELALNKFEKESGHIRNQRSDYSHTFSRKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHCTFE PAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLM EMKAYHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLKDRIQPEILEALLKHISFDKFVQISLKALRRIVPLM EQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPARIHIETAREVGKSFKDRKEIEKR QEENRKDREKAAAKFREYFPNFVGEPKSKDILKLRLYEQQHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSFNNKVLVLGS ENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQRILLQKFDEDGFKERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVF ASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGEVLHQKTHFPQPWEFFAQEV MIRVFGKPDGKPEFEEADTLEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSAKRLDEGVSVLRVPLTQLKLK DLEKMVNREREPKLYEALKARLEAHKDDPAKAFAEPFYKYDKAGNRTQQVKAVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEKGD KYYLVPIYSWQVAKGILPDRAVVQGKDEEDWQLIDDSFNFKFSLHPNDLVEVITKKARMFGYFASCHRGTGNINIRIHDLDHKIGKN GILEGIGVKTALSFQKYQIDELGKEIRPCRLKKRPPVRSGKRTADGSEFESPKKKRKVE	268
	Not Used	269

G502 guide RNA	mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	270
	Not Used	271
	Not Used	272
DNA coding sequence of eGFP	TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGA GCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGA GAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCA ACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGG TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCTAATACGACTCACTATAGGGTCCCGCAGTCGGCG TCCAGCGGCTCTGCTTGTTCGTGTGTTCGTTGCAGGCCTTATTCGGATCCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACG GCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGC AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCA TCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGG GCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACACGTCTATATCATGGCCGACA AGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA ACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGA AGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAATAGGAATTAT GCAGTCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAATGAAGATCAATAGCTTATTCATCTCTT TTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAAT TAATAAAAATGGAAAGAACCTCGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAATCTAGACTTAAGCTTGATGAGCTCTAGCTTGGCGTAATCATGGTCATA GCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTA ATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAAT CGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCT GCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAA AGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAA TCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCC TGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTA TCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAA CTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGT AGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCC AGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGCAAGCAGCA	273

	GATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTA AGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTAT ATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGT TGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCAT CCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCAT CGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTG CAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACT GCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTAT GCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAA ACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTC AGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAA ATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATG TATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGAC ATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCG	274
Modified sgRNA pattern, where N are nucleotides encoding a guide sequence	mN*mN*mN*NNNNNNNNNNNNNNNNGUUUUAG AmGmCmUmUmAmGmAmAmUmAmGmCAAGUUAAA AUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAm AmAmAmAmGmUmGmGmCmAmCnCnGmAmGmUmC mCmGmUmGmCmU*mU*mU*mU	275
CMV-1 5′ UTR	CAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCAT	276
CMV-2 5′ UTR	AGAAGACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACGG	277
CMV-3 5′ UTR	TGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACTCACCG	278
SV40 NLS	PKKKRKV	279
Exemplary NLS 1	LAAKRSRTT	280
Exemplary NLS 2	QAAKRSRTT	281
Exemplary NLS 3	PAPAKRERTT	282
Exemplary NLS 4	QAAKRPRTT	

Exemplary NLS 5	RAAKRPRTT	283
Exemplary NLS 6	AAAKRSWSMAA	284
Exemplary NLS 7	AAAKRVWSMAF	285
Exemplary NLS 8	AAAKRSWSMAF	286
Exemplary NLS 9	AAAKRKYFAA	287
Exemplary NLS 10	RAAKRKAFAA	288
Exemplary NLS 11	RAAKRKYFAV	289
Alternate SV40 NLS	PKKKRRV	290
Nucleoplasmin NLS	KRPAATKKAGQAKKKK	291
Exemplary coding sequence for SV40 NLS	CCGAAGAAGAAGAGAAAGGTC	292
Exemplary coding sequence for NLS1	CTGGCAGCAAAGAGAAGCAGAACAACA	293
Exemplary coding sequence for NLS2	CAGGCAGCAAAGAGAAGCAGAACAACA	294

Exemplary coding sequence for NLS3	CCGGCACCGGCAAAGAGAAAGAACAACA	295
Exemplary coding sequence for NLS4	CAGGCAGCAAAGACCGAGAACAACA	296
Exemplary coding sequence for NLS5	AGAGCAGCAAAGACCGAGAACAACA	297
Exemplary coding sequence for NLS6	GCAGCAGCAAAGAGAAGCTGGAGCATGGCAGCA	298
Exemplary coding sequence for NLS7	GCAGCAGCAAAGAGAGTCTGGAGCATGGCATTC	299
Exemplary coding sequence for NLS8	GCAGCAGCAAAGAGAAGCTGGAGCATGGCATTC	300
Exemplary coding sequence for NLS9	GCAGCAGCAAAGAGAAAGTACTTCGCAGCA	301

Exemplary coding sequence for NLS10	AGAGCAGCAAAGAGAAAGGCATTCGCAGCA	302
Exemplary coding sequence for NLS11	AGAGCAGCAAAGAGAAAGTACTTCGCAGTC	303
Exemplary coding sequence for alternate SV40 NLS	CCGAAGAAGAAGAGAAGAGTC	304
exemplary Kozak sequence	gccgccRccAUGG	305
	Not Used	306-310
Cas9 ORF using low A codons of Table 5, with start and stop codons	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCC AAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACC GCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCAC CCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCC ACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTG AACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCC TCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAG AAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGAC GCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTC CTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCC TCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCTGCCCGAGAAGTACAAG	311

GAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAG
CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGAC
AACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGAC
AACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTCCCGGTTCGCCTGG
ATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAG
CGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTACTTCACCGTGTACAAC
GAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCTG
CTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATC
TCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAAC
GAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACC
TACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCGAAGCTGATC
AACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTG
ATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGCACGAGCACATCGCC
AACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCAC
AAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTCCCGGGAGCGGATGAAGCGG
ATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTAC
CTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGTCCGACTACGACGTGGACCACATC
GTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTG
CCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAAC
CTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACC
AAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACC
CTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACGCCCACGAC
GCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTG
TACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCAACATCATGAAC
TTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTG
TGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACC
GGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTAC
GGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTG
AAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAG
GTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGC
GAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGC
TCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTC
TCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAG
GCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAG
CGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCC
CAGCTGGGCGGCGACGGCGGCGCTCCCCCAAGAAGAAGCGGAAGGTGTGA

		312
Cas9 ORF using low A/U codons of Table 5, with start and stop codons	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGC	
	AAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACC	
	GCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGTACACCCGGCGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGC	
	AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCAC	
	CCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGC	
	ACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTG	
	AACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCC	
	AGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAG	
	AAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGAC	
	GCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTC	
	CTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCC	
	AGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCTGGTGCGGCAGCAGCTGCCCGAGAAGTACAAG	
	GAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAG	
	CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGAC	
	AACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGAC	
	AACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGG	
	ATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAG	
	CGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAAC	
	GAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTG	
	CTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATC	
	AGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAAC	
	GAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACC	
	TACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGAGCCGGAAGCTGATC	
	AACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTG	
	ATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGACAGCCTGCACGAGCACATCGCC	
	AACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCAC	
	AAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGG	
	ATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTAC	
	CTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGAGCGACTACGACGTGGACCACATC	
	GTGCCCCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCAAGAGCGACAACGTG	
	CCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAAC	
	CTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACC	
	AAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACC	
	CTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACGCCCACGAC	
	GCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTG	
	TACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAAC	
	TTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTG	
	TGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACC	
	GGCGGCTTCAGCAAGGAGATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTAC	

	GGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTG	
	AAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAG	
	GTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGC	
	GAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGC	
	AGCCCCGAGGACAACGAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTC	
	AGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAG	
	GCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAG	
	CGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGC	
	CAGCTGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAGCGGAAGGTGTGA	313
Cas9 ORF using low A codons of Table 5, with two C-terminal NLS sequences and start and stop codons	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCC	
	AAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACC	
	GCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC	
	AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCAC	
	CCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCC	
	ACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTG	
	AACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCC	
	TCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAG	
	AAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGAC	
	GCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTC	
	CTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCC	
	TCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCTGGTGCGGCAGCAGCTGCCCGAGAAGTACAAG	
	GAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAG	
	CCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGAC	
	AACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCTTCCTGAAGGAC	
	AACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTCCCGGTTCGCCTGG	
	ATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAG	
	CGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTACTTCACCGTGTACAAC	
	GAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCTG	
	CTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATC	
	TCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAAC	
	GAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACC	
	TACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCGGAAGCTGATC	
	AACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTG	
	ATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGCACGAGCACATCGCC	
	AACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCAC	
	AAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTCCCGGGAGCGGATGAAGCGG	
	ATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTAC	
	CTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGTCCGACTACGACGTGGACCACATC	
	GTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTG	

	CCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAAC		
	CTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACC		
	AAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACC		
	CTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACGCCCACGAC		
	GCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTG		
	TACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCAACATCATGAAC		
	TTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTG		
	TGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACC		
	GGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTAC		
	GGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTG		
	AAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAG		
	GTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGC		
	GAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGC		
	TCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTC		
	TCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAG		
	GCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCTTCAAGTACTTCGACACCACCATCGACCGGAAG		
	CGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCC		
	CAGCTGGGCGGCGACGGTCCGGCTCCCCCAAGAAGAAGCGGAAGGTGGACGGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGC		
	TGA		
	Not Used	314-328	
Nme Cas9 ORF using low A codons of Table 5, with start and stop codons	ATGGCCGCCTTCAAGCCCAACTCCATCAACTACATCCTGGGCCTGGACATCGGCATCGCCTCCGTGGGCTGGGCCATGGTGGAGATC	328	
	GACGAGGAGGAGAACCCCATCCGGCTGATCGACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACTCCCTG		
	GCCATGGCCCGGCGCGGCTGGCCCGGTCCGTGCGGCGGCTGACCCGGCGCGGCCCACCGGCTGCTGCGGACCCGGCGGCTGCTGAAG		
	CGGGAGGGCGTGCTGCAGGCCGCCAACTTCGACGAGAACGGCCTGATCAAGTCCTGCCCCAACACCCCCTGGCAGCTGCGGGCCGCC		
	GCCCTGGACCGGAAGCTGACCCCCCTGGAGTGGTCCGCCGTGCTGCTGCACCTGATCAAGCACCGGGGCTACCTGTCCCAGCGGAAG		
	AACGAGGGCGACATCGCCGACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAGACCGGCGACTTC		
	CGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGTCCGGCCACATCCCGAACCAGCGGTCCGACTACTCCCACACCTTC		
	TCCCGGAAGGACCTGCAGGCCGAGCTGATCCTGCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCCACGTGTCCGGCGGCCTGAAG		
	GAGGGCATCGAGACCCTGTGATGACCCAGCGGCCCGCCTGTCCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACCTTCGAG		
	CCCGCCGAGCCCAAGGCCGCCAAGAACACCTACACCGCCGAGCGCGGTTCATCTGGCTGACCAAGCTGAACAACCTGCGGATCCTGGAG		
	CAGGGCTCCGAGCGGCCCTGACCGACACCGAGCGGGCCACCCTGATGGACGAGCCCTACCGGAAGTCCAAGCTGACCTACGCCCAG		
	GCCCGGAAGCTGCTGGGCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGCAAGGACAACGCCGAGGCCTCCACCCTGATG		
	GAGATGAAGGCCTACCACGCCATCTCCCGGGCCCTGAAGGAGGAGTACCCCGACCTGTTCAACGAGGACAAGAAGTCCCCCCTGAAC		
	CTGCAGGACGAGATCGGCACCGCCTTCTCCCTGTTCAAGACCGACGAGGACATCACCGGCCGGCTGAAGGACCGGATCCAGCCCGAG		
	ATCCTGGAGGCCCTGCTGAAGCACATCTCCTTCGACAAGTTCGTGCAGATCTCCCTGAAGGCCCTGCGGCGGATCGTGCCCCTGATG		
	GAGCAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGACCACTACGGCAAGAAGAACACCGAGGAGAAGATCTACCTG		

	CCCCCCATCCCCGCCGACAGATCCGGAACCCCGTGGTGCTGCGGGCCCTGTCCCAGGCCCGGAAGGTGATCAACGGCGTGGTGCGG	
	CGGTACGGCTCCCCCGCCCGATCCACATCGAGACCGCCCGGGAGGTGGGCAAGTCCTTCAAGGACCGGAAGGAGATCGAGAAGCGG	
	CAGGAGGAGAACCGGAAGGACCGGGAGAAGGCCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCGTGGGCGAGCCCAAGTCCAAG	
	GACATCCTGAAGCTGCGGCTGTACGAGCAGCAGCACGGCAAGTGCCTGTACTCCGGCAAGGAGATCAACCTGGGCCGGCTGAACGAG	
	AAGGGCTACGTGGAGATCGACCACGCCCTGCCCTTCTCCCGGACCTGGGACGACTCCTTCAACAACAAGGTGCTGGTGCTGGGCTCC	
	GAGAACCAGAACAAGGCAACCAGACCCCCTACGAGTACTTCAACGGCAAGGACAACTCCCGGGAGTGGCAGGAGTTCAAGGCCCGG	
	GTGGAGACCTCCCCGGTTCCCCCGGTCCAAGAAGCAGCGGATCCTGCTGCAGAAGTTCGACGAGGACGGCTTCAAGGAGCGGAACCTG	
	AACGACACCCGGTACGTGAACCGGTTCGTGTGCCAGTTCGTGGCCGACCGGATGCGGCTGACCGGCAAGGGCAAGAAGCGGGTGTTC	
	GCCTCCAACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGGCCTGCGGAAGGTGCGGCCGAGAACGACCGGCACCACGCCCTG	
	GACGCCGTGGTGGTGGCCTGCTCCACCGTGGCCATGCAGCAGAAGATCACCCGGTTCGTGCGTACAAGGAGATGAACGCCTTCGAC	
	GGCAAGACCATCGACAAGAGACCGGCGAGGTGCTGCACCAGAAGACCCACTTCCCCCAGCCCTGGGAGTTCTTCGCCCAGGAGGTG	
	ATGATCCGGGTGTTCGGCAAGCCCGACGGCAAGGCCGACACCCTGGAGAAGCTGCGGACCCTGCTGGCCGAG	
	AAGCTGTCCTCCCGGCCCGAGGCCGTGCACGAGTACGTGACCCCCCTGTTCGTGTCCGGGCCCCCAACCGGAAGATGTCCGGCCAG	
	GGCCACATGGAGAAGATGGTGAACCGCTCCGCCAAGCGGCTGGACGAGGGCGTGTCCGTGCTGCTGCGGGTGCCCCTGACCAGCTGAAGCTGAAG	
	GACCTGGAGAAGATGGTGAACCGGGAGGCGGAGCCCAAGCTGTACGAGGCCCTGAAGGCCCGGCTGGAGGCCCACAAGGACGACCCC	
	GCCAAGGCCTTCGCCGAGCCCTTCTACAAGTACGACAAGGCCGGCAACCGGACCCAGCAGGTGAAGGCCGTGCGGGTGGAGCAGGTG	
	CAGAAGACCGGCGTGTGGTGCGGAACCACAACGGCATCGCCGACACAACGCCACCATGGTGCGGGTGGACGTGTTCGAGAAGGGCGAC	
	AAGTACTACCTGGTGCCCATCTACTCCTGGCAGGTGGCCAAGGGCATCCTGCCCGACCGGGCCGTGGTGCAGGGCAAGGACGAGGAG	
	GACTGGCAGCTGATCGACGACTCCTTCAACTTCAAGTTCTCCCTGCACCCCAACGACCTGGTGGAGGTGATCACCAAGAAGGCCCGG	
	ATGTTCGGCTACTTCGCCTCCTGCCACCGGGCACCAACATCAACATCCGGATCCACGACCTGGACCACAAGATCGGCAAGAAC	
	GGCATCCTGGAGGGCATCGGCGTGAAGACCGCCCTGTCCTTCCAGAAGTACCAGATCGACGAGCTGGGCAAGGAGATCCGGCCCTGC	
	CGGCTGAAGAAGCGGCCCCCGTGCGGTCCGGCAAGCGGACCGCCGACGGCTCCGAGTTCGAGTCCCCCAAGAAGAAGCGGAAGGTG	
	GAGTGA	
Nme Cas9 ORF using low A/U codons of Table 5, with start and stop codons	ATGGCCGCCTTCAAGCCCAACAGCATCAACTACATCCTGGGCCTGGACATCGGCATCGGCAGCGTGGGCTGGGCCATGGTGGAGATC	329
	GACGAGGAGGAGAACCCCATCCGGCTGATCGACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACAGCCTG	
	GCCATGGCCCGGCGGCTGGCCCGAGCGTGCGGCGGCTGACCCGGCGGCGGCCCACCGGCTGCTGCGGACCCGGCTGCTGAAG	
	CGGGAGGGCGTGCTGCAGGCCGCCAACTTCGACGAGAACGGCCTGATCAAGAGCCTGCCCAACACCCCTGGCAGCTGCGGGCCGCC	
	GCCCTGGACCGGAAGCTGACCCCCCTGGAGTGGAGCGCCGTGCTGCTGCACCTGATCAAGCACCGGGGCTACCTGAGCCAGCGGAAG	
	AACGAGGGCGAGACCGCCGCAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAGACCGGCGACTTC	
	CGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGAGCGGCCACATCCGGAACCAGCGGAGCGACTACAGCCACACCTTC	
	AGCCGGAAGGACCTGCAGGCCGAGCTGATCCTGCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCCACGTGAGCGGCGGCCTGAAG	
	GAGGGCATCGAGACCCTGCTGATGACCCAGCGGCCCGCCCTGAGCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACCTTCGAG	
	CCCGCCGAGCCCAAGGCCGCCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAAGCTGAACAACCTGCGGATCCTGGAG	
	CAGGGCAGCGAGCGGCCCTGACCGACACCGAGCGGGCCACCCTGATGGACGAGCCCTACCGGAAGAGCAAGCTGACCTACGCCCAG	
	GCCCGGAAGCTGCTGGGCCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGCAAGGACACGCCGAGGCCAGCACCCTGATG	
	GAGATGAAGGCCTACCACGCCATCAGCCGGGCCCTGAAGGAGGAGCTGAAGGACAAGAAGAGCCCCCTGAACCTGAGCCCCGAG	
	CTGCAGGACGAGATCGGCACCGCCTTCAGCCTGTTCAAGACCGACGAGGACATCACCGGCCGGCTGAAGGACCGGATCCAGCCCGAG	
	ATCCTGGAGGCCCTGCTGAAGCACATCAGCTTCGACAAGTTCGTGCAGATCAGCCTGAAGGCCCTGCGGCGGATCGTGCCCCTGATG	
	GAGCAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGACCACTACGGCAAGAAGAACACCGAGGAGAAGATCTACCTG	

	CCCCCCATCCCCGCCGACGATCCGGAACCCCGTGTGCTGCGGGCCCTGAGCCAGCCCGGAAGGTGATCAACGGCGTGGTGCGG	
	CGGTACGGCAGCCCCGCCCGGATCCACATCGAGACCGCCCGGGAGGTGGGCAAGAGCTTCAAGGACCGGAAGGAGATCGAGAAGCGG	
	CAGGAGGAGAACCGGAAGGACCGGCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCGTGGGCGAGCCCAAGAGCAAG	
	GACATCCTGAAGCTGCGGCTGTACGAGCAGCAGCACGGCAAGTGCCTGTACAGCGGCAAGGAGATCAACCTGGGCCGGCTGAACGAG	
	AAGGGCTACGTGGAGATCGACCACGCCCTGCCCTTCAGCCGGACCTGGGACGACAGCTTCAACAACAAGGTGCTGGTGCTGGGCAGC	
	GAGAACCAGAACAAGGCAACCCCCTACGAGTACTTCAACGGCAAGGACAACAGCCGGGAGTGGCAGGAGTTCAAGGAGCGGAACCTG	
	GTGGAGACCAGCCGGTTCCCCCGGAGCAAGAAGCAGCGGATCCTGCTGCAGAAGTTCGACGAGGACGGCTTCAAGGAGCGGAACCTG	
	AACGACACCCGGTACGTGAACCGGTTCCTGTGCCAGTTCGTGGCCGACCGGATGCGGCTGACCGGCAAGGGCAAGAAGCGGGTGTTC	
	GCCAGCAACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGGCCTGCGGAAGGTGCGGGCCGAGAACGACCGGCACCACGCCCTG	
	GACGCCGTGGTGGTGGCCTGCAGCACCGTGGCCATGCAGAAGATCACCCGGTTCGTGCGGTACAAGGAGATGAACGCCTTCGAC	
	GGCAAGACCATCGACAAGAGACCGGCGAGGTGCTGCACCAGAAGACCCACTTCCCCCAGCCCTGGGAGTTCTTCGCCCAGGAGGTG	
	ATGATCCGGGTGTTCGGCAAGCCCGACGGCAAGGCCGACACCTGGAGAAGCTGCGGACCTGCTGGCCGAG	
	AAGCTGAGCAGCCGGCCCGAGGCCGTGCACGAGTACTGACCCCCCTGTTCGTGGCCGGGCCCCCAACCGGAAGATGAGCGGCCAG	
	GGCCACATGGAGACCGTGAAGAGCGCCAAGCGGCTGGACGAGGGCGTGAGCGTGCTGCGGGTGCCCCTGACCCAGCTGAAGCTGAAG	
	GACCTGGAGAAGATGGTGAACCGGGAGCGGGAGCCCAAGCTGTACGAGGCCCTGAAGGCCCGGCTGGAGGCCCACAAGGACGACCCC	
	GCCAAGGCCTTCGCCGAGCCCTTCTACAAGTACGACAAGGCCGGCAACCGGACCCAGCAGGTGAAGGCCGTGCGGGTGGAGCAGGTG	
	CAGAAGACCGGCGTGTGGGTGCGGAACCACAACGGCATCGCCGACACAACGCCACCATGGTGCGGGTGGACGTGTTCGAGAAGGGCGAC	
	AAGTACTACCTGGTGCCCATCTACAGCTGGCAAGGGCATCCTGCCCGACCGGGCCGTGGTGCAGGGCAAGGACGAGGAG	
	GACTGGCAGCTGATCGACGACAGCTTCAACTTCAAGTTCAGCCTGCACCCCAACGACCTGGTGGAGGTGATCACCAAGAAGGCCCGG	
	ATGTTCGGCTACTTCGCCAGCTGCCACCGGGGCACCAACATCAACATCCGGATCCACGACCTGGACCACAAGATCGGCAAGAAC	
	GGCATCCTGGAGGGCATCGGCGTGAAGACCGCCCTGAGCTTCCAGAAGTACCAGATCGACGAGCTGGGCAAGGAGATCCGGCCCTGC	
	CGGCTGAAGAAGCGGCCCCCGTGCGGAGCGGCAAGCGGACCGCCGACGGCAGCGAGTTCGAGAGCCCCAAGAAGAAGCGGAAGGTG	
	GAGTGA	
	Not Used	330-345
Cas9 ORF using low A codons of Table 5 (no start or stop codons; suitable	GACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAAG AAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCGCC GAGGCCACCCGGCTGAAGCGGACCGCCGGCGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAAC GAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCACCCC ATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACC GACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAAC CCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCC GGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAG	346

for inclusion in fusion protein coding sequence)	AAGAACGGCCTGTTCGGCAACCTGATCGCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCC
	AAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTG
	GCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCC
	ATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAGAAGTACAAGGAG
	ATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCC
	ATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAAC
	GGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAAC
	CGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTCCCGGTTCGCCTGGATG
	ACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCGG
	ATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTACTTCACCGTGTACAACGAG
	CTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTG
	TTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCC
	GGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAG
	GAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTAC
	GCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCGAAGCTGATCAAC
	GGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC
	CACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGCACGAGCACATCGCCAAC
	CTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAG
	CCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTCCCGGGAGCGGATGAAGCGGATC
	GAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTG
	TACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGTCCGACTACGACGTGGACCACATCGTG
	CCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCCC
	TCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTG
	ACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAG
	CACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTG
	AAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACGCCCACGACGCC
	TACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTAC
	GACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTC
	TTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGG
	GACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACGGGC
	GGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGC
	GGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAG
	GAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG
	AAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAG
	CTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC
	CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCC
	AAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCC
	GAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGG
	TACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAG

	CTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGGAAGGTG	
Cas9 ORF using low A/U codons of Table 5 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAG AAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCGCC GAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAAC GAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCACCCC ATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACC GACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAAC CCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGC GGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAG AAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCC AAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTG GCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGC ATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAGAAGTACAAGGAG ATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCC ATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAAC GGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAAC CGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATG ACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAGCGG ATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAG CTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTG TTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGC GGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAG GAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTAC GCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGAGCCGGAAGCTGATCAAC GGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGATTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGACAGCCTGCACGAGCACATCGCCAAC CTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAG CCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATC GAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTG TACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTG CCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCC AGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTG ACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGCCAGCTGGTGGAGACCCGCCAGATCACCAAG CACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTG AAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACGCCCACGACGCC	347

	TACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTAC	
	GACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTC	
	TTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGG	
	GACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACGGGC	
	GGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGC	
	GGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAG	
	GAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG	
	AAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAG	
	CTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGC	
	CCCGAGGACAACGAGCAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGC	
	AAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCC	
	GAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGG	
	TACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAG	
	CTGGGCGGCGACAAGCGGCGGCAGCCCCAAGAAGAAGCGGAAGGTG	
Cas9 ORF using low A codons of Table 5, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAAG	348
	AAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCGCC	
	GAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCCAAC	
	GAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCACCCC	
	ATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACC	
	GACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAAC	
	CCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCC	
	GGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAG	
	AAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCCGAGGACGCC	
	AAGCTGCAGCTGTCCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTG	
	GCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGTCCGCCTCC	
	ATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAGAAGTACAAGGAG	
	ATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCC	
	ATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAAC	
	GGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAAC	
	CGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTCCCGGTTCGCCTGGATG	
	ACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCAGTCCTTCATCGAGCGG	
	ATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTACTTCACCGTGTACAACGAG	
	CTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTG	
	TTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACTCCGTGGAGATCTCC	
	GGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAG	
	GAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTAC	
	GCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGTCCCGGAAGCTGATCAAC	
	GGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC	
	CACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGACTCCCTGCACGAGCACATCGCCAAC	

	CTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAG	
	CCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACTCCCGGGAGCGGATGAAGCGGATC	
	GAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTG	
	TACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGTCCGACTACGACGTGGACCACATCGTG	
	CCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGACAAGAACCGGGGCAAGTCCGACAACGTGCCC	
	TCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTG	
	ACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAG	
	CACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTG	
	AAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACGCCCACGACGCC	
	TACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTAC	
	GACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTC	
	TTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGG	
	GACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACGGGC	
	GGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGC	
	GGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGCCAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAG	
	GAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG	
	AAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAGAACGGCCGGAAGCGATGCTGGCCTCCGCCGGCGAG	
	CTGCAGAAGGGCAACGAGCTGGCCCTCCCAAGTACGTGAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCC	
	CCCGAGGACAACGAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCC	
	AAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCC	
	GAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGG	
	TACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAG	
	CTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGGAAGGTGGACGGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGC	
	Not Used	349-355
Cas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences (no start or stop codons;	GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAG	355
	AAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCGCC	
	GAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAAC	
	GAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCACCCC	
	ATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACC	
	GACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAAC	
	CCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGC	
	GGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAG	
	AAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCC	
	AAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTG	
	GCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGC	

suitable for inclusion in fusion protein coding sequence)	ATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAGAAGTACAAGGAG ATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCC ATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAAC GGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAAC CGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAGCCGTTCGCCTGGATG ACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGTTCATCGAGCGG ATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAG CTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTG TTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGC GGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAG GAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTAC GCCCACCTGTTCGACGACAAGGTGATGAAGCAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGAGCCGAAGCTGATCAAC GGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGATTCCTTCATCAAGGACGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGACAGCCTGCACGAGCACATCGCCAAC CTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAG CCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATC GAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTG TACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTG CCCCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCC AGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTG ACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAG CACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTG AAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACGCCCACGACGCC TACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTAC GACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTC TTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGG GACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGC GGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGC GGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAG GAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG AAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAGCTGGAGAACGGCCGTAAGCGTATGCTGGCCAGCGCCGGCGAG CTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGC CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGC AAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCC GAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGG TACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAG CTGGGCGGCGACGGCAAGCCCCAAGAAGAAGGGCGGAAGGTGGACGGCAAGCCCCAAGAAGAAGCGGAAGGTGGACAAGCGGC	356
Cas9 ORF using low	GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAG AAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCGCC	

A/U codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GAGGCCACCCGGCTGAAGCGGACCGCCGCGGCGGTACACCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAAC GAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCACCCC ATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACC GACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAAC CCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGC GGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAG AAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCC AAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTG GCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGC ATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAGAAGTACAAGGAG ATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCC ATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAAC GGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAAC CGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATG ACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAGCGG ATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAG CTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTG TTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGC GGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAG GAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAGACCTAC GCCCACCTGTTCGACGACAAGGTGATGAAGCAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGAGCCGAAGCTGATCAAC GGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGCCAACCGGAACTTCATGCAGCTGATC CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGACAGCCTGCACGAGCACATCGCCAAC CTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAG CCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATC GAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTG TACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGAGCGACTACGACGTGGACCACATCGTG CCCCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTGCTGACCCGGAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCC AGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTG ACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGCCAGCTGGTGGAGACCCGGCAGATCACCAAG CACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTG AAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACTACCACCACGCCCACGACGCC TACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTAC GACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTC TTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGCCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGG GACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGC GGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGC GGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAG GAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTG

	AAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAG	
	CTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGC	
	CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGC	
	AAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCC	
	GAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGG	
	TACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAG	
	CTGGGCGGCGAC	
	Not Used	357-362
Nme Cas9 ORF using low A codons of Table 5 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GCCGCCTTCAAGCCCAACTCCATCAACTACATCCTGGGCCTGGACATCGGCATCGCCTCCGTGGGCTGGGCCATGGTGGAGATCGAC	363
	GAGGAGGAGAACCCCATCCGGCTGATCGACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACTCCCTGGCC	
	ATGGCCCGGCGGCTGGCCCGGTCCGTGCGGCGGCTGACCCGGCTGCTGCGGACCCGGCTGCTGAAGCGG	
	GAGGGCGTGCTGCAGGCCGCCAACTTCGACGAGAACGGCCTGATCAAGTCCCTGCCCAACACCCCCTGGCAGCTGCGGCCGCCGCC	
	CTGGACCGGAAGCTGACCCCCTGGAGTGGTCCGCCGTGCTGCTGCACCTGATCAAGCACCGGGGCTACCTGTCCCAGCGGAAGAAC	
	GAGGGCGAGACCGCCGACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAGACCGGCGACTTCCGG	
	ACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGTCCGGCCACATCCGGAACCAGCGGTCCGACTACCTCCACACCTTCTCC	
	GGCTCCGAGCGGCGCCCCTGACCGACACCGAGCGGGCCACCCTGATGGACGAGCCCTACCGGAAGTCCAAGCTGACCTACGCCCAGGCC	
	CGGAAGGACCTGCAGGCCGAGCTGATCCTGCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCCACGTGTCCGGCGGCCTGAAGGAG	
	GGCATCGAGACCCTGCTGATGACCCAGCGGCCCGCCCTGTCCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACCTTCGAGCCC	
	GCCGAGCCCAAGGCCGCCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAAGCTGAACAACCTGCGGATCCTGGAGCAG	
	GGCTCCGAGCGGCGCCCCTGACCGACACCGAGCGGGCCACCCTGATGGACGAGCCCTACCGGAAGTCCAAGCTGACCTACGCCCAGGCC	
	CGGAAGCTGCTGGGCCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGCAAGGACAACGCCGAGGCCTCCACCCTGATGGAG	
	ATGAAGGCCTACCACGCCATCTCCCGGGCCCTGGAGAAGGAGGGCCTGAAGGACAAGAAGTCCCCCCTGAACCTGTCCCCCGAGCTG	
	CAGGACGAGATCGGCACCGCCTTCTCCCTGTTCAAGACCGACGAGGACATCACCGGCCGCTGAAGGACCGGATCCAGCCCGAGATC	
	CTGGAGGCCCTGCTGAAGCACATCTCCTTCGACAAGTTCGTGCAGATCTCCCTGAAGGCCCTGCGGCGGATCGTGCCCCTGATGGAG	
	CAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGACCACTACGGCAAGAAGAACACCGAGGAGAAGATCTACCTGCCC	
	CCCATCCCCGCCGACGAGATCCGGAACCCCGTGGTGCTGCGGGCCCTGTCCCAGGCCCGGAAGGTGATCAACGGCGTGGTGCGGCGG	
	TACGGCTCCCCCGCCCGGATCCACATCGAGACCGCCCGGGAGGTGGGCAAGTCCTTCAAGGACCGGAAGGAGATCGAGAAGCGGCAG	
	GAGGAGAACCGGAAGGACCGGGAGAAGGCCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCGTGGGCGAGCCCAAGTCCAAGGAC	
	ATCCTGAAGCTGCGGCTGTACGAGCAGCACGGCAAGTGCCTGTACTCCGGCAAGGAGATCAACCTGGGCCGGCTGAACGAGAAG	
	GGCTACGTGGAGATCGACCACGCCCTGCCCTTCTCCCGGACCTGGGACGACTCCTTCAACAACAAGGTGCTGGTGCTGGGCTCCGAG	
	AACCAGAACAAGGGCAACCAGACCCCCTACGAGTACTTCAACGGCAAGGACAACTCCCGGGAGTGGCAGGAGTTCAAGGCCCGGGTG	
	GAGACCTCCCGGTTCCCCAAGAAGCAGCGGATCCTGCTGCAGAAGTTCGACGAGGACGGCTTCAAGGAGCGGAACCTGAAC	
	GACACCCGGTACGTGAACCGGTTCCTGTGCCAGTTCGTGGCCGACCGGATGCGGCTGACCGGCAAGGGCAAGAAGCGGGTGTTCGCC	
	TCCAACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGCCTGCGGAAGGTGCGGCCGAGAACGACCGGCACCACGCCCTGGAC	
	GCCGTGGTGGTGGCCTGCTCCACCGTGGCCATGCAGCAGAAGATCACCCGGTTCGTGCGGTACAAGGAGATGAACGCCTTCGACGGC	
	AAGACCATCGACAAGGAGACCGGCGAGGTGCTGCACCAGAAGACCCACTTCCCCCAGCCCTGGGAGTTCTTCGCCCAGGAGGTGATG	
	ATCCGGGTGTTCGGCAAGCCCGACGGCAAGCCCGAGTTCGAGGAGGCCGACACCCTGGAGAAGCTGCGGACCCTGCTGGCCGAGAAG	

	CTGTCCTCCCGGCCCGAGGCCGTGCACGAGTACGTGACCCCCCTGTTCGTGTCCCGGCCCCCAACCGGAAGATGTCCGGCCAGGGC	
	CACATGGAGACCGTGAAGTCCGCCAAGCGGCTGGACGAGGGCGTGTCCGTGCTGCCCCTGACCCAGCTGAAGCTGAAGGAC	
	CTGGAGAAGATGGTGAACCGGGAGCGGGAGCCCAAGCTGTACGAGGCCCTGAAGGCCCGGCTGGAGGCCCACAAGGACGACCCGCC	
	AAGGCCTTCGCCGAGCCTTCTACAAGTACGACAAGGCCGGCAACCCGGACCCAGCAGGTGAAGGCCGTGCGGGTGGAGCAGGTGCAG	
	AAGACCGGCGTGTGGGTGCGGAACCACAACGGCATCGCCGACAACGCCACCATGGTGCGGGTGGACGTGTTCGAGAAGGGCGACCAAG	
	TACTACCTGGTGCCCATCTACTCCTGGCAGGTGGCCAAGGGCATCCTGCCCGACCGGCCGTGGTGCAGGGCAAGGACGAGGAGGAC	
	TGGCAGCTGATCGACGACTCCTTCAACTTCAAGTTCTCCCTGCACCCCAACGACCTGGTGGAGGTGATCACCAAGAAGGCCCGGATG	
	TTCGGCTACTTCGCCTCCTGCCACCGGGGCACCGGCAACATCAACATCCGGATCCACGACCTGGACCACAAGATCGGCAAGAACGGC	
	ATCCTGGAGGGCATCGGCGTGAAGACCGCCCTGTCCTTCCAGAAGTACCAGATCGACGAGCTGGGCAAGGAGATCCGGCCCTGCCGG	
	CTGAAGAAGCGGCCCCCCGTGCGGTCCGGCAAGCGGACCGCCGACGGCTCCGAGTTCGAGTCCCCCAAGAAGAAGCGGAAGGTGGAG	
Nme Cas9 ORF using low A/U codons of Table 5 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GCCGCCTTCAAGCCCAACAGCATCAACTACATCCTGGGCCTGGACATCGGCATCGCCAGCGTGGGCTATGGTATTATCGACTAC	364
	GAGGAGGAGAACCCCATCCGGCTGACCTGGGCGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACAGCCTGGCC	
	ATGGCCCGGCGGCTGGCCCGGAGCGTGCGGCGGCTGACCCGGCTGCTGCGGACCCGGCGTGCTGAAGCGG	
	GAGGGCGTGCTGCAGGCCGCCAACTTCGACGAGAACGGCCTGATCAAGAGCCTGCCCAACACCCCCTGGCAGCTGCGGGCCGCCGCC	
	CTGGACCGGAAGCTGACCCCCCTGGAGTGGAGCGCCGTGCTGCTGCACCTGATCAAGCACCGGGGCTACCTGAGCCAGCGCGAAGAAC	
	GAGGGCGAGACCGCCGACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAGACCGGCGACTTCCGG	
	ACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGCGGCCACATCCGGAACCAGCGGAGCGACTACACGACCACACCTTCAGC	
	CGGAAGGACCTGCAGGCCGAGCTGATCCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCCACGTGAGCGGCGGCCTGAAGGAG	
	GGCATCGAGACCCTGCTGATGACCCAGCGGCCCGCCCTGAGCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACCTTCGAGCCC	
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mRNA transcript with XBG UTRs and Cas9 ORF with low A codons of Table 5	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACAAGAAGUACUCCAUCGGCCUGGACAUCGGCACCAACU	377
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transcript with XBG UTRs and Cas9 ORF with low U/A codons of Table 5	GCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGAAGGUGCCCAGCAAGAAGGUUCAAGGUGCUGGGCAACACCGACCGGCACAGCAUCA
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mRNA transcript with ORF encoding Cas9 with HiBiT tag, XBG 5′ UTR and human ALB 3′ UTR	GGGAAGCTCAGAATAAACGCTCAACTTTGGCCGGATCTCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAAC	385
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Amino acid sequence for Cas9 with NLS1	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS	386
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	FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSLAAKRSRTT	387
Amino acid sequence for Cas9 with NLS2	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSQAAKRSRTT	388
Amino acid sequence for Cas9 with NLS3	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPAPAKRERTT	389
Amino acid sequence for Cas9	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED	

with NLS4	AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK	
	EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD	
	NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN	
	ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN	
	EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL	
	IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR	
	IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV	
	PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT	
	LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN	
	FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY	
	GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG	
	ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ	
	AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSQAAKRPRTT	390
Amino acid sequence for Cas9 with NLS5	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS	
	NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL	
	NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED	
	AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK	
	EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD	
	NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN	
	ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN	
	EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL	
	IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR	
	FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY	
	GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG	
	ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ	
	AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSRAAKRPRTT	391
Amino acid sequence for Cas9 with NLS6	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRYTRRKNRICYLQEIFS	
	NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL	
	NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED	
	AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK	
	EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD	
	NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN	
	ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN	
	EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL	
	IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR	
	IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV	

	PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAKRSWSMAA	392
Amino acid sequence for Cas9 with NLS7	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAKRVWSMAF	393
Amino acid sequence for Cas9 with NLS8	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAKRSWSMAF	394
Amino acid	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRYTRRKNRICYLQEIFS	

sequence for Cas9 with NLS9	NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAKRKYFAA	395
Amino acid sequence for Cas9 with NLS10	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSRAAKRKAFAA	396
Amino acid sequence for Cas9 with NLS11	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDN EENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQL	

IHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR	
IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV	
PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT	
LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMN	
FFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY	
GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG	
ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ	
AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSRAAKRKYFAV	

* = PS linkage; 'm' = 2'-O-Me nucleotide

What is Claimed is:

1. A composition comprising:

(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 93% identity to SEQ ID NO: 311; and/or

b. the open reading frame has at least 93% identity to SEQ ID NO: 311 over at least its first 50, 200, 250, or 300 nucleotides, or at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides; and/or

c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5; and/or

d. the open reading frame has an adenine content ranging from its minimum adenine content to 123% of the minimum adenine content; and/or

e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82.

2. A method of modifying the *TTR* gene and/or inducing a double-stranded break (DSB) within the *TTR* gene, comprising delivering a composition to a cell, wherein the composition comprises:

(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 93% identity to SEQ ID NO:311; and/or

b. the open reading frame has at least 93% identity to SEQ ID NO: 311over at least its first 50, 200, 250, or 300 nucleotides, or at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides; and/or

c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5; and/or
d. the open reading frame has an adenine content ranging from its minimum adenine content to 123% of the minimum adenine content; and/or
e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82.

3. A method of reducing TTR serum concentration, treating amyloidosis associated with TTR (ATTR), and/or reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, comprising administering a composition to a subject in need thereof, wherein the composition comprises:

(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 95% identity to SEQ ID NO:311; and/or
b. the open reading frame has at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides; and/or
c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5; and/or
d. the open reading frame has an adenine content ranging from its minimum adenine content to 150% of the minimum adenine content; and/or
e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82, thereby reducing TTR serum concentration, treating amyloidosis associated with TTR (ATTR), and/or reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in the subject.

4. The composition or method of any one of claims 1-3, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 22, 23, 27, 29, 30, 35, 36, 37, 38, 55, 61, 63, 65, 66, 68, or 69.

5. The composition of claim 1 or 4, for use in inducing a double-stranded break (DSB) within the *TTR* gene in a cell or subject, modifying the *TTR* gene in a cell or subject, treating amyloidosis associated with TTR (ATTR) in a subject, reducing TTR serum concentration in a subject, or reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

6. The composition for use or method of any one of claims 2-5, wherein the method comprises administering the composition by infusion for more than 30 minutes, such as about 45-75 minutes, 75-105 minutes, 105-135 minutes, 135-165 minutes, 165-195 minutes, 195-225 minutes, 225-255 minutes, 255-285 minutes, 285-315 minutes, 315-345 minutes, 345-375 minutes, for about 1.5-6 hours, for about 60 minutes, about 90 minutes, about 120 minutes, about 150 minutes, about 180 minutes, or about 240 minutes.

7. The method or composition for use of any one of claims 2-6, wherein the composition reduces serum TTR levels, such as at least 50% as compared to serum TTR levels before administration of the composition, or by 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, 95-98%, 98-99%, or 99-100% as compared to serum TTR levels before administration of the composition.

8. The method or composition for use of any one of claims 2-7, wherein the composition results in editing of the TTR gene, optionally wherein the editing is calculated as a percentage of the population that is edited (percent editing), further optionally wherein the percent editing is between 30 and 99% of the population, such as between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the population.

9. The method or the composition for use of any one of claims 2-8, wherein the composition reduces amyloid deposition in at least one tissue, optionally wherein the at least one tissue comprises one or more of stomach, colon, sciatic nerve, or dorsal root ganglion.

10. The method or composition for use of claim 9, wherein amyloid deposition is measured 8 weeks after administration of the composition.

11. The method or composition for use of any one of claims 9-10, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and

90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen before administration of the composition.

12. The method or composition for use of any one of claims 2-11, wherein the composition is administered or delivered at least two times.

13. The method or composition for use of claim 12, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days or at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks or at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 months.

14. The method or composition of any one of claims 1-13, wherein the guide RNA comprises a crRNA that comprises the guide sequence and further comprises a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end.

15. The method or composition of any one of claims 1-14, wherein the guide RNA is a single guide (sgRNA).

16. The method or composition of claim 15, wherein the sgRNA comprises a guide sequence that has the pattern of SEQ ID NO: 3.

17. The method or composition of claim 15 or 16, wherein the sgRNA comprises the sequence of SEQ ID NO: 3.

18. The method or composition of any one of claims 15-17, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82 and the nucleotides of SEQ ID NO: 126.

19. The method or composition of any one of claims 15-17, wherein the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID Nos: 87-113, 115-120, and 122-124.

20. The method or composition of claim 19, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-113, 115-120, and 122-124.

21. The method or composition of any one of claims 1-20, wherein the guide RNA comprises at least one modification.

22. The method or composition of claim 21, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide, a phosphorothioate (PS) bond between nucleotides, or a 2'-fluoro (2'-F) modified nucleotide.

23. The method or composition of any one of claims 21-22, wherein the at least one modification includes a modification at one or more of the first five nucleotides at the 5' end and/or one or more of the last five nucleotides at the 3' end.

24. The method or composition of any one of claims 21-23, wherein the at least one modification includes PS bonds between the first four nucleotides and/or between the last four nucleotides.

25. The method or composition of any one of claims 21-24, wherein the at least one modification includes 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end and/or 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

26. The method or composition of any one of claims 21-25, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

27. The method or composition of any one of claims 1-26, wherein the guide RNA and the nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent are associated with a lipid nanoparticle (LNP).

28. The method or composition of claim 27, wherein the LNP comprises a CCD lipid, optionally wherein the CCD lipid is Lipid A or Lipid B, further optionally wherein the CCD lipid is lipid A.

29. The method or composition of any one of claims 27-28, wherein the LNP comprises a helper lipid, optionally wherein the helper lipid is cholesterol.

30. The method or composition of any one of claims 27-29, wherein the LNP comprises a stealth lipid (e.g., a PEG lipid), optionally wherein the stealth lipid is PEG2k-DMG.

31. The method or composition of any one of claims 27-30, wherein:

(i) the LNP comprises a lipid component and the lipid component comprises: about 50-60 mol-% amine lipid such as Lipid A, about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 6;

(ii) the LNP comprises about 50-60 mol-% amine lipid such as Lipid A; about 27-39.5 mol-% helper lipid; about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% stealth lipid (e.g., a PEG lipid), wherein the N/P ratio of the LNP composition is about 5-7 (e.g., about 6);

(iii) the LNP comprises a lipid component and the lipid component comprises: about 50-60 mol-% amine lipid such as Lipid A; about 5-15 mol-% neutral lipid; and about 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-10;

(iv) the LNP comprises a lipid component and the lipid component comprises: about 40-60 mol-% amine lipid such as Lipid A; about 5-15 mol-% neutral lipid; and about 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 6;

(v) the LNP comprises a lipid component and the lipid component comprises: about 50-60 mol-% amine lipid such as Lipid A; about 5-15 mol-% neutral lipid; and about 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 6;

(vi) the LNP comprises a lipid component and the lipid component comprises: about 40-60 mol-% amine lipid such as Lipid A; about 0-10 mol-% neutral lipid; and about 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-10;

(vii) the LNP comprises a lipid component and the lipid component comprises: about 40-60 mol-% amine lipid such as Lipid A; less than about 1 mol-% neutral lipid; and about 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-10;

(viii) the LNP comprises a lipid component and the lipid component comprises: about 40-60 mol-% amine lipid such as Lipid A; and about 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, wherein the N/P ratio of the LNP composition is about 3-10, and wherein the LNP composition is essentially free of or free of neutral phospholipid; or

(ix) the LNP comprises a lipid component and the lipid component comprises: about 50-60 mol-% amine lipid such as Lipid A; about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-7.

32. The method or composition of any one of claims 26-31, wherein the LNP has an N/P ratio of about 6.

33. The method or composition of claim 26-32, wherein the LNP comprises a neutral lipid, optionally wherein the neutral lipid is DSPC.

34. The method or composition of any one of claim 32, wherein the LNP comprises a lipid component and the lipid component comprises: about 50 mol-% amine lipid such as Lipid A; about 9 mol-% neutral lipd such as DSPC; about 3 mol-% of stealth lipid such as a PEG lipid, such as PEG2k-DMG, and the remainder of the lipid component is helper lipid such as cholesterol wherein the N/P ratio of the LNP composition is about 6.

35. The method or composition of any one of claims 32, wherein the LNP comprises a lipid component and the lipid component comprises: about 50 mol-% Lipid A; about 9 mol-% DSPC; about 3 mol-% of PEG2k-DMG, and the remainder of the lipid component is cholesterol wherein the N/P ratio of the LNP composition is about 6.

36. The method or composition of any one of claims 1-35, wherein the RNA-guided DNA binding agent is a Cas cleavase.

37. The method or composition of claim 36, wherein the RNA-guided DNA binding agent is Cas9.

38. The method or composition of any one of claims 1-37, wherein the RNA-guided DNA binding agent is modified, optionally wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

39. The method or composition of any one of claims 1-38, wherein the RNA-guided DNA binding agent is a Cas from a Type-II CRISPR/Cas system.

40. The method or composition of any one of claims 1-39, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

41. The method or composition for use of claim 40, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the TTR gene that induces a frame shift or nonsense mutation in the *TTR* gene.

42. The method or composition for use of claim 41, wherein a frame shift or nonsense mutation is induced in the *TTR* gene of at least 50% of liver cells.

43. The method or composition for use of any one of claims 41-42, wherein a deletion or insertion of a nucleotide(s) occurs in the *TTR* gene at least 50-fold or more than in off-target sites.

44. The method or composition of any one of claims 1-43, wherein the sequence of the guide RNA is:

a) SEQ ID NO: 92 or 104;

b) SEQ ID NO: 87, 89, 96, or 113;

c) SEQ ID NO: 100, 102, 106, 111, or 112; or

d) SEQ ID NO: 88, 90, 91, 93, 94, 95, 97, 101, 103, 108, or 109,

optionally wherein the guide RNA does not produce indels at off-target site(s) that occur in a protein coding region in the genome of primary human hepatocytes.

45. The method or composition for use of any one of claims 2-44, wherein administering the composition reduces levels of TTR in the subject.

46. The method or composition for use of any one of claims 2-45, wherein the subject has ATTR, such as ATTRwt or hereditary ATTR.

47. The method or composition for use of any one of claims 2-46, wherein the subject is human.

48. The method or composition for use of any one of claims 2-47, wherein the subject has familial amyloid polyneuropathy, only or predominantly nerve symptoms of ATTR, familial amyloid cardiomyopathy, or only or predominantly cardiac symptoms of ATTR.

49. The method or composition for use of any one of claims 2-48, wherein the subject expresses TTR having a V30 mutation, such as V30A, V30G, V30L, or V30M; the subject expresses TTR having a T60 mutation, such as T60A; the subject expresses TTR having a V122 mutation, such as V122A, V122I, or V122(-); or the subject expresses wild-type TTR.

50. The method or composition for use of any one of claims 2-49, wherein after administration the subject has an improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy, and/or the subject has an improvement, stabilization, or slowing of change in symptoms of congestive heart failure.

51. The method or composition for use of any one of claims 2-50, wherein the composition or pharmaceutical formulation is administered via a viral vector.

52. The method or composition for use of any one of claims 2-50, wherein the composition or pharmaceutical formulation is administered via lipid nanoparticles.

53. The method or composition of any one of the preceding claims, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 5, 6, 9, 13, 14, 15, 16, 17, 22, 23, 27, 30, 35, 36, 37, 38, 55, 63, 65, 66, 68, or 69.

54. The composition or method of any one of claims 1-53, wherein the open reading frame comprises a sequence with at least 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 311.

55. The composition or method of any one of claims 1-54, wherein at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons of the open reading frame are codons listed in Table 4, Table 5, or Table 7.

56. The composition or method of any one of claims 1-55, wherein the open reading frame comprises a sequence with at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 377.

57. The composition or method of any of claims 1-56, wherein the nucleic acid is an mRNA in which at least 10% of the uridine is substituted with a modified uridine.

58. The composition or method of claim 57, wherein the modified uridine is one or more of N1-methyl-pseudouridine, pseudouridine, 5-methoxyuridine, or 5-iodouridine.

59. Use of a composition or formulation of any of claims 1 or 4-58 for the preparation of a medicament for treating a human subject having ATTR.