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(54) **Title:** COMPOSITIONS AND METHODS FOR TTR GENE EDITING AND TREATING ATTR AMYLOIDOSIS COMPRISING A CORTICOSTEROID OR USE THEREOF

(57) **Abstract:** Compositions and methods for editing, e.g., introducing double-stranded breaks, within the *TTR* gene in combination with administration of a corticosteroid are provided. Compositions and methods for treating subjects having amyloidosis associated with transthyretin (ATTR), in which a guide RNA and a corticosteroid are administered, are provided.

COMPOSITIONS AND METHODS FOR TTR GENE EDITING AND TREATING ATTR AMYLOIDOSIS COMPRISING A CORTICOSTEROID OR USE THEREOF

[0001] This patent application claims priority to United States provisional application 62/825,676 filed March 28, 2019 and United States provisional application 62/825,637 filed March 28, 2019, the content of each of which is incorporated herein by reference in their 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 20, 2020, is named 2020-03-20_01155-0029-00PCT_ST25.txt and is 967 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

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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] Administration of foreign RNA can cause innate immune responses which are undesirable in the context of gene editing and therapy. Accordingly, the present disclosure provides compositions and methods for gene editing that may reduce inflammation or immune responses. For example, coadministration of corticosteroids to subjects receiving guide RNAs may reduce such inflammation or immune responses.

[0012] Accordingly, the following embodiments are provided. In some embodiments, the present invention provides compositions and methods using a corticosteroid in combination with a guide RNA and optionally 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

[0013] The following embodiments are provided herein.

[0014] Embodiment 1 is a method of treating amyloidosis associated with TTR (ATTR), comprising administering a corticosteroid and a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. 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,

thereby treating ATTR.

[0015] Embodiment 2 is a method of reducing TTR serum concentration, comprising administering a corticosteroid and a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. 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,

thereby reducing TTR serum concentration.

[0016] Embodiment 3 is a method for reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, comprising administering a corticosteroid and a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. 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,

thereby reducing accumulation of amyloids or amyloid fibrils.

[0017] Embodiment 4 is a composition comprising a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. 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,

for use in combination with a corticosteroid in a method of 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.

[0018] Embodiment 5 is a composition comprising a vector encoding a guide RNA, wherein the guide RNA comprises:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. 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,

for use in combination with a corticosteroid in a method of 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.

[0019] Embodiment 6 is a composition comprising:

(i) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. 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, and

(ii) an mRNA that encodes an RNA-guided DNA binding agent, wherein:

the open reading frame comprises a sequence with at least 95% identity to SEQ ID NO: 311;

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;
the open reading frame consists of a set of codons of which at least 75% of the codons are codons listed in Table 1;
the open reading frame has an adenine content ranging from its minimum adenine content to 150% of the minimum adenine content; and/or
the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content;

for use in combination with a corticosteroid in a method of 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.

[0020] Embodiment 7 is a composition comprising:

(i) a vector encoding a guide RNA, wherein the guide RNA comprises:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. 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, and

(ii) an mRNA that encodes an RNA-guided DNA binding agent, wherein:

the open reading frame comprises a sequence with at least 95% identity to SEQ ID NO: 311;
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;
the open reading frame consists of a set of codons of which at least 75% of the codons are codons listed in Table 1;
the open reading frame has an adenine content ranging from its minimum adenine content to 150% of the minimum adenine content; and/or
the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content;

for use in combination with a corticosteroid in a method of 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.

[0021] Embodiment 8 is the composition for use or method of any one of embodiments 1-3 or 5-7, wherein the method comprises administering the composition by infusion for more than 30 minutes, e.g. more than 60 minutes or more than 120 minutes.

[0022] Embodiment 9 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: 5-72, 74-78, and 80-82.

[0023] Embodiment 10 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 .

[0024] Embodiment 11 is the composition of any one of embodiments 4-10, for use in inducing a double-stranded break (DSB) within the *TTR* gene in a cell or subject.

[0025] Embodiment 12 is the composition of any one of embodiments 4-11, for use in modifying the *TTR* gene in a cell or subject.

[0026] Embodiment 13 is the composition of any one of embodiments 4-12, for use in treating amyloidosis associated with TTR (ATTR) in a subject.

[0027] Embodiment 14 is the composition of any one of embodiments 4-13, for use in reducing TTR serum concentration in a subject.

[0028] Embodiment 15 is the composition of any one of embodiments 4-14, for use in reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

[0029] Embodiment 16 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is dexamethasone, betamethasone, prednisone, prednisolone, methylprednisolone, cortisone, hydrocortisone, triamcinolone, or ethamethasoneb.

[0030] Embodiment 17 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is dexamethasone.

[0031] Embodiment 18 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered before the composition.

[0032] Embodiment 19 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered after the composition.

[0033] Embodiment 20 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered simultaneously with the composition.

[0034] Embodiment 21 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered about 5 minutes to within about 168 hours before the composition is administered.

[0035] Embodiment 22 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered about 5 minutes to within about 168 hours after the composition is administered.

[0036] Embodiment 23 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 18 hours, 1 day, 1.5 days, 2 days, 3 days, 4 days, 5 days, 6 days, or one week before the composition is administered.

[0037] Embodiment 24 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 18 hours, 1 day, 1.5 days, 2 days, 3 days, 4 days, 5 days, 6 days, or one week after the composition is administered.

[0038] Embodiment 25 is the method or composition for use of any one of the preceding embodiments, wherein at least two doses of the corticosteroid are administered before or after the administration of the composition.

[0039] Embodiment 26 is the method or composition for use of any one of the preceding embodiments, wherein at least two doses of the corticosteroid and at least two doses of the composition are administered.

[0040] Embodiment 27 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered to the subject at a dose of 0.75 mg to 20 mg.

[0041] Embodiment 28 is the method or composition for use of embodiment 27, wherein the corticosteroid is administered to the subject at a dose of about 0.01 – 0.4 mg/kg, such as 0.1 – 0.35 mg/kg or 0.25 – 0.35 mg/kg.

[0042] Embodiment 29 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered to the subject parenterally or by injection.

[0043] Embodiment 30 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered to the subject via an intravenous injection.

[0044] Embodiment 31 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is administered to the subject intramuscularly or by infusion.

[0045] Embodiment 32 is the method or composition for use of any one of embodiments 1-31, wherein the corticosteroid is administered to the subject orally.

[0046] Embodiment 33 is the method or composition for use of any one of embodiment 32, wherein the corticosteroid is administered to the subject orally before the composition is administered to the subject by intravenous injection.

[0047] Embodiment 34 is the method or composition for use of any one of embodiment 32, wherein the corticosteroid is administered to the subject orally after the composition is administered to the subject by intravenous injection.

[0048] Embodiment 35 is the method or composition for use of any one of embodiments 32 and 33, wherein the corticosteroid is dexamethasone, and the dexamethasone is administered to the subject orally in the amount of 20 mg 6 to 12 hour before the composition is administered to the subject.

[0049] Embodiment 36 is the method or composition for use of any one of embodiments 32, 33 or 35, wherein the corticosteroid is dexamethasone, and the dexamethasone is administered to the subject intravenously in the amount of 20 mg for 30 minutes 6 to 12 hour before the composition is administered to the subject.

[0050] Embodiment 37 is the method or composition for use of any one of the preceding embodiments, 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. In some embodiments, the composition is administered by infusion for about 1.5-6 hours.

[0051] Embodiment 38 is the method or composition for use of any one of the preceding embodiments, 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.

[0052] Embodiment 39 is the method or composition for use of any one of the preceding embodiments, wherein the composition is administered by infusion for about 120 minutes.

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[0053] Embodiment 40 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is dexamethasone.

[0054] Embodiment 41 is the method or composition for use of any one of the preceding embodiments, wherein the method further comprises administering an infusion prophylaxis, wherein the infusion prophylaxis comprises one or more of acetaminophen, an H1 blocker, or an H2 blocker, optionally wherein the one or more of the acetaminophen, H1 blocker, or H2 blocker are concurrently administered with the corticosteroid and/or before the composition.

[0055] Embodiment 42 is the method or composition for use of embodiment 41, wherein each of the acetaminophen, H1 blocker, and H2 blocker are administered.

[0056] Embodiment 42a is the method or composition for use of embodiment 41 or 42, wherein the H1 blocker and/or the H2 blocker are administered orally.

[0057] Embodiment 42b is the method or composition for use of any one of embodiments 41-42a, wherein the infusion prophylaxis comprises an intravenous corticosteroid (such as dexamethasone 8-12 mg, or 10 mg or equivalent) and acetaminophen (such as oral acetaminophen 500 mg).

[0058] Embodiment 42c is the method or composition for use of any one of embodiments 41-42b, wherein the infusion prophylaxis is administered as a required premedication prior to administering a guide RNA-containing composition, e.g. an LNP composition.

[0059] Embodiment 43 is the method or composition for use of any one of embodiments 41-42c, wherein the H1 blocker is diphenhydramine.

[0060] Embodiment 44 is the method or composition for use of any one of embodiments 41-43, wherein the H2 blocker is ranitidine.

[0061] Embodiment 45 is the method or composition for use of any one of the preceding embodiments, wherein a first dose of the corticosteroid is administered at about 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered at about 1-2 hours before the composition is administered.

[0062] Embodiment 46 is the method or composition for use of any one of the preceding embodiments, wherein a first dose of the corticosteroid is administered orally and a second dose of the corticosteroid is administered intravenously before the composition is administered.

[0063] Embodiment 47 is the method or composition for use of any one of embodiments 45 and 46, wherein the method further comprises administering one or more of acetaminophen, an H1 blocker, or an H2 blocker, optionally wherein the one or more of the acetaminophen,

H1 blocker, or H2 blocker are concurrently administered with the second dose of the corticosteroid.

[0064] Embodiment 48 is the method or composition for use of any one of the preceding embodiments, wherein a first dose of the corticosteroid is administered orally at about 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered intravenously at about 1-2 hours before the composition is administered.

[0065] Embodiment 49 is the method or composition for use of any one of the preceding embodiments, wherein a first dose of the corticosteroid is administered orally at about 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered intravenously concurrently with administration of acetaminophen, H1 blocker and H2 blocker at about 1-2 hours before the composition is administered.

[0066] Embodiment 50 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of about 6-10 mg is administered to the subject orally at about 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the amount of about 8-12 mg is intravenously administered to the subject concurrently with oral administration of acetaminophen and intravenous administration of an H1 blocker and an H2 blocker, at about 1-2 hours before the composition is administered to the subject, optionally wherein the H1 blocker is diphenhydramine and the H2 blocker is ranitidine, and/or optionally wherein the subject is human.

[0067] Embodiment 51 is the method or composition for use of any one of the preceding embodiments, wherein the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of 8 mg is administered to the subject orally at about 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the amount of 10 mg is intravenously administered to the subject concurrently with oral administration of acetaminophen and intravenous administration of an H1 blocker and an H2 blocker, at about 1-2 hours before the composition is administered to the subject, optionally wherein the H1 blocker is diphenhydramine and the H2 blocker is ranitidine.

[0068] Embodiment 52 is the method or composition for use of any one of the preceding embodiments, wherein the composition is administered in the amount of 3 mg/kg by infusion for about 1.5-6 hours; a first dose of the corticosteroid is administered orally at about 8-24 hours before infusion of the composition; and a second dose of the corticosteroid is administered intravenously at about 1-2 hours before infusion of the composition.

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[0069] Embodiment 53 is the method or composition for use of any one of the preceding embodiments, wherein administering the corticosteroid improves tolerability of the composition comprising the guide RNA.

[0070] Embodiment 54 is the method or composition for use of any one of the preceding embodiments, wherein administering the corticosteroid reduces the incidence or severity of one or more of inflammation, nausea, vomiting, elevated ALT concentration in blood, hyperthermia, and/or hyperalgesia in response to the composition comprising the guide RNA.

[0071] Embodiment 55 is the method or composition for use of any one of the preceding embodiments, wherein administering the corticosteroid reduces or inhibits production or activity of one or more interferons and/or inflammatory cytokines in response to the composition comprising the guide RNA.

[0072] Embodiment 56 is the method or composition for use of any one of the preceding embodiments, wherein the composition reduces serum TTR levels.

[0073] Embodiment 57 is the method or composition for use of embodiment 56, wherein the serum TTR levels are reduced by at least 50% as compared to serum TTR levels before administration of the composition.

[0074] Embodiment 58 is the method or composition for use of embodiment 56, 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.

[0075] Embodiment 59 is the method or composition for use of any one of the preceding embodiments, wherein the composition results in editing of the TTR gene.

[0076] Embodiment 60 is the method or composition for use of embodiment 59, wherein the editing is calculated as a percentage of the population that is edited (percent editing).

[0077] Embodiment 61 is the method or composition for use of embodiment 60, wherein the percent editing is between 30 and 99% of the population.

[0078] Embodiment 62 is the method or composition for use of embodiment 61, 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.

[0079] Embodiment 63 is the method or composition for use of any one of the preceding embodiments, wherein the composition reduces amyloid deposition in at least one tissue.

[0080] Embodiment 64 is the method or composition for use of embodiment 63, wherein the at least one tissue comprises one or more of stomach, colon, sciatic nerve, or dorsal root ganglion.

[0081] Embodiment 65 is the method or composition for use of any one of embodiments 63 and 64, wherein amyloid deposition is measured 8 weeks after administration of the composition.

[0082] Embodiment 66 is the method or composition for use of any one of embodiments 63-65, wherein amyloid deposition is compared to a negative control or a level measured before administration of the composition.

[0083] Embodiment 67 is the method or composition for use of any one of embodiments 63-66, wherein amyloid deposition is measured in a biopsy sample and/or by immunostaining.

[0084] Embodiment 68 is the method or composition for use of any one of embodiments 63-67, 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.

[0085] Embodiment 69 is the method or composition for use of any one of embodiments 63-68, 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.

[0086] Embodiment 70 is the method or composition for use of any one of the preceding embodiments, wherein the composition is administered or delivered at least two times.

[0087] Embodiment 71 is the method or composition for use of embodiment 70, wherein the composition is administered or delivered at least three times.

[0088] Embodiment 72 is the method or composition for use of embodiment 70, wherein the composition is administered or delivered at least four times.

[0089] Embodiment 73 is the method or composition for use of embodiment 70, wherein the composition is administered or delivered up to five, six, seven, eight, nine, or ten times.

[0090] Embodiment 74 is the method or composition for use of any one of embodiments 70-73, 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.

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[0091] Embodiment 75 is the method or composition for use of any one of embodiments 70-73, 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.

[0092] Embodiment 76 is the method or composition for use of any one of embodiments 70-73, 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.

[0093] Embodiment 77 is the method or composition of any one of the preceding embodiments, wherein the guide sequence is selected from SEQ ID NOs: 5-82.

[0094] Embodiment 78 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is at least partially complementary to a target sequence present in the human *TTR* gene.

[0095] Embodiment 79 is the method or composition of embodiment 78, wherein the target sequence is in exon 1, 2, 3, or 4 of the human *TTR* gene.

[0096] Embodiment 80 is the method or composition of embodiment 78, wherein the target sequence is in exon 1 of the human *TTR* gene.

[0097] Embodiment 81 is the method or composition of embodiment 78, wherein the target sequence is in exon 2 of the human *TTR* gene.

[0098] Embodiment 82 is the method or composition of embodiment 78, wherein the target sequence is in exon 3 of the human *TTR* gene.

[0099] Embodiment 83 is the method or composition of embodiment 78, wherein the target sequence is in exon 4 of the human *TTR* gene.

[00100] Embodiment 84 is the method or composition for use of any one of the preceding embodiments, wherein the guide sequence is complementary to a target sequence in the positive strand of *TTR*.

[00101] Embodiment 85 is the method or composition of any one of embodiments 1-83, wherein the guide sequence is complementary to a target sequence in the negative strand of *TTR*.

[00102] Embodiment 86 is the method or composition of any one of embodiments 1-83, wherein the first guide sequence is complementary to a first target sequence in the positive strand of the *TTR* gene, and wherein the composition further comprises a second guide sequence that is complementary to a second target sequence in the negative strand of the *TTR* gene.

[00103] Embodiment 87 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is a dual guide (dgRNA).

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[00104] Embodiment 88 is the method or composition of any one of embodiments 1-86, wherein the guide RNA is a single guide (sgRNA).

[00105] Embodiment 89 is the method or composition of embodiment 88, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-82 and nucleotides 21-100 of SEQ ID NO: 3.

[00106] Embodiment 90 is the method or composition of any one of embodiments 88 and 89, wherein the sgRNA 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: 87-124.

[00107] Embodiment 91 is the method or composition of embodiment 88, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-124.

[00108] Embodiment 92 is the method or composition of any one of the preceding embodiments, wherein the guide RNA comprises at least one modification.

[00109] Embodiment 93 is the method or composition of embodiment 92, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

[00110] Embodiment 94 is the method or composition of embodiment 92 or 93, wherein the at least one modification includes a phosphorothioate (PS) bond between nucleotides.

[00111] Embodiment 95 is the method or composition of any one of embodiments 92-94, wherein the at least one modification includes a 2'-fluoro (2'-F) modified nucleotide.

[00112] Embodiment 96 is the method or composition of any one of embodiments 92-95, wherein the at least one modification includes a 5' end modification, a 3' end modification, or 5' and 3' end modifications.

[00113] Embodiment 97 is the method or composition of any one of embodiments 92-96, wherein the at least one modification includes a modification at one or more of the first five nucleotides at the 5' end.

[00114] Embodiment 98 is the method or composition of any one of embodiments 92-97, wherein the at least one modification includes a modification at one or more of the last five nucleotides at the 3' end.

[00115] Embodiment 99 is the method or composition of any one of embodiments 92-98, wherein the at least one modification includes PS bonds between the first four nucleotides.

[00116] Embodiment 100 is the method or composition of any one of embodiments 92-99, wherein the at least one modification includes PS bonds between the last four nucleotides.

[00117] Embodiment 101 is the method or composition of any one of embodiments 92-100, wherein the at least one modification includes 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

[00118] Embodiment 102 is the the method or composition of any one of embodiments 92-101, wherein the at least one modification includes 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

[00119] Embodiment 103 is the method or composition of any one of embodiments 92-102, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

[00120] Embodiment 104 is the method or composition of any one of the preceding embodiments, wherein the composition further comprises a pharmaceutically acceptable excipient.

[00121] Embodiment 105 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is associated with a lipid nanoparticle (LNP).

[00122] Embodiment 106 is the method or composition of embodiment 105, wherein the LNP comprises an ionizable lipid.

[00123] Embodiment 107 is the method or composition of embodiment 106, wherein the LNP comprises a biodegradable ionizable lipid.

[00124] Embodiment 108 is the method or composition of any one of embodiments 105-017, wherein the LNP comprises an amine lipid, e.g., a CCD lipid.

[00125] Embodiment 109 is the method or composition of any one of embodiments 105-108, wherein the LNP comprises a helper lipid.

[00126] Embodiment 110 is the method or composition of any one of embodiments 105-109, wherein the LNP comprises a stealth lipid, optionally 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;

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(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.

[00127] Embodiment 111 is the method or composition of any one of embodiments 105-110, wherein the LNP comprises a neutral lipid.

[00128] Embodiment 112 is the method or composition of any one of embodiments 105-111, wherein the amine lipid is present at about 50 mol-%.

[00129] Embodiment 113 is the method or composition of any one of embodiments 105-112, wherein the neutral lipid is present at about 9 mol-%.

[00130] Embodiment 114 is the method or composition of any one of embodiments 105-113, wherein the stealth lipid is present at about 3 mol-%.

[00131] Embodiment 115 is the method or composition of any one of embodiments 105-114, wherein the helper lipid is present at about 38 mol-%.

[00132] Embodiment 116 is the method or composition of any one of embodiments 105-115, wherein the N/P ratio of the LNP composition is about 6.

[00133] Embodiment 117 is the method or composition of any one of embodiments 105-116, 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 lipid 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.

[00134] Embodiment 118 is the method or composition of any one of embodiments 105-117, wherein the amine lipid is Lipid A.

[00135] Embodiment 119 is the method or composition of any one of embodiments 105-118, wherein the neutral lipid is DSPC.

[00136] Embodiment 120 is the method or composition of any one of embodiments 105-119, wherein the stealth lipid is PEG2k-DMG.

[00137] Embodiment 121 is the method or composition of any one of embodiments 105-120, wherein the helper lipid is cholesterol.

[00138] Embodiment 122 is the method or composition of any one of embodiments 105-121, 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.

[00139] Embodiment 123 is the method or composition of any one of the preceding embodiments, wherein the composition further comprises an RNA-guided DNA binding agent.

[00140] Embodiment 124 is the method or composition of any one of the preceding embodiments, wherein the composition further comprises a polynucleotide that encodes an RNA-guided DNA binding agent.

[00141] Embodiment 125 is the method or composition of embodiment 124, wherein the polynucleotide is an mRNA.

[00142] Embodiment 126 is the method or composition of any one of embodiments 123-125, wherein the RNA-guided DNA binding agent is a Cas cleavase.

[00143] Embodiment 127 is the method or composition of any one of embodiments 123-126, wherein the RNA-guided DNA binding agent is a Cas from a Type-II CRISPR/Cas system.

[00144] Embodiment 128 is the method or composition of any one of embodiments 123-127, wherein the RNA-guided DNA binding agent is a Cas9.

[00145] Embodiment 129 is the method or composition of embodiment 128, wherein the RNA-guided DNA binding agent is an *S. pyogenes* Cas9 nuclease.

[00146] Embodiment 130 is the method or composition of any one of embodiments 124-129, wherein the polynucleotide comprises 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;
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;
c. the open reading frame consists of a set of codons of which at least 75% of the codons are codons listed in Table 4;
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.

[00147] Embodiment 131 is the composition or method of embodiment 130, 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.

[00148] Embodiment 132 is the composition or method of embodiment 130 or 131, 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: 311.

[00149] Embodiment 133 is the composition or method of any one of embodiments 130-132, wherein at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons of the open reading frame are codons listed in Table 4.

[00150] Embodiment 134 is the composition or method of any one of embodiments 130-133, wherein the open reading frame has an adenine content ranging from its minimum adenine content to 101%, 102%, 103%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, or 150% of the minimum adenine content.

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[00151] Embodiment 135 is the composition or method of any one of embodiments 130-134, 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.

[00152] Embodiment 136 is the composition or method of any one of embodiments 124-135, wherein the polynucleotide comprises a 5' UTR with at least 90% identity to any one of SEQ ID NOs: 232, 234, 236, 238, 241, or 275-277.

[00153] Embodiment 137 is the composition or method of any one of embodiments 124-136, wherein the polynucleotide comprises a 3' UTR with at least 90% identity to any one of SEQ ID NOs: 233, 235, 237, 239, or 240.

[00154] Embodiment 138 is the composition or method of any one of embodiments 124-137, wherein the polynucleotide comprises a 5' UTR and a 3' UTR from the same source.

[00155] Embodiment 139 is the composition or method of any one of embodiments 124-138, wherein the polynucleotide comprises a 5' cap selected from Cap0, Cap1, and Cap2.

[00156] Embodiment 140 is the composition or method of any one of embodiments 124-139, 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: 311.

[00157] Embodiment 141 is the composition or method of any of embodiments 125-140, wherein at least 10% of the uridine in the mRNA is substituted with a modified uridine.

[00158] Embodiment 142 is the composition or method of embodiment 141, wherein the modified uridine is one or more of N1-methyl-pseudouridine, pseudouridine, 5-methoxyuridine, or 5-iodouridine.

[00159] Embodiment 143 is the composition or method of embodiment 141, wherein the modified uridine is one or both of N1-methyl-pseudouridine or 5-methoxyuridine.

[00160] Embodiment 144 is the composition or method of embodiment 141, wherein the modified uridine is N1-methyl-pseudouridine.

[00161] Embodiment 145 is the composition or method of embodiment 141, wherein the modified uridine is 5-methoxyuridine.

[00162] Embodiment 146 is the composition or method of any one of embodiments 141-145, wherein 15% to 45% of the uridine is substituted with the modified uridine.

[00163] Embodiment 147 is the composition or method of any one of embodiments 141-146, wherein at least 20% or at least 30% of the uridine is substituted with the modified uridine.

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[00164] Embodiment 148 is the composition or method of embodiment 147, wherein at least 80% or at least 90% of the uridine is substituted with the modified uridine.

[00165] Embodiment 149 is the composition or method of embodiment 147, wherein 100% of the uridine is substituted with the modified uridine.

[00166] Embodiment 150 is the method or composition of any one of embodiments 123-149, wherein the RNA-guided DNA binding agent is modified.

[00167] Embodiment 151 is the method or composition of embodiment 150, wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

[00168] Embodiment 152 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.

[00169] Embodiment 153 is the method or composition for use of any one of the preceding embodiments, wherein the composition reduces or prevents amyloids or amyloid fibrils comprising TTR.

[00170] Embodiment 154 is the method or composition for use of embodiment 153, wherein the amyloids or amyloid fibrils are in the nerves, heart, or gastrointestinal track.

[00171] Embodiment 155 is the method or composition for use of any one of the preceding embodiments, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the *TTR* gene.

[00172] Embodiment 156 is the method or composition for use of embodiment 155, wherein NHEJ leads to a deletion or insertion of a nucleotide(s) during repair of a DSB in the *TTR* gene.

[00173] Embodiment 157 is the method or composition for use of embodiment 156, wherein the deletion or insertion of a nucleotide(s) induces a frame shift or nonsense mutation in the *TTR* gene.

[00174] Embodiment 158 is the method or composition for use of embodiment 155 or 156, wherein a frame shift or nonsense mutation is induced in the *TTR* gene of at least 50% of liver cells.

[00175] Embodiment 159 is the method or composition for use of embodiment 158, 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.

[00176] Embodiment 160 is the method or composition for use of any one of embodiments 156-159, 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.

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[00177] Embodiment 161 is the method or composition for use of embodiment 160, 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.

[00178] Embodiment 162 is the method or composition for use of any one of embodiments 156-161, 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.

[00179] Embodiment 163 is the method or composition for use of embodiment 162, 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.

[00180] Embodiment 164 is the method or composition for use of embodiment 163, wherein the Cas9-overexpressing cells are HEK293 cells stably expressing Cas9.

[00181] Embodiment 165 is the method or composition for use of any one of embodiments 162-164, 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.

[00182] Embodiment 166 is the method or composition for use of any one of embodiments 162-164, 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.

[00183] Embodiment 167 is the method or composition of any one of the preceding embodiments, wherein the sequence of the guide RNA is:

a) SEQ ID NO: 92 or 104;

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

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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,

[00184] 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.

[00185] Embodiment 168 is the method or composition for use of any one of the preceding embodiments, wherein administering the composition reduces levels of TTR in the subject.

[00186] Embodiment 169 is the method or composition for use of embodiment 168, wherein the levels of TTR are reduced by at least 50%.

[00187] Embodiment 170 is the method or composition for use of embodiment 169, wherein the levels of TTR are reduced by 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100%.

[00188] Embodiment 171 is the method or composition for use of embodiment 168 or 169, wherein the levels of TTR are measured in serum, plasma, blood, cerebral spinal fluid, or sputum.

[00189] Embodiment 172 is the method or composition for use of embodiment 168 or 169, wherein the levels of TTR are measured in liver, choroid plexus, and/or retina.

[00190] Embodiment 173 is the method or composition for use of any one of embodiments 168-172, wherein the levels of TTR are measured via enzyme-linked immunosorbent assay (ELISA).

[00191] Embodiment 174 is the method or composition for use of any one of the preceding embodiments, wherein the subject has ATTR.

[00192] Embodiment 175 is the method or composition for use of any one of the preceding embodiments, wherein the subject is human.

[00193] Embodiment 176 is the method or composition for use of embodiment 174 or 175, wherein the subject has ATTRwt.

[00194] Embodiment 177 is the method or composition for use of embodiment 174 or 175, wherein the subject has hereditary ATTR.

[00195] Embodiment 178 is the method or composition for use of any one of the preceding embodiments, wherein the subject has a family history of ATTR.

[00196] Embodiment 179 is the method or composition for use of any one of the preceding embodiments , wherein the subject has familial amyloid polyneuropathy.

[00197] Embodiment 180 is the method or composition for use of any one of the preceding embodiments , wherein the subject has only or predominantly nerve symptoms of ATTR.

[00198] Embodiment 181 is the method or composition for use of any one of embodiments 1-179, wherein the subject has familial amyloid cardiomyopathy.

[00199] Embodiment 182 is the method or composition for use of any one of embodiments 1-179 or 181, wherein the subject has only or predominantly cardiac symptoms of ATTR.

[00200] Embodiment 183 is the method or composition for use of any one of the preceding embodiments, wherein the subject expresses TTR having a V30 mutation.

[00201] Embodiment 184 is the method or composition for use of embodiment 183, wherein the V30 mutation is V30A, V30G, V30L, or V30M.

[00202] Embodiment 185 is the method or composition for use of embodiment any one of the preceding embodiments, wherein the subject expresses TTR having a T60 mutation.

[00203] Embodiment 186 is the method or composition for use of embodiment 185, wherein the T60 mutation is T60A.

[00204] Embodiment 187 is the method or composition for use of embodiment any one of the preceding embodiments, wherein the subject expresses TTR having a V122 mutation.

[00205] Embodiment 188 is the method or composition for use of embodiment 187, wherein the V122 mutation is V122A, V122I, or V122(-).

[00206] Embodiment 189 is the method or composition for use of any one of the preceding embodiments, wherein the subject expresses wild-type TTR.

[00207] Embodiment 190 is the method or composition for use of any one of embodiments 1-182 or 189, wherein the subject does not express TTR having a V30, T60, or V122 mutation.

[00208] Embodiment 191 is the method or composition for use of any one of embodiments 1-182 or 189-190, wherein the subject does not express TTR having a pathological mutation.

[00209] Embodiment 192 is the method or composition for use of any one of embodiments 190-192, wherein the subject is homozygous for wild-type *TTR*.

[00210] Embodiment 193 is the method or composition for use of any one of the preceding embodiments, wherein after administration the subject has an improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy.

[00211] Embodiment 194 is the method or composition for use of embodiment 193, wherein the improvement, stabilization, or slowing of change in sensory neuropathy is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

[00212] Embodiment 195 is the method or composition for use of any one of the preceding embodiments, wherein the subject has an improvement, stabilization, or slowing of change in symptoms of congestive heart failure.

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[00213] Embodiment 196 is the method or composition for use of embodiment 195, 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.

[00214] Embodiment 197 is the method or composition for use of any one of the preceding embodiments, wherein the composition or pharmaceutical formulation is administered via a viral vector.

[00215] Embodiment 198 is the method or composition for use of any one of the preceding embodiments, wherein the composition or pharmaceutical formulation is administered via lipid nanoparticles.

[00216] Embodiment 199 is the method or composition for use of any one of the preceding embodiments, wherein the subject is tested for specific mutations in the *TTR* gene before administering the composition or formulation.

[00217] Embodiment 200 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 5.

[00218] Embodiment 201 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 6.

[00219] Embodiment 202 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 7.

[00220] Embodiment 203 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 8.

[00221] Embodiment 204 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 9.

[00222] Embodiment 205 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 10.

[00223] Embodiment 206 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 11.

[00224] Embodiment 207 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 12.

[00225] Embodiment 208 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 13.

[00226] Embodiment 209 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 14.

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[00227] Embodiment 210 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 15.

[00228] Embodiment 211 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 16.

[00229] Embodiment 212 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 17.

[00230] Embodiment 213 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 18.

[00231] Embodiment 214 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 19.

[00232] Embodiment 215 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 20.

[00233] Embodiment 216 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 21.

[00234] Embodiment 217 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 22.

[00235] Embodiment 218 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 23.

[00236] Embodiment 219 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 24.

[00237] Embodiment 220 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 25.

[00238] Embodiment 221 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 26.

[00239] Embodiment 222 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 27.

[00240] Embodiment 223 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 28.

[00241] Embodiment 224 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 29.

[00242] Embodiment 225 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 30.

[00243] Embodiment 226 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 31.

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[00244] Embodiment 227 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 32.

[00245] Embodiment 228 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 33.

[00246] Embodiment 229 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 34.

[00247] Embodiment 230 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 35.

[00248] Embodiment 231 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 36.

[00249] Embodiment 232 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 37.

[00250] Embodiment 233 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 38.

[00251] Embodiment 234 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 39.

[00252] Embodiment 235 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 40.

[00253] Embodiment 236 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 41.

[00254] Embodiment 237 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 42.

[00255] Embodiment 238 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 43.

[00256] Embodiment 239 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 44.

[00257] Embodiment 240 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 45.

[00258] Embodiment 241 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 46.

[00259] Embodiment 242 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 47.

[00260] Embodiment 243 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 48.

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[00261] Embodiment 244 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 49.

[00262] Embodiment 245 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 50.

[00263] Embodiment 246 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 51.

[00264] Embodiment 247 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 52.

[00265] Embodiment 248 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 53.

[00266] Embodiment 249 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 54.

[00267] Embodiment 250 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 55.

[00268] Embodiment 251 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 56.

[00269] Embodiment 252 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 57.

[00270] Embodiment 253 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 58.

[00271] Embodiment 254 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 59.

[00272] Embodiment 255 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 60.

[00273] Embodiment 256 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 61.

[00274] Embodiment 257 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 62.

[00275] Embodiment 258 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 63.

[00276] Embodiment 259 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 64.

[00277] Embodiment 260 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 65.

[00278] Embodiment 261 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 66.

[00279] Embodiment 262 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 67.

[00280] Embodiment 263 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 68.

[00281] Embodiment 264 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 69.

[00282] Embodiment 265 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 70.

[00283] Embodiment 266 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 71.

[00284] Embodiment 267 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 72.

[00285] Embodiment 268 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 73.

[00286] Embodiment 269 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 74.

[00287] Embodiment 270 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 75.

[00288] Embodiment 271 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 76.

[00289] Embodiment 272 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 77.

[00290] Embodiment 273 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 78.

[00291] Embodiment 274 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 79.

[00292] Embodiment 275 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 80.

[00293] Embodiment 276 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 81.

[00294] Embodiment 277 is the method or composition of any one of embodiments 1-199, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 82.

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[00295] Embodiment 278 is a use of a composition or formulation of any of the preceding embodiments for the preparation of a medicament for treating a human subject having ATTR.

BRIEF DESCRIPTION OF THE DRAWINGS

[00296] FIG. 1 shows a schematic of chromosome 18 with the regions of the TTR gene that are targeted by the guide sequences provided in Table 1.

[00297] FIG. 2 shows off-target analysis in HEK293_Cas9 cells of certain dual guide RNAs targeting TTR. The on-target site is designated by a filled square for each dual guide RNA tested, whereas closed circles represent a potential off-target site.

[00298] FIG. 3 shows off-target analysis in HEK_Cas9 cells of certain single guide RNAs targeting TTR. The on-target site is designated by a filled square for each single guide RNA tested, whereas open circles represent a potential off-target site.

[00299] FIG. 4 shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary human hepatocytes.

[00300] FIG. 5 shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary cyno hepatocytes.

[00301] FIG. 6 shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary cyno hepatocytes.

[00302] FIG. 7 shows percent editing (% edit) of TTR and reduction of secreted TTR following administration of the guide in HUH7 cells sequences provided on the x-axis. The values are normalized to the amount of alpha-1-antitrypsin (AAT) protein.

[00303] FIG. 8 shows western blot analysis of intracellular TTR following administration of targeted guides (listed in Table 1) in HUH7 cells.

[00304] FIG. 9 shows percentage liver editing of TTR observed following administration of LNP formulations to mice with humanized (G481-G499) or murine (G282) TTR. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and 3.

[00305] FIGS. 10A-B show serum TTR levels observed following the dosing regimens indicated on the horizontal axis as µg/ml (FIG. 10A) or percentage of TSS control (FIG. 10B). MPK = mg/kg throughout.

[00306] FIGS. 11A-B show serum TTR levels observed following the dosing regimens indicated on the horizontal axis for 1 mg/kg (FIG. 11A) or 0.5 mg/kg dosages (FIG. 11B). Data for a single 2 mg/kg dose is included as the right column in both panels.

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[00307] FIGS. 12A-B show percentage liver editing observed following the dosing regimens indicated on the horizontal axis for 1 mg/kg (FIG. 12A) or 0.5 mg/kg dosages (FIG. 12B). FIG. 12C shows percentage liver editing observed following a single dose at 0.5, 1, or 2 mg/kg.

[00308] FIG. 13 shows percent liver editing observed following administration of LNP formulations to mice humanized with respect to the TTR gene. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and 3.

[00309] FIGS. 14A-B show that there is correlation between liver editing (FIG. 14A) and serum human TTR levels (FIG. 14B) following administration of LNP formulations to mice humanized with respect to the TTR gene. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and 3.

[00310] FIGS. 15A-B show that there is a dose response with respect to percent editing (FIG. 15A) and serum TTR levels (FIG. 15B) in wild type mice following administration of LNP formulations comprising guide G502, which is cross homologous between mouse and cyno.

[00311] FIG. 16 shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary cyno hepatocytes.

[00312] FIG. 17 shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary human hepatocytes.

[00313] FIG. 18 shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary cyno hepatocytes.

[00314] FIGS. 19A-D show serum TTR (% TSS; FIGs. 19A and 19C) and editing results following dosing of LNP formulations at the indicated ratios and amounts (FIGs. 19B and 19D).

[00315] FIG. 20 shows off-target analysis of certain single guide RNAs in Primary Human Hepatocytes (PHH) targeting TTR. In the graph, filled squares represent the identification of the on-target cut site, while open circles represent the identification of potential off-target sites.

[00316] FIGS. 21A-B show percent editing on-target (ONT, FIG. 21A) and at two off-target sites (OT2 and OT4) in primary human hepatocytes following administration of lipid nanoparticle formulated G000480. FIG. 21B is a re-scaled version of the OT2, OT4, and negative control (Neg Cont) data in FIG. 21A.

[00317] FIGS. 22A-B show percent editing on-target (ONT, FIG. 22A) and at an off-target site (OT4) in primary human hepatocytes following administration of lipid nanoparticle formulated G000486. FIG. 22B is a re-scaled version of the OT4 and negative control (Neg Cont) data in FIG. 22A.

[00318] FIGS. 23A-B show percent editing (FIG. 23A) and number of insertion and deletion events at the TTR locus (FIG. 23B). FIG. 23A shows percent editing at the TTR locus in control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) groups. FIG. 23B shows the number of insertion and deletion events at the TTR locus when editing was observed in the treatment group of FIG. 23A.

[00319] FIGS. 24A-B show TTR levels in circulating serum (FIG. 24A) and cerebrospinal fluid (CSF) (FIG. 24B), respectively, in μg/mL for control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) groups. Treatment resulted in >99% knockdown of TTR levels in serum.

[00320] FIGS. 25A-D show immunohistochemistry images with staining for TTR in stomach (FIG. 25A), colon (FIG. 25B), sciatic nerve (FIG. 25C), and dorsal root ganglion (DRG) (FIG. 25D) from control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) mice. At right, bar graphs show reduction in TTR staining 8 weeks after treatment in treated mice as measured by percent occupied area for each tissue type.

[00321] FIGS. 26A-C show liver TTR editing (FIG. 26A) and serum TTR results (in μg/mL (FIG. 26B) and as percentage of TSS-treated control (FIG. 26C)), respectively, from humanized TTR mice dosed with LNP formulations across a range of doses with guides G000480, G000488, G000489 and G000502 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide.

[00322] FIGS. 27A-C show liver TTR editing (FIG. 27A) and serum TTR results (in μg/mL (FIG. 27B) and as percentage of TSS-treated control (FIG. 27C)), respectively, from humanized TTR mice dosed with LNP formulations across a range of doses with guides G000481, G000482, G000486 and G000499 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide.

[00323] FIGS. 28A-C show liver TTR editing (FIG. 28A) and serum TTR results (in μg/mL (FIG. 28B) and as percentage of TSS-treated control (FIG. 28C)), respectively, from humanized TTR mice dosed with LNP formulations across a range of doses with guides G000480, G000481, G000486, G000499 and G000502 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:2 ratio by weight to the guide.

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[00324] FIG. 29 shows relative expression of TTR mRNA in primary human hepatocytes (PHH) after treatment with LNPs comprising Cas9 mRNA and a gRNA as indicated, as compared to negative (untreated) controls.

[00325] FIG. 30 shows relative expression of TTR mRNA in primary human hepatocytes (PHH) after treatment with LNPs comprising Cas9 mRNA and a gRNA as indicated, as compared to negative (untreated) controls.

[00326] FIGS. 31A-C show serum TTR levels (FIG. 31A), liver TTR editing (FIG 31B), and circulating ALT levels (FIG. 31C) in an *in vivo* study in nonhuman primates comparing 30' administration of LNPs to a long dosing protocol.

DETAILED DESCRIPTION

[00327] 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.

[00328] 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.

[00329] 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.

[00330] 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.

[00331] 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

[00332] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[00333] "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,

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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.

[00334] "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.

[00335] "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.

[00336] 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. For example, in some embodiments, the guide sequence comprises at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82. In some embodiments, the target sequence is in a gene or on a chromosome, for example,

and is complementary to the guide sequence. In some embodiments, the degree of complementarity or identity between a guide sequence and its corresponding target sequence may be about 75%, 80%, 85%, 88%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. For example, in some embodiments, the guide sequence comprises a sequence with about 75%, 80%, 85%, 88%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82. 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.

[00337] 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.

[00338] 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).

[00339] "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.

[00340] "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.

[00341] 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.

[00342] "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.

[00343] 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.

[00344] 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

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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.

[00345] 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.

[00346] 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.

[00347] As used herein, "TTR" refers to transthyretin, which is the gene product of a *TTR* gene.

[00348] 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.

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[00349] 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.

[00350] 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.

[00351] As used herein, "ATTR," "TTR-related amyloidosis," "TTR amyloidosis," "ATTR amyloidosis," or "amyloidosis associated with TTR" refers to amyloidosis associated with deposition of TTR.

[00352] 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.

[00353] 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.

[00354] 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.

[00355] Guide sequences useful in the guide RNA compositions and methods described herein are shown in Table 1 and throughout the application.

[00356] 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(-).

[00357] 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.

[00358] 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).

[00359] As used herein, "knockout" refers to a loss of expression of a particular protein in a cell. Knockout can be measured either by detecting the amount of protein secretion from a tissue or population of cells (e.g., in serum or cell media) or by detecting total cellular amount of a protein a tissue or a population of cells. In some embodiments, methods are provided to "knockout" TTR in one or more cells (e.g., in a population of cells including *in vivo* populations such as those found in tissues). In some embodiments, a knockout is not the formation of mutant TTR protein, for example, created by indels, but rather the complete loss of expression of TTR protein in a cell.

[00360] 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(-).

[00361] As used herein, "mutant *TTR*" or "mutant *TTR* allele" refers to a *TTR* sequence having a change in the nucleotide sequence of *TTR* compared to the wildtype sequence (NCBI Gene ID: 7276; Ensembl: ENSG00000118271).

[00362] 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.

[00363] 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.

[00364] 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.

[00365] 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.

[00366] 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. Emulsions, micelles, and suspensions may be suitable compositions for local and/or topical delivery. 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.

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[00367] 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.

[00368] 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.

[00369] 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.

[00370] 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.

[00371] 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.

[00372] 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.

II. Methods and Compositions Targeting the *TTR* gene

[00373] Disclosed herein are methods for 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. A corticosteroid, guide RNA, RNA-guided DNA binding agent, or polynucleotide encoding an RNA-guided DNA binding agent, such as any of those described herein, is also provided for use in a method disclosed herein. For example, in some embodiments, the disclosed compositions such as LNP compositions comprise a guide RNA targeting TTR and, optionally, an RNA-guided DNA binding agent or a nucleic acid comprising an open reading frame encoding such 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.

[00374] The dosage, frequency and mode of administration of the corticosteroid, infusion prophylaxis, and the guide-RNA containing composition described herein can be controlled independently.

[00375] 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.

[00376] The guide RNA-containing composition, e.g. an LNP composition comprising a guide RNA and optionally a polynucleotide encoding an RNA-guided DNA binding agent, may be administered by infusion. 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.

[00377] In some embodiments, the corticosteroid is administered about 5 minutes to within about 168 hours before the administration of the guide RNA-containing composition described herein. In some embodiments, the corticosteroid is administered about 5 minutes to within about 168 hours after the administration of the guide RNA-containing composition described herein. In some embodiments, the corticosteroid is administered 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 18 hours, 24 hours, 36 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, 168 hours, or an amount of time in a range bounded by any two of the preceding values before the administration of the guide RNA-containing composition described herein. In some embodiments, the corticosteroid is administered 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 18 hours, 24 hours, 36 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours 168 hours, or an amount of time in a range bounded by any two of the preceding values after the administration of the guide RNA-containing composition described herein. In certain embodiments, a corticosteroid is delivered about 8-24 hours before administration of the guide RNA-containing composition and an infusion prophylaxis is administered 1-2 hours prior to administration of the guide RNA-containing composition. The corticosteroid may be administered with or at about the same time as the administration of the guide RNA-

containing composition described herein.

[00378] 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.

[00379] 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 parenteral and a second or subsequent dose may be by oral administration.

[00380] 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.

A. Corticosteroid; Infusion Prophylaxis

[00381] The corticosteroid used in the disclosed methods and compositions is useful for treating subjects undergoing gene editing and/or therapy with gene editing compositions. Without wishing to be bound to any particular theory, corticosteroids may be useful for reducing inflammation or immune responses to foreign RNAs (guide RNA or mRNAs encoding RNA-guided DNA binding agent). The corticosteroid used in the disclosed methods and compositions may be any of those known in the art and/or commercially available from a number of sources.

[00382] In some embodiments, an infusion prophylaxis is administered to a subject before the gene editing composition, e.g., at a time 1-2 hours prior to the administration of the gene editing composition. In some embodiments, the infusion prophylaxis comprises one or more, or all, of an intravenous corticosteroid (e.g., dexamethasone 8-12 mg, such as 10 mg or equivalent, or any of the other corticosteroids described elsewhere herein), an antipyretic (e.g. oral acetaminophen (also called paracetamol) 500 mg), an H1 blocker (e.g., diphenhydramine 50 mg or equivalent), an H2 blocker (e.g., ranitidine 50 mg or equivalent). 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 intravenous H1 blocker and/or an intravenous H2 blocker is substituted with an equivalent, e.g., an orally administered equivalent. Additionally or alternatively, an oral corticosteroid (e.g., dexamethasone 6-10 mg, such as 8 mg or equivalent, or any of the other corticosteroids described elsewhere herein) may be administered, e.g., 8-24 hours prior to treatment. These dosages may be used, e.g., when the subject is a human, e.g., an adult human. In some embodiments, the infusion prophylaxis consists of the following: an intravenous corticosteroid (e.g., dexamethasone 10 mg or equivalent) which may reduce the severity of inflammation, oral acetaminophen 500 mg which may reduce pain and fever and/or inhibit COX enzymes and/or prostaglandins, intravenous H1 blocker (e.g., diphenhydramine 50 mg or equivalent), and intravenous H2 blocker (e.g., ranitidine 50 mg, or equivalent) which act to block the action of histimine at the H1 and H2 receptors respectively, and may optionally be

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preceded by administration of oral dexamethasone (such as in the amount of 8 mg or equivalent), e.g., at 8-24 hours prior to the administration of the gene editing composition. The infusion prophylaxis may function to reduce adverse reactions associated with administering a guide RNA-containing composition, e.g. an LNP composition. In some embodiments, the corticosteroid and/or infusion prophylaxis is administered as a required premedication prior to administering a guide RNA-containing composition, e.g. an LNP composition.

[00383] In some embodiments, the corticosteroid is concurrently administered with one or more of acetaminophen, H1 blocker, or H2 blocker. In some embodiments, the corticosteroid is concurrently administered with acetaminophen and H1 blocker. In some embodiments, the the corticosteroid is concurrently administered with acetaminophen and H2 blocker. In some embodiments, the the corticosteroid is concurrently administered with H1 blocker and H2 blocker. In some embodiments, an H1 blocker and/or an H2 blocker are administered orally. In some embodiments, the composition is concurrently administered with acetaminophen, H1 blocker, and H2 blocker.

[00384] Many H1 and H2 blockers are known in the art. In some embodiments, the H1 blocker is diphenhydramine, clemastine, cetirizine, terfenadine, doxylamine, mirtazapine, dexbrompheniramine, triprolidine, cyproheptadine, loratadine, hydroxyzine, cinnarizine, astemizole, azatadine, meclizine, carbinoxamine, epinastine, olopatadine, tripelennamine, brompheniramine, ketotifen, fexofenadine, desloratadine, azelastine, dimenhydrinate, promethazine, mequitazine, emedastine, levocabastine, chlorpheniramine, cyclizine, alimemazine, phenindamine, pheniramine, methapyrilene, flunarizine, mianserin, levocetirizine, esmirtazapine, mepyramine, alcaftadine, antazoline, chloropyramine, dimetindene, dimetotiazine, acrivastine, dexchlorpheniramine maleate, ebastine, mizolastine, gsk-1004723, oxatomide, dexchlorpheniramine, bepotastine, buclizine, risperidone, methdilazine, maprotiline, diphenylpyraline, bromodiphenhydramine, ziprasidone, olanzapine, clozapine, promazine, trazodone, doxepin, desipramine, orphenadrine, methotrimeprazine, clofedanol, chlorprothixene, quetiapine, asenapine, benzatropine, aripiprazole, amitriptyline, imipramine, nortriptyline, trimipramine, isothipendyl, chlorpromazine, iloperidone, zuclopenthixol, chlorcyclizine, amoxapine, butriptyline, cariprazine, bilastine, dosulepin, rupatadine, pizotifen, thonzylamine, benzquinamide, propiomazine, aceprometazine, aripiprazole lauroxil, or deptropine.

[00385] In some embodiments, the H2 blocker is ranitidine, nizatidine, cimetidine, or famotidine. Equivalent corticosteroids and dosages can be found, for example, in Liu et al.,

Allergy, Asthma & Clinical Immunology, 2013, 9:30. Equivalent antihistamines (H1 blockers and/or H2 blockers) and dosages include the customary dose for a suitable member of the class, as known in the art.

[00386] In some embodiments, at least two doses of the corticosteroid are administered before the administration of the composition. In some embodiments, a first dose of the corticosteroid is administered before a second dose of the corticosteroid is administered before the composition is administered. In some embodiments, a first dose of the corticosteroid is administered within 8-24 hours before the composition is administered. In some embodiments, a first dose of the corticosteroid is administered orally within 8-24 hours before the composition is administered. In some embodiments, a second dose of the corticosteroid is administered within 1-2 hours before the composition is administered. In some embodiments, a second dose of the corticosteroid is administered intravenously within 1-2 hours before the composition is administered. In some embodiments, a first dose of the corticosteroid is administered within 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered within 1-2 hours before the composition is administered.

[00387] In some embodiments, a first dose of the corticosteroid is administered orally and a second dose of the corticosteroid is administered intravenously before the composition is administered. In some embodiments, a first dose of the corticosteroid is administered orally within 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered intravenously within 1-2 hours before the composition is administered.

[00388] In some embodiments, a first dose of the corticosteroid is administered orally and a second dose of the corticosteroid is concurrently administered with one or more of acetaminophen, H1 blocker, or H2 blocker before the composition is administered. In some embodiments, a first dose of the corticosteroid is administered orally and a second dose of the corticosteroid is concurrently administered with acetaminophen, H1 blocker and H2 blocker before the composition is administered. In some embodiments, a first dose of the corticosteroid is administered orally within 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered intravenously concurrently administered with one or more of acetaminophen, H1 blocker or H2 blocker within 1-2 hours before the composition is administered. In some embodiments, a first dose of the corticosteroid is administered orally within 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered intravenously concurrently

administered with acetaminophen, H1 blocker and H2 blocker within 1-2 hours before the composition is administered. In some embodiments, a first dose of the corticosteroid is administered orally within 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered intravenously concurrently administered with acetaminophen, H1 blocker and H2 blocker within 1-2 hours before the composition is administered, wherein the acetaminophen is administered orally and the H1 blocker and H2 blocker are administered intravenously.

[00389] In some embodiments, administering the corticosteroid improves tolerability of the composition comprising the guide RNA. For example, compared to administration of the composition comprising the guide RNA without the corticosteroid, administering the corticosteroid may reduce the incidence or severity of one or more adverse effects, such as inflammation, nausea, vomiting, elevated ALT concentration in blood, hyperthermia, and/or hyperalgesia. In some embodiments, administering the corticosteroid reduces or inhibits production or activity of one or more interferons and/or inflammatory cytokines in response to the composition comprising the guide RNA.

[00390] Exemplary corticosteroids include, but are not limited to, dexamethasone, betamethasone, prednisone, prednisolone, methylprednisolone, cortisone, hydrocortisone, triamcinolone, or ethamethasone, or a pharmaceutically acceptable salt thereof. Exemplary corticosteroids include, but are not limited to, dexamethasone, betamethasone, prednisone (Rayos®, Horizon Pharma), prednisolone (Pred Forte®, Allergan; Omnipred™, Novartis) methylprednisolone (Medrol®, Pharmacia&Upjohn; Solu-Medrolx®, Pharmacia&Upjohn), cortisone, hydrocortisone, triamcinolone, ethamethasone, budesonide (ENTOCORT®, Perrigo Pharma Intl.; Rhinocort®, Symbicort®, Astrazeneca Pharms; Ulceris®, Valeant Pharms), paramethasone, and deflazacort. In some embodiments, the corticosteroid is dexamethasone.

[00391] The corticosteroid used in the disclosed methods may be administered according to regimens known in the art, e.g., US FDA-approved regimens. Suitable modes of administration include, but are not limited to, enteral, topical, and parenteral administration. The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral (which includes oral) and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion. In some embodiments, the

corticosteroid is administered to the subject parenterally or by injection. In some embodiments, the corticosteroid is administered to the subject by intravenous injection. In some embodiments, the corticosteroid is administered to the subject orally or enterally. In some embodiments, the corticosteroid is administered to the subject topically.

[00392] 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.

[00393] In one example, dexamethasone is administered orally in the amount of 20 mg or 25 mg 6 to 12 hours before intravenous administration of the guide RNA. In another example, dexamethasone is administered intravenously in the amount of 20 mg or 25 mg for 30 minutes 6 to 12 hour before intravenous administration of the guide RNA. In another example, dexamethasone is administered orally in the amount of 8-12 mg, such as 10 mg, 8 to 24 hours before infusion of the guide RNA composition. In another example, dexamethasone is administered intravenously in the amount of 8-12 mg, such as 10 mg, 1-2 hour before infusion of the guide RNA composition. In another example, dexamethasone is administered orally in the amount of 8-12 mg, such as 10 mg, 8 to 24 hours before infusion of the guide RNA composition and dexamethasone is administered intravenously in the amount of 8-12 mg, such as 10 mg, 1-2 hour before infusion of the guide RNA composition.

[00394] In some embodiments, the corticosteroid is dexamethasone, and the dexamethasone is administered to the subject orally in the amount of 8 mg 8-24 hours before the composition is administered to the subject. In some embodiments, the corticosteroid is dexamethasone, and the dexamethasone is administered to the subject orally in the amount of 8 mg 8-24 hours before the composition is administered to the subject.

[00395] In some embodiments, the corticosteroid is dexamethasone, and the dexamethasone is administered to the subject intravenously in the amount of 10 mg 1-2 hours before the composition is administered to the subject. In some embodiments, the corticosteroid is dexamethasone, and the dexamethasone is administered to the subject intravenously in the amount of 10 mg 1-2 hours before the composition is administered to the subject.

[00396] In some embodiments, the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of 8 mg is administered to the subject orally 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the

amount of 10 mg is administered to the subject intravenously 1-2 hours before the composition is administered to the subject.

[00397] In some embodiments, the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of 8 mg is administered to the subject orally 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the amount of 10 mg is administered to the subject intravenously 1-2 hours before the composition is administered to the subject, wherein the second dose of the corticosteroid is concurrently administered with one or more of acetaminophen, H1 blocker or H2 blocker.

[00398] In some embodiments, the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of 8 mg is administered to the subject orally 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the amount of 10 mg is administered to the subject intravenously 1-2 hours before the composition is administered to the subject, wherein the second dose of the corticosteroid is concurrently administered with acetaminophen, H1 blocker and H2 blocker.

[00399] In some embodiments, the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of 8 mg is administered to the subject orally 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the amount of 10 mg is administered to the subject intravenously, concurrently with oral administration of acetaminophen and intravenous administration of H1 blocker and H2 blocker, 1-2 hours before the composition is administered to the subject.

[00400] In some embodiments, the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of 8 mg is administered to the subject orally 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the amount of 10 mg is administered to the subject intravenously, concurrently with oral administration of acetaminophen in the amount of 500 mg and intravenous administration of H1 blocker in the amount of 50 mg and H2 blocker in the amount of 50 mg, 1-2 hours before the composition is administered to the subject.

[00401] Further, it is recognized by those having ordinary skill in the art that the dose of corticosteroid is easily adjustable depending on the choice of particular corticosteroid. For example, for the purpose of comparison, the following are approximate equivalent mg dosages of corticosteroids: hydrocortisone 20 mg; cortisone 25 mg; predisone or prednisolone 5 mg; deflazacort 6 mg; methylprednisolone 4 mg; dexamethasone or betamethasone 0.75 mg; triamcinolone 4 mg. Therefore, although the doses of corticosteroid presented in the above examples are based on dexamethasone, when another corticosteroid is to be

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administered to the patient, one of ordinary skill in the art would use the above conversion information to calculate equivalent doses of the other corticosteroid.

B. Guide RNA (gRNAs)

[00402] 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 (Exon 2)	Human	chr18:31592974-31592994	AGCCUUUCUGAACACAUGCA
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

[00403] 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.

[00404] 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*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmU 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.

[00405] 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 UAUCAmAmCmUmUmGmAmAmAmAmAm 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 UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
96	G000489	TTR sgRNA modified sequence	Human	mU*mU*mU*GACCAUCAGAGGACACUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

97	G000490	TTR sgRNA modified sequence	Human	mA*mA*mA*UAGACACCAAAUCUUACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
98	G000491	TTR sgRNA modified sequence	Human	mA*mU*mA*CCAGUCCAGCAAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
99	G000492	TTR sgRNA modified sequence	Human	mC*mU*mU*CUCUACACCCAGGGCACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
100	G000493	TTR sgRNA modified sequence	Human	mA*mA*mG*UGCCUUCCAGUAAGAUUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
101	G000494	TTR sgRNA modified sequence	Human	mG*mU*mG*AGUCUGGAGAGCUGCAUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
102	G000495	TTR sgRNA modified sequence	Human	mC*mA*mG*AGGACACUUGGAUUCACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
103	G000496	TTR sgRNA modified sequence	Human	mG*mG*mC*CGUGCAUGUGUUCAGAAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
104	G000497	TTR sgRNA modified sequence	Human	mC*mU*mG*CUCCUCCUCUGCCUUGCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

105	G000498	TTR sgRNA modified sequence	Human	mA*mG*mU*GAGUCUGGAGAGCUGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
106	G000499	TTR sgRNA modified sequence	Human	mU*mG*mA*AUCCAAGUGUCCUCUGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
107	G000500	TTR sgRNA modified sequence	Human	mC*mC*mA*GUCCAGCAAGGCAGAGGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
108	G000501	TTR sgRNA modified sequence	Human	mU*mC*mA*CAGAAACACUCACCGUAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
109	G000567	TTR sgRNA modified sequence	Human	mG*mA*mA*AGGCUGCUGAUGACACCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
110	G000568	TTR sgRNA modified sequence	Human	mG*mG*mC*UGUCGUCACCAAUCCCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
111	G000570	TTR sgRNA modified sequence	Human	mC*mA*mU*UGAUGGCAGGACUGCCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
112	G000571	TTR sgRNA modified sequence	Human	mG*mU*mC*ACAGAAACACUCACCGUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

113	G000572	TTR sgRNA modified sequence	Human	mC*mC*mC*CUACUCCUAUUCCACCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
114	G000502	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mC*mA*CAAAUACCAGUCCAGCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
115	G000503	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*AGGCUGCUGAUGAGACCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
116	G000504	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*GGCUGCUGAUGAGACCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
117	G000505	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mA*mU*UGACAGCAGGACUGCCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
118	G000506	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mU*mA*CCAGUCCAGCGAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAmAm 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

[00406] 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		

[00407] 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.

[00408] 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.

[00409] 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.

[00410] 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.

[00411] In some embodiments, the composition comprises one or more guide RNAs comprising a guide sequence selected from SEQ ID NOs: 5-82.

[00412] 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.

[00413] 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.

[00414] 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 a sequence selected from SEQ ID NOs: 5-82.

[00415] 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.

[00416] 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.

[00417] 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 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.

[00418] 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.

[00419] In some embodiments, the selection of the one or more guide RNAs is determined based on target sequences within the *TTR* gene.

[00420] 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*.

[00421] In some embodiments, the guide sequence is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a target sequence present in the human *TTR* gene. In some embodiments, the target sequence may be complementary to the guide sequence of the guide RNA. In some embodiments, the degree of complementarity or identity between a guide sequence of a guide RNA and its corresponding target sequence may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the target sequence and the guide sequence of the gRNA may be 100% complementary or identical. In other embodiments, the target sequence and the guide sequence of the gRNA may contain at least one mismatch. For example, the target sequence and the guide sequence of the gRNA may contain 1, 2, 3, or 4 mismatches, where the total length of the guide sequence is 20. In some embodiments, the target sequence and the guide sequence of the gRNA may contain 1-4 mismatches where the guide sequence is 20 nucleotides.

C. Modifications of gRNAs

[00422] 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).

[00423] 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.

[00424] 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.

[00425] 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.

[00426] 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.

[00427] 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.

[00428] 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.

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[00429] 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.

[00430] 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.

[00431] 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).

[00432] "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.

[00433] 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.

[00434] 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.

[00435] 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.

[00436] 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

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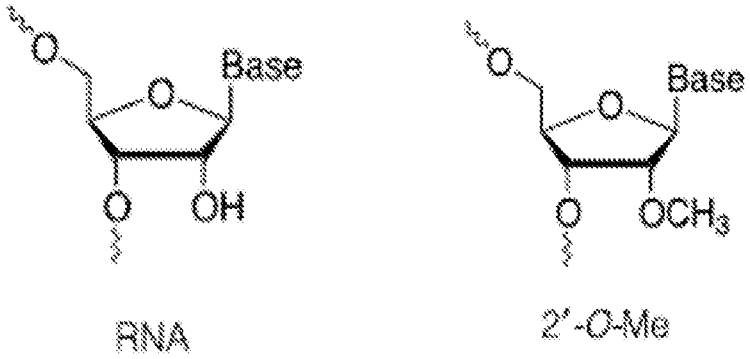
"Chemically Modified Guide RNAs," the contents of which are hereby incorporated by reference in their entirety.

[00437] 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.

[00438] The terms "mA," "mC," "mU," or "mG" may be used to denote a nucleotide that has been modified with 2'-O-Me.

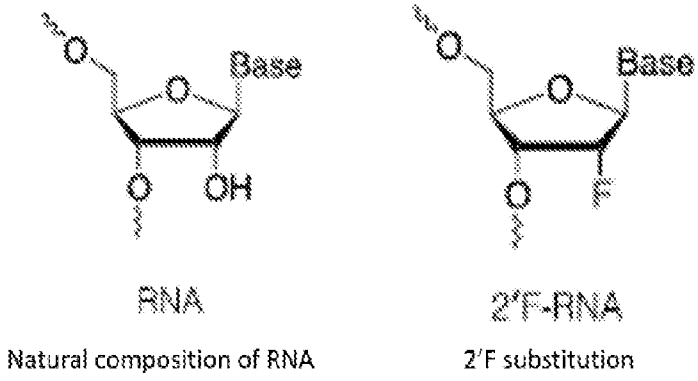
[00439] Modification of 2'-*O*-methyl can be depicted as follows:



[00440] 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.

[00441] In this application, the terms "fA," "fC," "fU," or "fG" may be used to denote a nucleotide that has been substituted with 2'-F.

[00442] Substitution of 2'-F can be depicted as follows:



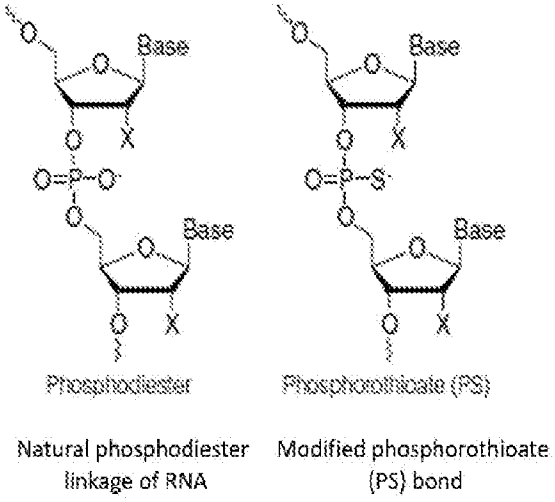
[00443] Phosphorothioate (PS) linkage or bond refers to a bond where a sulfur is substituted for one nonbridging phosphate oxygen in a phosphodiester linkage, for example

in the bonds between nucleotides bases. When phosphorothioates are used to generate oligonucleotides, the modified oligonucleotides may also be referred to as S-oligos.

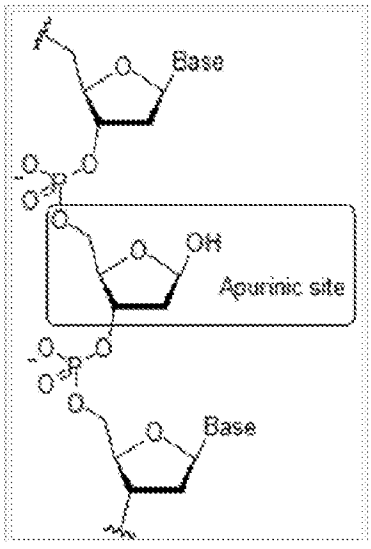
[00444] A "*" may be used to depict a PS modification. In this application, the terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (e.g., 3') nucleotide with a PS bond.

[00445] 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.

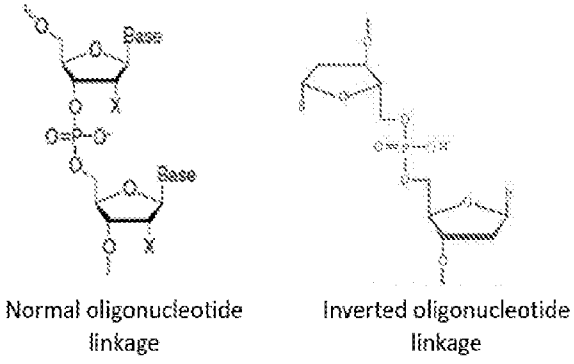
[00446] The diagram below shows the substitution of S- into a nonbridging phosphate oxygen, generating a PS bond in lieu of a phosphodiester bond:



[00447] 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:



[00448] 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:



[00449] 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.

[00450] 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.

[00451] 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.

[00452] 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.

[00453] 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.

[00454] 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

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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.

[00455] 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 nucleotides 21-100 of SEQ ID No: 3, wherein the nucleotides of SEQ ID No: 3 are on the 3' end of the guide sequence, and wherein the guide sequence may be modified as shown in SEQ ID No: 3.

D. RNA-Guided DNA Binding Agent

[00456] 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 binding agent is a Cas cleavase, e.g. a Cas9 cleavase. 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 a Cas9 nuclease, such as a cleavase or nickase. In some embodiments, the RNA-guided DNA binding agent is an *S. pyogenes* Cas9 nuclease, e.g. a cleavase.

[00457] 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*

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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.*

[00458] 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*.

[00459] 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: 210.

[00460] 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.

[00461] 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.

[00462] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a "nick." In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. See, e.g., US Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain. An exemplary Cas9 nickase amino acid sequence is provided as SEQ ID NO: 206. An exemplary Cas9 nickase mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 207. An exemplary Cas9 nickase mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 211.

[00463] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclease domain. For example, the agent protein may be modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a

RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[00464] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the S. *pyogenes* Cas9 protein). *See*, *e.g.*, Zetsche et al. (2015) *Cell* Oct 22:163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the S. *pyogenes* Cas9 protein). *See*, *e.g.*, Zetsche et al. (2015). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB - A0Q7Q2 (CPF1_FRATN)).

[00465] In some embodiments, a nucleic acid encoding a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, use of double nicking may improve specificity and reduce off-target effects. In some embodiments, a nickase is used together with two separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[00466] In some embodiments, the RNA-guided DNA-binding agent lacks cleavase and nickase activity. In some embodiments, the RNA-guided DNA-binding agent comprises a dCas DNA-binding polypeptide. A dCas polypeptide has DNA-binding activity while essentially lacking catalytic (cleavase/nickase) activity. In some embodiments, the dCas polypeptide is a dCas9 polypeptide. In some embodiments, the RNA-guided DNA-binding agent lacking cleavase and nickase activity or the dCas DNA-binding polypeptide is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which its endonucleolytic active sites are inactivated, e.g., by one or more alterations (e.g., point mutations) in its catalytic domains. See, e.g., US 2014/0186958 A1; US 2015/0166980 A1. An exemplary dCas9 amino

acid sequence is provided as SEQ ID NO: 208. An exemplary dCas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 209. An exemplary dCas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 346.

a) Heterologous functional domains; nuclear localization signals

[00467] 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).

[00468] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent 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: 91). 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.

[00469] In some embodiments, the heterologous functional domain may be capable of modifying the intracellular half-life of the RNA-guided DNA binding agent. 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).

[00470] 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.

[00471] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent 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.

[00472] 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. Nucleic Acid Comprising an Open Reading Frame Encoding an RNA-Guided DNA Binding Agent

[00473] Any nucleic acid comprising an ORF encoding an RNA-guided DNA binding agent disclosed herein, e.g. a Cas9 nuclease such as an *S. pyogenes* Cas9, may be optionally 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

[00474] 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.

[00475] 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.

[00476] 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.

[00477] 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.

[00478] 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%,

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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.

[00479] 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

[00480] 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

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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.

[00481] 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

[00482] 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%,

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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.

[00483] Alternatively, codons corresponding to highly expressed tRNAs in an organism (e.g., human) in general may be used.

[00484] 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).

[00485] 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 1, low A, or low A/U codon set). The codons in the low U 1, low G, low C, 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 1 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.

[00486] Table 5. Exemplary Codon Sets

Amino Acid	Low U 1	Low U 2	High U	Low G	Low C	Low A	Low A/U	Long Half Life
Gly	GGC	GGG	GGT	GGC	GGA	GGC	GGC	GGT
Glu	GAG	GAA	GAA	GAA	GAG	GAG	GAG	GAA
Asp	GAC	GAC	GAT	GAC	GAT	GAC	GAC	GAC
Val	GTG	GTA	GTT	GTC	GTG	GTG	GTG	GTC

Ala	GCC	GCG	GCT	GCC	GCT	GCC	GCC	GCC
Arg	AGA	CGA	CGT	AGA	AGA	CGG	CGG	AGA
Ser	AGC	AGC	TCT	TCC	AGT	TCC	AGC	TCT
Lys	AAG	AAA	AAA	AAA	AAG	AAG	AAG	AAG
Asn	AAC	AAC	AAT	AAC	AAT	AAC	AAC	AAC
Met	ATG	ATG	ATG	ATG	AGT	ATG	ATG	ATG
Ile	ATC	ATA	ATT	ATC	ATT	ATC	ATC	ATC
Thr	ACC	ACG	ACT	ACC	ACA	ACC	ACC	ACC
Trp	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG
Cys	TGC	TGC	TGT	TGC	TGT	TGC	TGC	TGC
Tyr	TAC	TAC	TAT	TAC	TAT	TAC	TAC	TAC
Leu	CTG	CTA	TTA	CTC	TTG	CTG	CTG	TTG
Phe	TTC	TTC	TTT	TTC	TTT	TTC	TTC	TTC
Gln	CAG	CAA	CAA	CAA	CAG	CAG	CAG	CAA
His	CAC	CAC	CAT	CAC	CAT	CAC	CAC	CAC

3. Exemplary sequences

[00487] 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 1; 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.

[00488] In some embodiments, the polynucleotide 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.

[00489] 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: 201, 204, 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375. 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, 206, 208, 213, 216, 219, 222, 225, 228, 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, 206, 208, 213, 216, 219, 222, 225, 228, 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, the mRNA comprises a sequence with at least 90% identity to any one of SEQ ID NOs: 243, 244, 251, 253, 255-261, or 267, wherein the sequence comprises an ORF encoding an RNA-guided DNA binding agent. In some embodiments, the mRNA comprises a sequence with at least 90% identity to any one of SEQ ID NOs: 243, 244, 251, 253, 255-261, or 267, wherein the sequence comprises an ORF encoding an RNA-guided DNA binding agent, wherein the first three nucleotides of SEQ ID NOs: 243, 244, 251, 253, 255-261, or 267are omitted. In some embodiments, any of the foregoing levels of identity is at least 95%, at least 98%, at least 99%, or 100%.

[00490] In some embodiments, the ORF encoding an RNA-guided DNA binding agent has at least 90% identity to any one of SEQ ID NO: 201, 204, 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375over 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

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(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: 201, 204, 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375over 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.

[00491] 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: 243in 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: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00492] 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: 244in 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: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00493] 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: 256in which the ORF of SEQ ID NO: 256 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00494] 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: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00495] 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: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

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[00496] 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: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00497] 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: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00498] 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: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00499] 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 in which the ORF of SEQ ID NO: 376 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00500] 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 in which the ORF of SEQ ID NO: 377 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00501] 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 in which the ORF of SEQ ID NO: 378 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00502] 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 (i.e., SEQ ID NO: 204) is substituted with

the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00503] 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 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00504] 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 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00505] 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 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00506] 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 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00507] 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 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00508] 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 (i.e., SEQ ID NO: 204) is substituted with the ORF of any one of SEQ ID NO: 207, 209, 210, 211, 212, 214, 215, 217, 218, 220, 221, 223, 224, 226, 227, 229, 230, 250, 252, 254, 265, 266, or 307-375.

[00509] In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID Nos: 243, 244, 256-61, 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, 256-61, 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, 256-61, or 376-385 is at least 99%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs: 243, 244, 256-61, or 376-385 is 100%.

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

[00510] 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

[00511] 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

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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.

[00512] 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

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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.

[00513] 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.

[00514] 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.

[00515] 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.

[00516] 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

[00517] 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

[00518] 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 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.

[00519] 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

[00520] 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.

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c) UTRs; Kozak sequences

[00521] 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 polynucleotide (e.g., mRNA), for example, human alpha globin (HBA) polynucleotide (e.g., mRNA), human beta globin (HBB) polynucleotide (e.g., mRNA), or Xenopus laevis beta globin (XBG) polynucleotide (e.g., mRNA). In some embodiments, the polynucleotide (e.g., mRNA) comprises a 5' UTR, 3' UTR, or 5' and 3' UTRs from a globin polynucleotide (e.g., 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).

[00522] In some embodiments, the polynucleotide (e.g., mRNA) comprises 5' and 3' UTRs that are from the same source, e.g., a constitutively expressed polynucleotide (e.g., mRNA) such as actin, albumin, or a globin such as HBA, HBB, or XBG.

[00523] 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.

[00524] 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

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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.

[00525] 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.

d) Poly-A tail

[00526] 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 polynucleotide (e.g., mRNA) comprise non-consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA-

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binding agent or a sequence of interest, wherein non-adenine nucleotides interrupt the adenine nucleotides at regular or irregularly spaced intervals.

[00527] 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.

[00528] 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.

[00529] 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.

[00530] 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.

[00531] 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)

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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.

e) Modified nucleotides

[00532] 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.

[00533] 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

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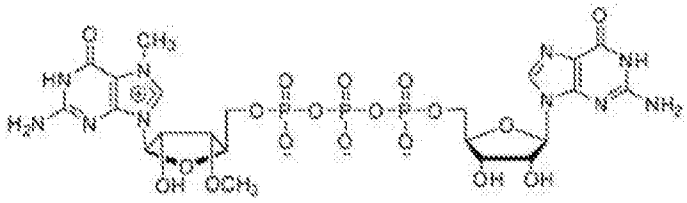
some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the nucleic acid are 5-iodouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in the 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.

f) 5' Cap

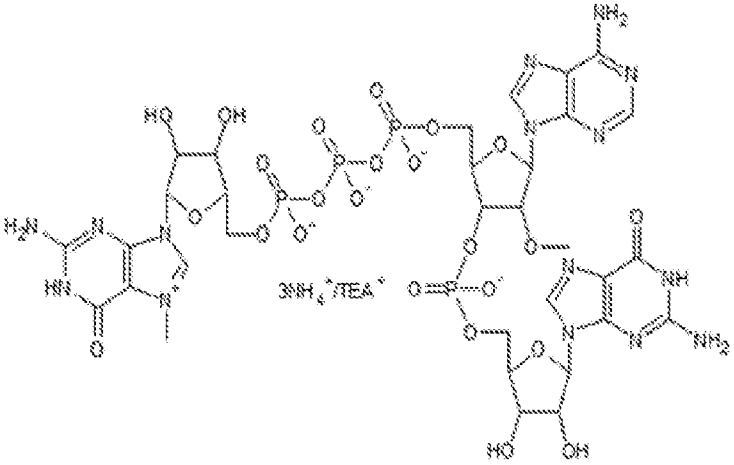
[00534] In some embodiments, the nucleic acid (e.g., mRNA) comprising an ORF encoding an RNA-guided DNA-binding agent comprises a 5' cap, such as a Cap0, Cap1, or Cap2. A 5' cap is generally a 7-methylguanine ribonucleotide (which may be further modified, as discussed below e.g. with respect to ARCA) 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.

[00535] 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.



[00536] 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).



[00537] 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-A130.

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F. Determination of efficacy of RNAs

[00538] 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 corticosteroid and a gRNA, and optionally an RNA-guided DNA binding agent or nucleic acid encoding such an agent is determined.

[00539] As described herein, use of an RNA-guided DNA binding agent 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.

[00540] In some embodiments, the efficacy of particular gRNAs, compositions, or treatments comprising administering a gRNA, corticosteroid, and optionally an RNA-guided DNA binding agent or nucleic acid encoding such an agent 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.

[00541] In some embodiments, the efficacy of particular gRNAs, compositions, or treatments comprising administering a gRNA, corticosteroid, and optionally an RNA-guided DNA binding agent or nucleic acid encoding such an agent 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.

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[00542] In some embodiments, the efficacy of particular gRNAs, compositions, or treatments comprising administering a gRNA, corticosteroid, and optionally an RNA-guided DNA binding agent or nucleic acid encoding such an agent 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.

[00543] In some embodiments, the efficacy of a guide RNA, compositions, or treatments comprising administering a gRNA, corticosteroid, and optionally an RNA-guided DNA binding agent or nucleic acid encoding such an agent 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.

[00544] In some embodiements, the efficacy of a gRNA, compositions, or treatments comprising administering a gRNA, corticosteroid, and optionally an RNA-guided DNA binding agent or nucleic acid encoding such an agent 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 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 which do not exhibit any off target indel formation in the target cell type (e.g., hepatocyte). In some embodiments, guide RNAs 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 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.

[00545] 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

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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).

[00546] In some embodiments, the method comprises isolating cellular DNA from a cell that has been induced to have a double strand break (DSB) and optionally that has been provided with an HDR template to repair the DSB; performing at least one cycle of linear amplification of the DNA with a tagged primer; isolating the linear amplification products that comprise tag, thereby discarding any amplification product that was amplified with a non-tagged primer; optionally further amplifying the isolated products; and analyzing the linear amplification products, or the further amplified products, to determine the presence or absence of an editing event such as, for example, a double strand break, an insertion, deletion, or HDR template sequence in the target DNA. In some instances, the editing event can be quantified. Quantification and the like as used herein (including in the context of HDR and non-HDR editing events such as indels) includes detecting the frequency and/or type(s) of editing events in a population.

[00547] In some embodiments, only one cycle of linear amplification is conducted.

[00548] In some instances, the tagged primer comprises a molecular barcode. In some embodiments, the tagged primer comprises a molecular barcode, and only one cycle of linear amplification is conducted.

[00549] 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.

[00550] 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.

[00551] 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.

[00552] 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.

[00553] 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

[00554] In some embodiments, a method of treating ATTR is provided comprising administering a corticosteroid and 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

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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 may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease. In some embodiments, the RNA-guided DNA nuclease is a Cas cleavase. In some embodiments, the RNA-guided DNA nuclease is a Cas from a Type-II CRISPR/Cas system. In some embodiments, the RNA-guided DNA nuclease is a Cas9. In some embodiments, the RNA-guided DNA nuclease is an *S. pyogenes* Cas9 nuclease. 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).

[00555] In some embodiments, a method of treating ATTR is provided comprising administering a corticosteroid and 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: 87-113, 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 treat ATTR. The guide RNA is optionally administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease. In some embodiments, the RNA-guided DNA nuclease is a Cas cleavase. In some embodiments, the RNA-guided DNA nuclease is a Cas from a Type-II CRISPR/Cas system. In some embodiments, the RNA-guided DNA nuclease is a Cas9. In some embodiments, the RNA-guided DNA nuclease is an *S. pyogenes* Cas9 nuclease. 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).

[00556] In some embodiments, a method of reducing TTR serum concentration is provided comprising administering a corticosteroid and 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). In some embodiments, the RNA-guided DNA nuclease is a Cas cleavase. In some embodiments, the RNA-guided DNA nuclease is a Cas from a Type-II CRISPR/Cas system. In some embodiments, the RNA-guided DNA nuclease is a Cas9. In some embodiments, the RNA-guided DNA nuclease is an *S. pyogenes* Cas9 nuclease. 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).

[00557] 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 guide RNA is optionally administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease. In some embodiments, the RNA-guided DNA nuclease is a Cas cleavase. In some embodiments, the RNA-guided DNA nuclease is a Cas from a Type-II CRISPR/Cas system. In some embodiments, the RNA-guided DNA nuclease is a Cas9. In some embodiments, the RNA-guided DNA nuclease is an *S. pyogenes* Cas9 nuclease. 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).

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[00558] 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 corticosteroid and 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 corticosteroid and 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 optionally administered together with a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9). In some embodiments, the RNA-guided DNA nuclease is a Cas cleavase. In some embodiments, the RNA-guided DNA nuclease is a Cas from a Type-II CRISPR/Cas system. In some embodiments, the RNA-guided DNA nuclease is a Cas9. In some embodiments, the RNA-guided DNA nuclease is an *S. pyogenes* Cas9 nuclease. 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).

[00559] 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 guide RNA is optionally administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or a nucleic acid or vector described herein encoding an RNA-guided DNA nuclease. In some

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embodiments, the RNA-guided DNA nuclease is a Cas cleavase. In some embodiments, the RNA-guided DNA nuclease is a Cas from a Type-II CRISPR/Cas system. In some embodiments, the RNA-guided DNA nuclease is a Cas9. In some embodiments, the RNA-guided DNA nuclease is an *S. pyogenes* Cas9 nuclease. 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).

[00560] 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.

[00561] In some embodiments, administering the corticosteroid and the guide RNA (and optionally an RNA-guided DNA binding agent or a 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.

[00562] 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.

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[00563] 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.

[00564] 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.

[00565] 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.

[00566] In some embodiments, a single administration of a composition comprising a guide RNA (and optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent) 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 optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent) 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 optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent) 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.

[00567] 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 mpk, such as 2.5-3.5 mpk. In some embodiments, a composition is administered in the amount of about 3 mpk. As reported herein, for an LNP composition, the dosage or effective amount is assessed by total RNA administered.

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[00568] 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.

[00569] In some embodiments, treatment slows or halts disease progression.

[00570] 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.

[00571] 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.

[00572] In some embodiments, efficacy of treatment is measured by increased survival time of the subject. In some embodiments, efficacy of treatment is measured by increased tolerability of the treatment. In some embodiments, increased tolerability, e.g. cytokine, complement, or other immune response is measured.

[00573] 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.

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[00574] 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

[00575] In some embodiments, the invention comprises combination therapies comprising administering a corticosteroid and 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 optionally an RNA-guided DNA binding agent or a nucleic acid described herein encoding an RNA-guided DNA binding agent, such as a nucleic acid (e.g. mRNA) or vector described herein encoding an *S. pyogenes* Cas9) (e.g., in a composition provided herein) 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).

[00576] 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.

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[00577] 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.

[00578] In some embodiments, the combination therapy comprises administering a corticosteroid and 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 optionally an RNA-guided DNA binding agent or a nucleic acid described herein 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.

[00579] In some embodiments, the combination therapy comprises administering a corticosteroid and 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 optionally an RNA-guided DNA binding agent or a nucleic acid described herein encoding an RNA-guided DNA binding agent) (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

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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.

[00580] In some embodiments, the combination therapy comprises administering a corticosteroid and 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 optionally an RNA-guided DNA binding agent or a nucleic acid described herein 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.

[00581] 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.

B. Delivery of Nucleic Acid Compositions

[00582] In some embodiments, the nucleic acid compositions described herein, comprising a gRNA, and optionally 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 published October 5, 2017 and WO2019067992A1 published April 4, 2019, 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 optionallythe nucleic acid encoding an RNA-guided DNA nuclease.

[00583] 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

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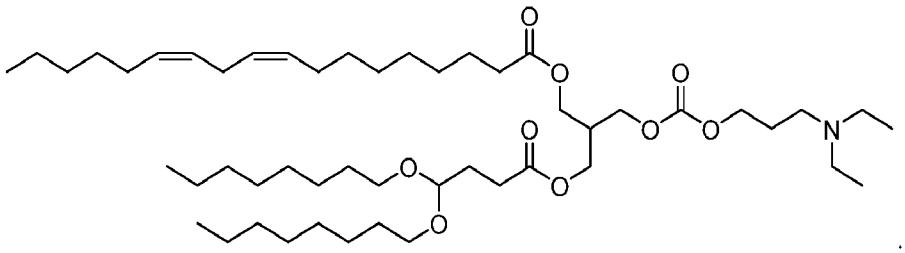
helper lipid, a neutral lipid, and a stealth lipid such as a PEG lipid. In some embodiments, the LNP formulations include less than 1 percent neutral phospholipid. In some embodiments, the LNP formulations include less than 0.5 percent neutral phospholipid. By "lipid nanoparticle" is meant a particle that comprises a plurality of (*i.e.* more than one) lipid molecules physically associated with each other by intermolecular forces.

[00584] CCD Lipids

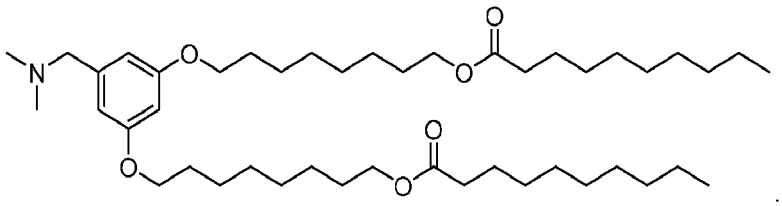
[00585] Lipid compositions for delivery of CRISPR/Cas mRNA and guide RNA components to a target cell, such as a liver cell comprise a CCD Lipid.

[00586] 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:



[00587] Lipid A may be synthesized according to WO2015/095340 (e.g., pp. 84-86).

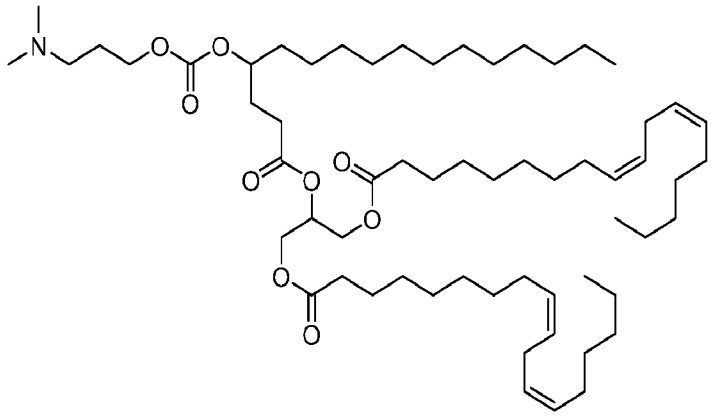
[00588] In some embodiments, the CCD lipid is Lipid B, which is ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate), also called ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl) bis(decanoate). Lipid B can be depicted as:



[00589] Lipid B may be synthesized according to WO2014/136086 (e.g., pp. 107-09).

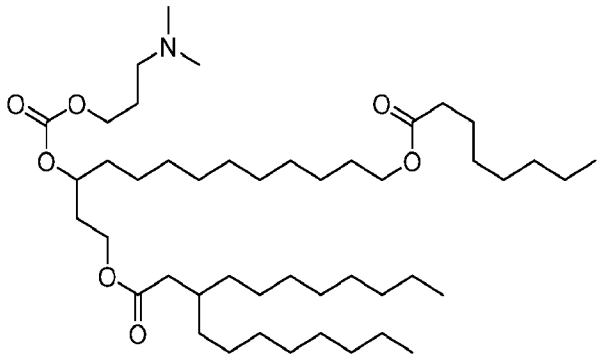
[00590] In some embodiments, the CCD lipid is Lipid C, which is 2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1,3-diyl

(9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate). Lipid C can be depicted as:



[00591] In some embodiments, the CCD lipid is Lipid D, which is 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl 3-octylundecanoate.

[00592] Lipid D can be depicted as:



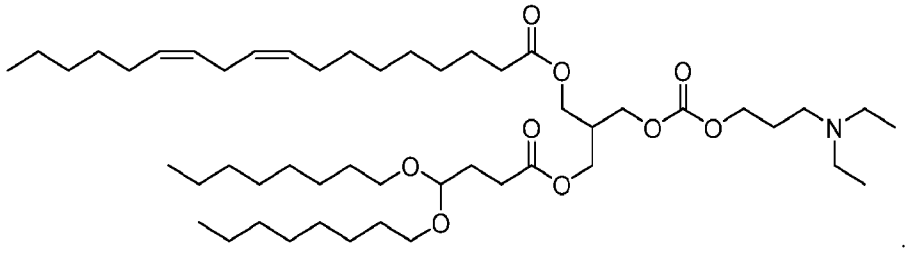
[00593] Lipid C and Lipid D may be synthesized according to WO2015/095340.

[00594] 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.

[00595] Amine Lipids

[00596] 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.

[00597] 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:



[00598] 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.

[00599] 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.

[00600] 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.

[00601] 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.

[00602] 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.

[00603] 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.

[00604] 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,

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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.

[00605] Additional Lipids

[00606] "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

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(DSPC). In another embodiment, the neutral phospholipid may be dipalmitoylphosphatidylcholine (DPPC).

[00607] "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.

[00608] "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.

[00609] 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.

[00610] 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].

[00611] In one embodiment, the PEG lipid comprises a polymer moiety based on PEG (sometimes referred to as poly(ethylene oxide)).

[00612] 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.

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In some embodiments, the alkyl chail 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.

[00613] 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.

[00614] 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. 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.

[00615] 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)

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(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.

[00616] LNP Formulations

[00617] 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.

[00618] 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, e.g. *S. pyogenes* Cas9, 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

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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.

[00619] 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%.

[00620] 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-%.

[00621] 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

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mol-%. In one embodiment, the mol-% of the neutral lipid, e.g., neutral phospholipid, may be about 9 mol-%.

[00622] 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-%.

[00623] 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).

[00624] 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%.

[00625] 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%.

[00626] 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%.

[00627] 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.

[00628] 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

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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.

[00629] 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%.

[00630] In some embodiments, the RNA component may comprise a nucleic acid, such as a nucleic acid disclosed herein, e.g., encoding a Cas nuclease. In one embodiment, RNA component may comprise a Cas9 mRNA. In some compositions comprising a nucleic acid encoding a Cas nuclease, the LNP further comprises a gRNA nucleic acid, such as a gRNA. In some embodiments, the RNA component comprises a Cas nuclease mRNA and a gRNA. In some embodiments, the RNA component comprises a Class 2 Cas nuclease mRNA and a gRNA. In any of the foregoing embodiments, the gRNA may be an sgRNA described herein, such as a chemically modified sgRNA described herein.

[00631] In certain embodiments, an LNP composition may comprise a nucleic acid disclosed herein, e.g., encoding a Cas nuclease, such as a Class 2 Cas nuclease, a gRNA, 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.

[00632] In one embodiment, an LNP composition may comprise an sgRNA. In one embodiment, an LNP composition may comprise a Cas9 sgRNA. In one embodiment, an LNP composition may comprise a Cpf1 sgRNA. In some compositions comprising an sgRNA, the LNP includes an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In certain compositions comprising an sgRNA, the helper lipid is cholesterol. In other compositions comprising an sgRNA, the neutral lipid is DSPC. In additional embodiments comprising an sgRNA, 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.

[00633] In certain embodiments, the LNP compositions include a Cas nuclease mRNA, such as a Class 2 Cas mRNA and at least one gRNA. 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.

[00634] 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.

[00635] 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

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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.

[00636] 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.

[00637] 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.

[00638] 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.

[00639] 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).

[00640] 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%.

[00641] 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.

[00642] 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.

[00643] 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.

[00644] 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%.

[00645] In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for treating ATTR. In some embodiments, LNPs associated with the gRNAs 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 are for use in preparing a medicament for reducing serum TTR concentration. In some embodiments, LNPs associated with the gRNAs 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 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 are for use in reducing serum TTR concentration in a subject, such as a mammal, e.g., a primate such as a human. In any of the foregoing embodiments, the LNPs may be associated with the gRNAs disclosed herein and

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nucleic acids (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein.

[00646] 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 optionally an RNA-guided DNA nuclease such as Cas9 or a nucleic acid encoding an RNA-guided DNA nuclease such as Cas9.

[00647] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to an ex vivo cell, wherein the gRNA is associated with an LNP or not associated with an LNP. In some embodiments, the gRNA/LNP or gRNA is also optionally associated with an RNA-guided DNA nuclease such as Cas9 or a nucleic acid encoding an RNA-guided DNA nuclease, e.g., a nucleic acid (e.g., mRNA) encoding an RNA-guided DNA binding agent (e.g. Cas9, Spy Cas9) disclosed herein.

[00648] 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 optionallynucleic acids described herein 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 optionally a nucleic acid described herein 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 optionally a nucleic acid described herein 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

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consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

[00649] 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.

[00650] 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.

[00651] 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

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vector system described herein may also encode the viral components required for virus amplification and packaging.

[00652] 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.

[00653] 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.

[00654] 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

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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.

[00655] 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).

[00656] In some embodiments, the promoter may be a tissue-specific promoter, e.g., a promoter specific for expression in the liver.

[00657] 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.*, $tRNA^{Lys3}$, 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.

[00658] In some embodiments, the vector may optionally further 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.

[00659] 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.

[00660] 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.

[00661] 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).

[00662] In additional embodiments, the vector system may comprise tissue-specific promoters to start expression only after it is delivered into a specific tissue.

[00663] 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, 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.

[00664] 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.

[00665] 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.

[00666] In some embodiments, the vector may be delivered systemically. In some embodiments, the vector may be delivered into the hepatic circulation.

[00667] 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.

[00668] 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

[00669] 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.

Example 1. Materials and Methods

In vitro transcription ("IVT") of nuclease mRNA

[00670] Capped and polyadenylated *Streptococcus pyogenes* ("Spy") Cas9 mRNA containing N1-methyl pseudo-U was generated by *in vitro* transcription using a linearized plasmid DNA template and T7 RNA polymerase. Plasmid DNA containing a T7 promoter, a sequence for transcription according to SEQ ID NO: 1, 2, or another sequence disclosed herein, and a 90-100 nt poly (A/T) region was 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 was inactivated by heating the reaction at 65°C for 20 min. The linearized plasmid was purified from enzyme and buffer salts. The IVT reaction to generate Cas9 modified mRNA was 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) was added to a final concentration of 0.01 U/μL, and the reaction was incubated for an additional 30 minutes to remove the DNA

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template. The Cas9 mRNA was purified using a MegaClear Transcription Clean-up kit (ThermoFisher) or a RNeasy Maxi kit (Qiagen) per the manufacturers' protocols. Alternatively, the mRNA was purified through a precipitation protocol, which in some cases was followed by HPLC-based purification. Briefly, after the DNase digestion, mRNA is purified using LiCl precipitation, ammonium acetate precipitation and sodium acetate precipitation. For HPLC purified mRNA, after the LiCl precipitation and reconstitution, the mRNA was purified by RP-IP HPLC (see, e.g., Kariko, et al. *Nucleic Acids Research*, 2011, Vol. 39, No. 21 e142). The fractions chosen for pooling were combined and desalted by sodium acetate/ethanol precipitation as described above. In a further alternative method, mRNA was purified with a LiCl precipitation method followed by further purification by tangential flow filtration. RNA concentrations were determined by measuring the light absorbance at 260 nm (Nanodrop), and transcripts were analyzed by capillary electrophoresis by Bioanlayzer (Agilent).

[00671] When SEQ ID NOs: 1 and 2 are referred to below with respect to RNAs, it is understood that Ts should be replaced with Us (which were N1-methyl pseudouridines as described above). Cas9 mRNAs used in the Examples include a 5' cap and a 3' poly-A tail, e.g., up to 100 nts, and are identified by SEQ ID NO.

Human TTR guide design and human TTR with cynomolgus monkey homology guide design

[00672] Initial guide selection was performed *in silico* using a human reference genome (e.g., hg38) and user defined genomic regions of interest (e.g., TTR protein coding exons), for identifying PAMs in the regions of interest. For each identified PAM, analyses were performed and statistics reported. gRNA molecules were further selected and rank ordered based on a number of criteria (e.g., GC content, predicted on-target activity, and potential off-target activity).

[00673] A total of 68 guide RNAs were designed toward TTR (ENSG00000118271) targeting the protein coding regions within Exon 1, 2, 3 and 4. Of the total 68 guides, 33 were 100% homologous in cynomolgus monkey ("cyno"). In addition, for 10 of the human TTR guides which were not perfectly homologous in cyno, "surrogate" guides were designed and made in parallel to perfectly match the corresponding cyno target sequence. These "surrogate" or "tool" guides may be screened in cyno, e.g., to approximate the activity and function of the homologous human guide sequence. Guide sequences and corresponding

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genomic coordinates are provided (Table 1). All of the guide RNAs were made as dual guide RNAs, and a subset of the guide sequences were made as modified single guide RNA (Table 2). Guide ID alignment across dual guide RNA (dgRNA) IDs, modified single guide RNA (sgRNA) IDs, the number of mismatches to the cyno genome as well as the cyno exact matched IDs are provided (Table 3). Where dgRNAs are used in the experiments detailed throughout the Examples, SEQ ID NO: 270 was used.

[00674] The sgRNAs in the following examples were chemically synthesized by known methods using phosphoramidites.

Cas9 mRNA and guide RNA delivery in vitro

[00675] *HEK293_Cas9 cell line.* The human embryonic kidney adenocarcinoma cell line HEK293 constitutively expressing Spy Cas9 ("HEK293_Cas9") was cultured in DMEM media supplemented with 10% fetal bovine serum and 500 μg/ml G418. Cells were plated at a density of 10,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with a lipoplex containing individual crRNA (25 nM), trRNA (25 nM), Lipofectamine RNAiMAX (0.3 μL/well) and OptiMem.

[00676] *HUH7 cell line.* The human hepatocellular carcinoma cell line HUH7 (Japanese Collection of Research Bioresources Cell Bank, Cat. JCRB0403) was cultured in DMEM media supplemented with 10% fetal bovine serum. Cells were plated on at a density of 15,000 cells/well in a 96-well plate 20 hours prior to transfection. Cells were transfected with Lipofectamine MessengerMAX (ThermoFisher, Cat. LMRNA003) per the manufacturer's protocol. Cells were sequentially transfected with a lipoplex containing Spy Cas9 mRNA (100 ng), MessengerMAX (0.3 μL/well) and OptiMem followed by a separate lipoplex containing individual crRNA (25 nM), tracer RNA (25 nM), MessengerMAX (0.3 μL/well) and OptiMem.

[00677] *HepG2 cell line.* The human hepatocellular carcinoma cell line HepG2 (American Type Culture Collection, Cat. HB-8065) was cultured in DMEM media supplemented with 10% fetal bovine serum. Cells were counted and plated on Bio-coat collagen I coated 96-well plates (ThermoFisher, Cat. 877272) at a density of 10,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine 2000 (ThermoFisher, Cat. 11668019) per the manufacturer's protocol. Cells were sequentially transfected with lipoplex containing Spy Cas9 mRNA (100 ng), Lipofectamine 2000 (0.2

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µL/well) and OptiMem followed by a separate lipoplex containing individual crRNA (25 nM), tracer RNA (25 nM), Lipofectamine 2000 (0.2 µL/well) and OptiMem.

[00678] *Primary liver hepatocytes*. Primary human liver hepatocytes (PHH) and primary cynomolgus liver hepatocytes (PCH) (Gibco) were cultured per the manufacturer's protocol (Invitrogen, protocol 11.28.2012). In brief, the cells were thawed and resuspended in hepatocyte thawing medium with supplements (Gibco, Cat. CM7000) followed by centrifugation at 100 g for 10 minutes for human and 80g for 4 minutes for cyno. 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 human or 60,000 cells/well for cyno (or 65,000 cells/well when assaying effects on TTR protein, described further below). Plated cells were allowed to settle and adhere for 6 or 24 hours in a tissue culture incubator at 37°C and 5% CO_2 atmosphere. After incubation cells were checked for monolayer formation and media was replaced with hepatocyte culture medium with serum-free supplement pack (Invitrogen, Cat. A1217601 and CM4000).

[00679] Lipofectamine RNAiMax (ThermoFisher, Cat. 13778150) based transfections were conducted as per the manufacturer's protocol. Cells were sequentially transfected with a lipoplex containing Spy Cas9 mRNA (100 ng), Lipofectamine RNAiMax (0.4 µL/well) and OptiMem followed by a separate lipoplex containing crRNA (25 nM) and tracer RNA (25 nM) or sgRNA (25nM), Lipofectamine RNAiMax (0.4 µL/well) and OptiMem.

[00680] Ribonucleotide formation was performed prior to electroporation or transfection of Spy Cas9 protein loaded with guide RNAs (RNPs) onto cells. For dual guide (dgRNAs), individual crRNA and trRNA was pre-annealed by mixing equivalent amounts of reagent and incubating at 95°C for 2 min and cooling to room temperature. Single guide (sgRNAs) were boiled at 95°C for 2 min and cooling to room temperature. The boiled dgRNA or sgRNA was incubated with Spy Cas9 protein in Optimem for 10 minutes at room temperature to form a ribonucleoprotein (RNP) complex.

[00681] For RNP electroporation into primary human and cyno hepatocytes, the cells are thawed and resuspended in Lonza electroporation Primary Cell P3 buffer at a concentration of 2500 cells per µL for human hepatocytes and 3500 cells per µL for cyno hepatocytes. A volume of 20 µL of resuspended cells and 5 µL of RNP are mixed together per guide. 20 µL of the mixture is placed into a Lonza electroporation plate. The cells were electroporated

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using the Lonza nucleofector with the preset protocol EX-147. Post electroporation, the cells are transferred into a Biocoat plate containing pre-warmed maintenance media and placed in a tissue culture incubator at 37°C and 5% CO_2.

[00682] For RNP lipoplex transfections, cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with an RNP containing Spy Cas9 (10nM), individual guide (10 nM), tracer RNA (10 nM), Lipofectamine RNAiMAX (1.0 μL/well) and OptiMem. RNP formation was performed as described above.

[00683] LNPs were formed either by by microfluidic mixing of the lipid and RNA solutions using a Precision Nanosystems NanoAssemblr™ Benchtop Instrument, per the manufacturer's protocol, or cross-flow mixing.

LNP formulation - NanoAssemblr

[00684] In general, the lipid nanoparticle components were dissolved in 100% ethanol with the lipid component of various molar ratios. The RNA cargos were dissolved in 25 mM citrate, 100 mM NaCl, pH 5.0, resulting in a concentration of RNA cargo of approximately 0.45 mg/mL. The LNPs were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 4.5 or about 6, with the ratio of mRNA to gRNA at 1:1 by weight.

[00685] The LNPs were formed by microfluidic mixing of the lipid and RNA solutions using a Precision Nanosystems NanoAssemblr™ Benchtop Instrument, according to the manufacturer's protocol. A 2:1 ratio of aqueous to organic solvent was maintained during mixing using differential flow rates. After mixing, the LNPs were collected, diluted in water (approximately 1:1 v/v), held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v) before final buffer exchange. The final buffer exchange into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS) was completed with PD-10 desalting columns (GE). If required, formulations were concentrated by centrifugation with Amicon 100 kDa centrifugal filters (Millipore). The resulting mixture was then filtered using a 0.2 μm sterile filter. The final LNP was stored at -80 °C until further use.

LNP Formulation – Cross-Flow

[00686] For LNPs prepared using the cross-flow technique, the LNPs were formed by impinging jet mixing of the lipid in ethanol with two volumes of RNA solutions 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* WO2016010840 FIG.2.) 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). Alternatively, the final buffer exchange into TSS was completed with PD-10 desalting columns (GE). If required, formulations were concentrated by centrifugation with Amicon 100 kDa centrifugal filters (Millipore). 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.

Formulation Analytics

[00687] 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. Average particle size and polydispersity are measured by dynamic light scattering (DLS) using a Malvern Zetasizer DLS instrument. LNP samples were diluted 30X in PBS prior to being measured by DLS. Z-average diameter which is an intensity based measurement of average particle size was 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 (50uL into 800uL) in 0.1X PBS, pH 7.4 prior to measurement.

[00688] Electrophoretic 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.

[00689] Asymmetric-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).

[00690] 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.

[00691] Cryo-electron microscopy ("cryo-EM") can be used to determine the particle size, morphology, and structural characteristics of an LNP.

[00692] Lipid compositional analysis of the LNPs can be determined from liquid chromatography followed by charged aerosol detection (LC-CAD). This analysis can provide a comparison of the actual lipid content versus the theoretical lipid content.

[00693] 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.

[00694] A fluorescence-based assay (Ribogreen®, ThermoFisher Scientific) is used to determine total RNA concentration and free 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.

[00695] 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

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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.

[00696] 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.

[00697] 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.

[00698] Typically, when preparing LNPs, encapsulation was >80%, particle size was <120 nm, and pdi was <0.2.

***LNP Delivery* In Vivo**

[00699] Unless otherwise noted, CD-1 female mice, ranging from 6-10 weeks of age were used in each study. Animals were weighed and grouped according to body weight for preparing dosing solutions based on group average weight. LNPs were dosed via the lateral tail vein in a volume of 0.2 mL per animal (approximately 10 mL per kilogram body weight). The animals were observed at approximately 6 hours post dose for adverse effects. Body weight was measured at twenty-four hours post-administration, and animals were euthanized at various time points by exsanguination via cardiac puncture under isoflourane anesthesia. Blood was collected into serum separator tubes or into tubes containing buffered sodium citrate for plasma as described herein. For studies involving in vivo editing, liver tissue was typically collected from the median lobe or from three independent lobes (e.g., the right median, left median, and left lateral lobes) from each animal for DNA extraction and analysis.

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Transthyretin (TTR) ELISA analysis used in animal studies

[00700] Blood was collected and the serum was isolated as indicated. The total mouse TTR serum levels were determined using a Mouse Prealbumin (Transthyretin) ELISA Kit (Aviva Systems Biology, Cat. OKIA00111); rat TTR serum levels were measured using a rat specific ELISA kit (Aviva Systems Biology catalog number OKIA00159); human TTR serum levels were measured using a human specific ELISA kit (Aviva Systems Biology catalog number OKIA00081); each according to manufacture's protocol. Briefly, sera were serial diluted with kit sample diluent to a final dilution of 10,000-fold, or 5,000-fold when measuring human TTR in mouse sera. 100ul of the prepared standard curve or diluted serum samples were added to the ELISA plate, incubated for 30 minutes at room temperature then washed 3 times with provided wash buffer. 100uL of detection antibody was then added to each well and incubated for 20 minutes at room temperature followed by 3 washes. 100uL of substrate is added then incubated for 10 minutes at room temperature before the addition of 100uL stop solution. The absorbance of the contents was measured on the Spectramax M5 plate reader with analysis using SoftmaxPro version 7.0 software. Serum TTR levels were quantitated off the standard curve using 4 parameter logistic fit and expressed as ug/mL of serum or percent knockdown relative control (vehicle treated) animals.

Genomic DNA isolation

[00701] Transfected cells were harvested post-transfection at 24, 48, or 72 hours. The genomic DNA was extracted from each well of a 96-well plate using 50 µL/well BuccalAmp DNA Extraction solution (Epicentre, Cat. QE09050) per manufacturer's protocol. All DNA samples were subjected to PCR and subsequent NGS analyses, as described herein.

Next-generation sequencing ("NGS") analysis

[00702] To quantitatively determine the efficiency of editing at the target location in the genome, sequencing was utilized to identify the presence of insertions and deletions introduced by gene editing.

[00703] Primers were designed around the target site within the gene of interest (e.g. *TTR*), and the genomic area of interest was amplified.

[00704] Additional PCR was performed per the manufacturer's protocols (Illumina) to add chemistry for sequencing. The amplicons were sequenced on an Illumina MiSeq instrument. The reads were aligned to a reference genome (e.g., the human reference genome (hg38), the cynomologus reference genome (mf5), the rat reference genome (rn6), or the mouse reference genome (mm10)) after eliminating those having low quality scores. The resulting files

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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.

[00705] The editing percentage (e.g., the "editing efficiency" or "percent editing" or "indel frequency") is defined as the total number of sequence reads with insertions/deletions ("indels") or substitutions over the total number of sequence reads, including wild type.

Analysis of secreted transthyretin ("TTR") protein by Western Blot

[00706] Secreted levels of TTR protein in media were determined using western blotting methods. HepG2 cells were transfected as previously described with select guides from Table 1. Media changes were performed every 3 days post transfection. Six days post-transfection, the media was removed, and cells were washed once with media that did not contain fetal bovine serum (FBS). Media without serum was added to the cells and incubated at 37°C. After 4hrs the media was removed and centrifuged to pellet any debris; cell number for each well was estimated based on the values obtained from a CTG assay on remaining cells and comparison to the plate average. After centrifugation, the media was transferred to a new plate and stored at -20°C. An acetone precipitation of the media was performed to precipitate any protein that had been secreted into the media. Four volumes of ice cold acetone were added to one volume of media. The solution was mixed well and kept at -20°C for 90min. The acetone:media mixture was centrifuged at 15,000xg and 4°C for 15min. The supernatant was discarded and the retained pellet was air dried to eliminate any residual acetone. The pellet was resuspended in 15µL RIPA buffer (Boston Bio Products, Cat. BP-115) plus freshly added protease inhibitor mixture consisting of complete protease inhibitor cocktail (Sigma, Cat. 11697498001) and 1mM DTT. Lysates were mixed with Laemmli buffer and denatured at 95°C for 10 minutes. Western blots were run using the NuPage system on 12% Bis-Tris gels (ThermoFisher) per the manufacturer's protocol followed by wet transfer onto 0.45 µm nitrocellulose membrane (Bio-Rad, Cat. 1620115). Blots were blocked using 5% Dry Milk in TBS for 30 minutes on a lab rocker at room temperature. Blots were rinsed with TBST and probed with rabbit α-TTR monoclonal antibody (Abcam, Cat. Ab75815) at 1:1000 in TBST. Alpha-1 antitrypsin was used as a loading control (Sigma, Cat. HPA001292) at 1:1000 in TBST and incubated simultaneously with the TTR primary antibody. Blots were sealed in a bag and kept overnight at 4°C on a lab rocker. After incubation, blots were rinsed 3 times for 5min each in TBST and probed with secondary antibodies to Rabbit (ThermoFisher, Cat.

PISA535571) at 1:25,000 in TBST for 30min at room temperature. After incubation, blots were rinsed 3 times for 5min each in TBST and 2 times with PBS. Blots were visualized and analyzed using a Licor Odyssey system.

Analysis of intracellular TTR by Western Blot

[00707] The hepatocellular carcinoma cell line, HUH7, was transfected as previously described with select guides from Table 1. Six-days post-transfection, the media was removed and the cells were lysed with 50 µL/well RIPA buffer (Boston Bio Products, Cat. BP-115) plus freshly added protease inhibitor mixture consisting of complete protease inhibitor cocktail (Sigma, Cat. 11697498001), 1 mM DTT, and 250 U/ml Benzonase (EMD Millipore, Cat. 71206-3). Cells were kept on ice for 30 minutes at which time NaCl (1 M final concentration) was added. Cell lysates were thoroughly mixed and retained on ice for 30 minutes. The whole cell extracts ("WCE") were transferred to a PCR plate and centrifuged to pellet debris. A Bradford assay (Bio-Rad, Cat. 500-0001) was used to assess protein content of the lysates. The Bradford assay procedure was completed per the manufacturer's protocol. Extracts were stored at minus 20°C prior to use. Western blots were performed to assess intracellular TTR protein levels. Lysates were mixed with Laemmli buffer and denatured at 95°C for 10min. Western blots were run using the NuPage system on 12% Bis-Tris gels (ThermoFisher) per the manufacturer's protocol followed by wet transfer onto 0.45 µm nitrocellulose membrane (Bio-Rad, Cat. 1620115). After transfer membranes were rinsed thoroughly with water and stained with Ponceau S solution (Boston Bio Products, Cat. ST-180) to confirm complete and even transfer. Blots were blocked using 5% Dry Milk in TBS for 30 minutes on a lab rocker at room temperature. Blots were rinsed with TBST and probed with rabbit α-TTR monoclonal antibody (Abcam, Cat. Ab75815) at 1:1000 in TBST. β-actin was used as a loading control (ThermoFisher, Cat. AM4302) at 1:2500 in TBST and incubated simultaneously with the TTR primary antibody. Blots were sealed in a bag and kept overnight at 4°C on a lab rocker. After incubation, blots were rinsed 3 times for 5 minutes each in TBST and probed with secondary antibodies to Mouse and Rabbit (ThermoFisher, Cat. PI35518 and PISA535571) at 1:25,000 each in TBST for 30min at room temperature. After incubation, blots were rinsed 3 times for 5min each in TBST and 2 times with PBS. Blots were visualized and analyzed using a Licor Odyssey system.

Example 2. Screening of dgRNA sequences

[00708] Cross screening of TTR dgRNAs in multiple cell types

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[00709] Guides in dgRNA format targeting human *TTR* and the cynomologus matched sequences were delivered to HEK293_Cas9, HUH7 and HepG2 cell lines, as well as primary human hepatocytes and primary cynomolgus monkey hepatocytes as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % edit. The screening data for the guide sequences in Table 1 in all five cell lines are listed below (Table 4 through 11).

[00710] Table 6 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the *TTR* crRNAs in the human kidney adenocarcinoma cell line, HEK293_Cas9, which constitutively over expresses Spy Cas9 protein.

Table 6: TTR editing data in Hek_Cas9 cells transfected with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	26.59	4.73	4.73	0.65	21.87	4.09
CR003336	29.09	4.57	3.31	0.24	25.78	4.32
CR003337	42.72	1.72	5.24	1.62	37.48	0.70
CR003338	52.42	3.28	4.76	0.03	47.66	3.30
CR003339	56.37	4.13	49.39	3.23	6.98	0.91
CR003340	42.38	8.43	27.88	4.31	14.50	4.13
CR003341	20.04	5.26	6.73	1.86	13.31	3.41
CR003342	36.57	5.80	1.19	0.22	35.38	5.59
CR003343	24.36	1.51	4.82	0.43	19.53	1.39
CR003344	33.87	2.93	4.32	0.58	29.54	2.37
CR003345	35.02	7.05	19.00	3.58	16.01	3.48
CR003346	48.33	5.81	33.03	3.12	15.30	2.72
CR003347	21.45	5.57	0.95	0.33	20.50	5.26
CR003348	35.53	5.81	22.32	3.79	13.21	2.03
CR003349	13.19	4.46	8.03	2.81	5.16	1.66
CR003350	22.31	4.25	5.54	0.74	16.77	3.51
CR003351	49.67	3.77	28.42	1.69	21.24	2.22
CR003352	27.90	7.55	4.91	1.35	22.99	6.26
CR003353	25.03	5.16	3.71	0.75	21.32	4.42
CR003354	18.46	2.02	2.56	0.21	15.90	1.89
CR003355	30.60	2.53	6.99	0.80	23.61	1.75
CR003356	32.21	4.71	10.03	1.39	22.19	3.36
CR003357	43.23	6.71	5.38	0.87	37.85	5.88
CR003358	5.44	0.86	1.29	0.16	4.14	0.84
CR003359	37.75	7.50	18.35	3.73	19.40	3.78
CR003360	22.68	3.16	2.70	0.56	19.98	2.60

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CR003361	34.45	8.97	8.66	1.66	25.78	7.32
CR003362	9.90	2.66	1.48	0.33	8.41	2.33
CR003363	31.03	10.74	14.77	4.21	16.26	6.54
CR003364	35.65	7.90	19.17	4.24	16.48	3.76
CR003365	36.43	6.20	11.83	1.88	24.61	4.45
CR003366	47.36	6.59	10.10	1.28	37.26	5.32
CR003367	47.11	15.43	28.44	9.11	18.67	6.33
CR003368	40.35	10.13	3.73	0.96	36.61	9.17
CR003369	33.10	7.26	9.06	1.12	24.04	6.16
CR003370	34.22	5.69	4.49	0.67	29.73	5.06
CR003371	25.60	8.33	3.84	1.41	21.76	6.92
CR003372	15.24	7.92	3.25	1.61	11.99	6.31
CR003373	13.55	2.40	1.31	0.21	12.25	2.19
CR003374	10.91	0.88	0.81	0.10	10.10	0.81
CR003375	11.63	3.18	0.78	0.17	10.85	3.05
CR003376	28.16	4.49	1.35	0.18	26.81	4.52
CR003377	24.70	4.44	2.71	0.54	21.99	3.91
CR003378	20.97	2.67	4.49	0.49	16.48	2.18
CR003379	26.32	2.91	5.34	0.61	20.98	2.30
CR003380	47.64	5.74	3.64	0.24	44.00	5.52
CR003381	22.04	5.74	3.82	1.26	18.23	4.64
CR003382	29.95	3.13	4.46	0.45	25.49	2.73
CR003383	40.47	0.64	25.12	0.45	15.35	0.66
CR003384	17.45	1.32	1.45	0.23	16.00	1.42
CR003385	26.19	5.62	7.36	1.57	18.82	4.06
CR003386	33.12	10.65	2.94	0.63	30.18	10.03
CR003387	24.68	5.93	7.75	1.99	16.92	3.94
CR003388	19.23	4.41	1.41	0.39	17.82	4.07
CR003389	34.18	5.09	10.30	2.12	23.87	3.02
CR003390	28.02	3.77	4.31	0.25	23.71	3.61
CR003391	44.81	4.67	0.61	0.07	44.19	4.63
CR003392	21.67	7.52	0.85	0.26	20.82	7.27

[00711] Table 7 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR crRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in the human hepatocellular carcinoma cell line, HUH7.

Table 7: TTR editing data in HUH7 cells transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	31.95	4.50	4.62	0.83	27.57	4.08

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CR003336	30.05	4.25	4.14	1.07	26.56	3.55
CR003337	55.72	3.12	8.34	0.93	48.95	2.24
CR003338	75.64	2.03	10.22	1.42	67.06	2.79
CR003339	79.97	4.73	60.55	3.94	20.13	1.02
CR003340	46.93	7.12	33.33	6.01	14.23	1.65
CR003341	20.58	5.98	7.78	1.64	13.20	4.44
CR003342	45.14	7.16	1.23	0.91	44.66	7.68
CR003343	76.13	7.04	9.58	3.49	66.97	6.10
CR003344	64.02	3.33	10.76	1.35	54.40	2.71
CR003345	72.43	2.17	41.33	0.96	32.18	1.37
CR003346	18.07	1.02	13.17	1.39	6.97	3.06
CR003347	32.16	5.50	1.64	0.42	30.79	5.11
CR003348	57.14	10.98	36.08	6.97	22.71	4.42
CR003349	14.14	4.99	9.73	3.26	4.82	1.91
CR003350	52.91	7.61	13.43	2.00	41.64	6.03
CR003351	63.51	4.61	36.87	2.49	27.49	2.14
CR003352	39.68	9.53	7.62	7.42	32.79	7.37
CR003353	69.18	4.59	7.73	2.46	62.87	3.13
CR003354	12.27	3.38	1.25	0.40	11.46	3.23
CR003355	38.83	5.31	9.40	1.81	30.31	3.56
CR003356	49.63	5.55	18.98	2.67	31.31	3.04
CR003357	36.31	5.72	6.37	1.17	30.82	4.68
CR003358	36.50	6.17	10.53	1.56	26.60	4.49
CR003359	66.75	5.84	21.73	2.30	45.97	3.93
CR003360	58.62	8.73	5.01	0.60	55.13	8.19
CR003361	28.68	6.52	6.84	1.26	22.44	5.31
CR003362	26.43	0.83	3.43	0.32	23.76	0.85
CR003363	41.01	7.16	17.83	3.32	23.78	3.97
CR003364	47.13	10.61	24.68	5.15	23.03	5.74
CR003365	60.68	5.25	17.77	1.57	43.82	3.73
CR003366	69.98	8.84	20.77	3.10	50.32	5.69
CR003367	66.29	4.48	33.62	4.14	33.48	0.51
CR003368	31.57	11.73	3.08	0.92	29.69	11.32
CR003369	24.19	6.89	7.12	2.27	17.38	4.76
CR003370	39.16	11.59	4.83	1.79	35.55	10.35
CR003371	40.47	7.68	6.07	0.89	35.65	7.01
CR003372	21.52	6.02	4.89	1.66	17.25	4.58
CR003373	27.29	4.45	3.31	0.66	25.12	4.12
CR003374	3.10	0.68	0.45	0.24	2.87	0.54
CR003375	2.38	0.22	0.26	0.14	2.25	0.12
CR003376	19.42	5.60	1.37	0.45	18.55	5.28
CR003377	34.93	5.47	5.59	0.88	29.89	4.71
CR003378	40.73	4.63	9.73	1.85	32.27	2.91
CR003379	19.18	5.17	3.38	0.77	16.48	4.32

CR003380	31.76	5.81	3.29	0.57	29.29	5.42
CR003381	99.70	0.17	1.92	0.20	99.70	0.17
CR003382	34.47	5.71	0.14	0.16	34.47	5.71
CR003383	42.89	10.14	2.14	0.56	41.19	9.67
CR003384	17.03	1.95	0.84	0.30	16.29	1.84
CR003386	69.40	19.41	0.53	0.23	69.34	19.32
CR003387	25.64	3.69	0.23	0.07	25.55	3.62
CR003388	59.48	4.29	3.88	0.68	56.45	4.45
CR003389	62.32	1.97	13.19	1.18	50.90	1.02
CR003390	18.97	4.82	3.31	0.91	16.49	3.98
CR003391	61.31	13.21	2.10	0.51	59.70	12.76
CR003392	28.37	8.58	1.93	0.73	26.98	7.94

[00712] Table 8 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR and control crRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in the human hepatocellular carcinoma cell line, HepG2.

Table 8: TTR editing data in HepG2 cells transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261 (control)	49.16	7.45	16.46	3.46	32.71	4.06
CR001262 (control)	63.33	5.66	59.88	4.92	3.45	0.86
CR001263 (control)	39.19	6.98	37.59	8.01	1.60	1.92
CR001264 (control)	57.09	12.14	47.47	9.25	9.61	2.89
CR003335	37.19	2.12	32.96	1.67	4.23	0.59
CR003336	31.31	5.47	30.48	5.10	0.83	0.75
CR003337	61.93	2.68	59.28	2.11	2.65	1.39
CR003338	68.00	6.09	65.40	6.78	2.60	1.17
CR003339	68.21	7.67	12.37	1.47	55.84	6.31
CR003340	37.76	6.01	6.12	1.95	31.65	4.07
CR003341	15.60	5.49	9.94	3.38	5.66	2.13
CR003342	11.06	6.71	10.78	6.69	0.28	0.03
CR003343	45.41	15.20	40.05	10.79	5.36	5.20
CR003344	33.43	6.11	29.81	5.09	3.62	1.13
CR003345	10.58	9.25	6.12	5.38	4.45	3.87
CR003346	0.13	0.05	0.07	0.02	0.05	0.03
CR003347	22.57	10.94	21.08	11.19	1.49	0.90
CR003348	38.44	10.45	17.04	5.04	21.40	5.89
CR003349	8.36	2.19	4.46	1.75	3.91	0.76

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CR003350	29.60	5.17	25.16	4.56	4.44	0.67
CR003351	57.54	5.67	31.98	2.63	25.57	3.08
CR003352	44.28	8.71	39.51	7.10	4.77	1.79
CR003353	60.40	11.37	56.71	9.95	3.68	1.45
CR003354	5.36	3.94	4.84	3.41	0.53	0.71
CR003355	15.80	5.38	12.36	4.23	3.44	1.16
CR003356	9.39	1.82	5.67	1.03	3.72	0.92
CR003357	45.83	10.66	42.37	8.47	3.46	2.28
CR003358	35.93	7.34	28.66	7.76	7.27	1.77
CR003359	64.44	14.90	48.79	14.32	15.65	1.94
CR003360	41.31	12.23	38.94	10.60	2.38	1.78
CR003361	14.05	4.79	11.47	4.35	2.58	0.43
CR003362	17.44	4.34	16.50	4.86	0.94	0.52
CR003363	42.65	9.90	28.58	6.95	14.07	3.01
CR003364	51.88	7.67	31.03	2.67	20.85	5.03
CR003365	46.88	15.78	35.77	13.49	11.11	2.30
CR003366	54.69	9.10	46.20	8.98	8.49	1.11
CR003367	45.55	8.19	24.28	6.57	21.27	1.62
CR003368	51.55	8.60	48.34	9.87	3.22	1.36
CR003369	22.62	4.01	17.11	4.47	5.51	2.52
CR003370	28.51	6.94	24.88	6.17	3.62	1.45
CR003371	15.91	4.17	14.07	4.02	1.84	0.22
CR003372	14.57	2.47	12.14	2.08	2.42	0.40
CR003373	17.69	8.41	15.92	6.44	1.77	1.97
CR003374	5.43	0.53	5.12	0.62	0.31	0.36
CR003375	2.06	0.04	1.96	0.06	0.10	0.03
CR003376	14.41	3.01	14.16	2.93	0.24	0.10
CR003377	16.30	2.85	15.29	2.59	1.02	0.59
CR003378	8.16	3.83	6.82	3.43	1.34	0.61
CR003379	19.74	4.24	17.70	4.30	2.04	0.33
CR003380	17.08	2.48	14.78	1.18	2.30	1.36
CR003381	6.81	3.48	6.18	3.82	0.63	0.44
CR003382	1.73	0.14	1.58	0.12	0.15	0.03
CR003383	6.35	1.67	6.19	1.68	0.16	0.04
CR003384	3.37	0.88	3.12	0.94	0.25	0.09
CR003385	53.94	9.41	46.32	10.66	7.62	1.29
CR003386	2.71	0.76	2.15	0.77	0.56	0.53
CR003387	1.39	0.15	1.27	0.17	0.12	0.02
CR003388	9.33	4.47	7.76	4.56	1.56	0.10
CR003389	31.84	6.09	27.27	5.96	4.57	1.21
CR003390	24.88	4.96	22.44	3.41	2.44	2.25
CR003391	48.78	14.41	48.28	14.44	0.50	0.52
CR003392	14.64	5.25	14.32	4.95	0.33	0.36
CR005298	42.65	10.94	21.29	8.16	21.36	2.87
CR005299	38.61	5.57	36.32	3.99	2.30	2.11
CR005300	64.34	9.55	53.20	6.59	11.15	3.33

CR005301	37.04	5.32	33.39	3.85	3.65	1.89
CR005302	33.21	2.19	30.93	2.43	2.29	0.24
CR005303	21.63	6.05	20.55	5.80	1.08	0.25
CR005304	62.82	3.28	8.07	1.22	54.75	4.27
CR005305	13.51	3.58	12.30	3.49	1.21	0.84
CR005306	24.07	5.24	21.20	5.03	2.87	1.10
CR005307	22.03	3.86	7.70	1.35	14.33	4.15

[00713] Table 9 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* dgRNAs electroporated with Spy Cas9 protein (RNP) in primary human hepatocytes.

Table 9: TTR editing data in primary human hepatocytes electroporated with Spy Cas9 protein loaded with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	72.20	4.53	69.70	4.36	2.50	0.30
CR003336	39.17	3.04	38.43	3.20	0.70	0.17
CR003337	54.27	2.70	53.23	3.05	1.30	0.26
CR003338	83.03	4.84	80.87	4.63	2.13	0.25
CR003339	43.00	2.66	8.93	1.86	34.07	1.72
CR003340	12.03	1.55	5.60	1.32	6.50	0.53
CR003341	11.43	0.71	7.03	0.50	4.40	1.21
CR003342	32.77	3.63	31.87	3.28	0.90	0.35
CR003343	77.10	2.21	75.63	2.01	1.50	0.36
CR003344	39.40	3.86	33.30	2.52	6.10	1.31
CR003345	48.07	6.24	34.53	2.95	13.57	3.74
CR003346	35.67	1.80	20.83	1.65	14.83	1.66
CR003347	82.30	5.93	81.97	5.98	0.43	0.15
CR003348	28.53	1.79	11.30	2.46	17.27	0.86
CR003349	4.10	0.17	2.33	0.46	1.87	0.25
CR003350	28.13	3.50	22.40	2.41	5.73	1.22
CR003351	51.77	5.11	30.83	3.32	20.97	2.43
CR003352	29.83	4.18	25.63	3.67	4.30	0.56
CR003353	84.83	4.68	82.23	4.05	2.63	0.74
CR003354	2.50	0.36	2.43	0.32	0.03	0.06
CR003355	12.53	1.54	10.60	2.36	1.97	1.17
CR003356	9.97	2.68	7.80	2.01	2.23	0.85
CR003357	36.23	4.02	35.47	4.11	0.77	0.61
CR003358	5.70	1.42	4.93	1.36	0.80	0.26
CR003359	63.77	7.07	56.33	5.81	7.50	1.35
CR003360	32.23	3.09	31.67	2.97	0.63	0.31
CR003361	4.10	0.36	3.73	0.42	0.37	0.06
CR003362	7.03	1.30	6.87	1.20	0.20	0.20

CR003363	9.43	8.22	7.80	6.86	1.63	1.44
CR003364	23.30	5.20	16.93	4.96	6.53	0.55
CR003365	42.37	3.88	35.57	1.88	6.83	2.00
CR003366	34.70	3.26	31.63	2.98	3.10	1.15
CR003367	39.20	5.31	22.93	4.14	16.37	1.46
CR003368	28.47	3.29	27.63	2.90	0.80	0.66
CR003369	3.67	1.16	3.30	1.06	0.40	0.20
CR003370	15.27	1.75	14.43	1.72	0.90	0.20
CR003371	16.20	2.13	14.47	2.37	1.87	0.81
CR003372	12.17	2.69	10.47	2.63	1.77	0.12
CR003373	0.87	0.21	0.83	0.25	0.07	0.12
CR003374	0.80	0.17	0.70	0.26	0.10	0.10
CR003375	1.33	1.10	1.27	1.08	0.07	0.06
CR003376	1.90	1.06	1.87	1.00	0.03	0.06
CR003377	10.23	1.53	10.13	1.51	0.10	0.10
CR003378	4.60	1.92	3.87	1.19	0.73	0.67
CR003379	6.57	1.00	6.30	0.70	0.27	0.31
CR003380	5.37	2.57	5.27	2.54	0.10	0.10
CR003381	6.20	2.74	5.83	2.61	0.50	0.10
CR003382	8.40	2.07	8.10	1.87	0.43	0.21
CR003383	8.57	0.75	3.37	0.67	5.27	0.46
CR003384	1.87	0.67	1.73	0.57	0.23	0.12
CR003385	40.87	6.86	38.43	6.41	2.53	0.45
CR003386	4.90	1.20	4.47	1.14	0.47	0.25
CR003387	1.87	0.25	1.70	0.26	0.20	0.10
CR003388	5.70	0.40	5.47	0.40	0.27	0.12
CR003389	27.67	2.76	27.20	2.88	0.50	0.36
CR003390	15.97	3.86	15.80	3.99	0.23	0.15
CR003391	29.77	3.85	29.57	3.85	0.27	0.06
CR003392	4.13	1.21	4.00	1.15	0.17	0.06
CR005298	39.90	2.92	22.37	3.04	17.57	0.42
CR005299	8.65	0.78	8.30	0.99	0.35	0.21
CR005300	57.47	1.69	53.47	1.86	4.10	0.92
CR005301	25.37	1.65	24.00	2.26	1.60	0.82
CR005302	61.10	5.20	60.10	4.77	1.00	0.46
CR005303	53.57	8.52	53.07	8.36	0.53	0.47
CR005304	67.00	5.80	5.53	1.37	61.63	6.98
CR005305	3.83	0.78	3.53	0.61	0.40	0.17
CR005306	9.43	1.63	8.07	2.17	1.37	0.72
CR005307	8.17	1.20	5.20	0.87	3.00	0.82

[00714] Table 10 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* and control dgRNAs transfected with Spy Cas9 protein (RNP) in primary human hepatocytes.

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Table 10: TTR editing data in primary human hepatocytes transfected with Spy Cas9 loaded with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261	32.51	1.00	12.50	0.47	20.01	0.59
CR001262	50.09	1.48	45.25	1.69	4.83	0.31
CR001263	15.25	2.41	14.83	2.37	0.42	0.10
CR001264	45.30	3.48	23.87	2.09	21.43	1.68
CR003335	51.14	4.27	49.51	4.04	1.63	0.25
CR003336	30.70	2.41	30.11	2.48	0.58	0.11
CR003337	49.43	4.75	47.54	4.49	1.88	0.47
CR003338	61.34	3.55	59.13	3.44	2.22	0.11
CR003339	45.06	9.83	8.85	1.65	36.21	8.34
CR003340	10.44	2.44	5.94	1.34	4.50	1.16
CR003341	19.66	3.67	14.64	3.31	5.02	0.37
CR003342	20.66	2.55	19.85	2.54	0.81	0.15
CR003343	43.25	4.47	41.61	4.26	1.63	0.33
CR003344	35.45	13.12	30.97	11.72	4.48	1.51
CR003345	28.90	6.33	21.00	5.23	7.91	1.81
CR003346	4.11	1.36	2.27	0.53	1.84	0.85
CR003347	66.35	4.48	66.11	4.51	0.24	0.08
CR003348	23.18	2.16	13.74	1.17	9.44	0.99
CR003349	10.83	1.57	9.00	1.41	1.83	0.32
CR003350	24.84	2.74	19.77	1.91	5.07	0.89
CR003351	40.28	1.31	23.92	0.70	16.36	0.78
CR003352	30.48	1.93	27.27	2.31	3.21	0.38
CR003353	61.54	4.13	59.38	4.04	2.16	0.11
CR003354	10.31	1.47	10.07	1.50	0.23	0.11
CR003355	19.11	0.92	17.69	0.79	1.42	0.44
CR003356	7.53	1.78	6.24	1.51	1.29	0.32
CR003357	49.35	2.53	48.45	2.54	0.90	0.13
CR003358	31.62	5.97	25.95	5.03	5.67	1.04
CR003359	59.47	6.05	50.96	5.69	8.51	0.54
CR003360	31.47	4.12	30.27	4.21	1.19	0.22
CR003361	13.08	1.48	12.52	1.45	0.56	0.18
CR003362	11.65	1.24	11.10	1.06	0.56	0.36
CR003363	27.65	2.84	21.47	2.39	6.18	0.61
CR003364	35.29	3.50	23.93	2.63	11.36	1.16
CR003365	47.78	3.67	40.24	3.12	7.54	0.72
CR003366	42.74	3.41	37.95	2.88	4.79	0.60
CR003367	31.19	4.60	16.06	2.66	15.13	1.94
CR003368	34.83	5.05	33.83	5.09	1.00	0.10
CR003369	12.98	0.26	11.67	0.21	1.31	0.11
CR003370	20.06	1.79	18.80	1.65	1.26	0.28
CR003371	18.80	2.73	17.23	2.34	1.57	0.43

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CR003372	17.56	2.26	15.74	2.16	1.81	0.10
CR003373	3.64	0.29	3.44	0.30	0.19	0.07
CR003374	2.65	0.33	2.52	0.33	0.14	0.02
CR003375	5.04	0.66	4.93	0.66	0.11	0.01
CR003376	5.00	1.10	4.86	1.10	0.14	0.03
CR003377	12.77	2.00	12.45	1.84	0.31	0.18
CR003378	8.66	1.90	8.24	1.74	0.42	0.19
CR003379	16.86	2.62	16.51	2.62	0.34	0.08
CR003380	8.17	1.42	7.71	1.47	0.46	0.10
CR003381	7.15	0.73	6.88	0.67	0.27	0.07
CR003382	2.44	0.06	2.28	0.05	0.15	0.03
CR003383	4.76	0.40	4.52	0.42	0.24	0.09
CR003384	3.56	0.26	3.39	0.26	0.17	0.01
CR003385	41.15	6.06	38.15	5.59	3.00	0.48
CR003386	3.22	0.25	2.97	0.27	0.25	0.02
CR003387	1.79	0.11	1.68	0.09	0.11	0.04
CR003388	5.43	1.03	4.38	1.00	1.05	0.25
CR003389	19.87	4.39	19.19	4.52	0.68	0.24
CR003390	16.09	2.84	15.85	2.91	0.24	0.09
CR003391	34.72	8.29	34.46	8.35	0.26	0.06
CR003392	10.07	1.06	9.93	1.02	0.14	0.04
CR005298	32.07	1.02	21.12	1.02	10.95	0.15
CR005299	19.37	0.61	18.79	0.51	0.58	0.13
CR005300	57.23	6.24	53.62	5.44	3.61	0.87
CR005301	31.37	3.02	29.53	2.88	1.84	0.15
CR005302	48.29	5.22	47.32	5.32	0.97	0.14
CR005303	36.45	4.83	36.06	4.72	0.39	0.12
CR005304	49.45	6.85	4.32	0.31	45.13	6.74
CR005305	7.07	1.43	6.73	1.30	0.34	0.17
CR005306	18.81	1.82	16.24	1.57	2.57	0.35
CR005307	18.73	1.68	10.18	0.92	8.55	0.88

[00715] Table 11 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* and control dgRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in primary human hepatocytes.

Table 11: TTR editing data in primary human hepatocytes transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261	32.33	4.95	5.83	1.63	26.47	3.30
CR001262	41.50	4.71	34.43	3.31	7.13	1.42
CR001263	10.23	3.61	9.40	3.20	0.90	0.44
CR001264	42.80	0.50	11.90	1.32	30.90	1.80

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CR003335	36.43	2.98	33.03	2.31	3.40	0.70
CR003336	16.93	3.78	16.20	3.41	0.80	0.44
CR003337	19.30	1.57	18.10	1.44	1.23	0.15
CR003338	36.30	9.55	33.73	9.27	2.73	0.49
CR003339	36.43	1.21	2.27	0.15	34.23	1.31
CR003340	24.97	2.78	1.83	0.23	23.17	2.66
CR003341	15.83	1.38	6.80	0.53	9.07	0.81
CR003342	22.10	1.27	20.60	0.57	1.50	0.71
CR003343	55.03	0.38	52.40	0.53	2.60	0.44
CR003344	31.50	1.30	22.40	1.31	9.20	0.10
CR003345	50.65	2.90	32.30	1.56	18.45	1.20
CR003346	19.97	1.94	5.63	0.55	14.33	1.72
CR003347	41.47	3.59	41.33	3.63	0.17	0.06
CR003348	18.00	0.87	2.30	0.66	15.80	0.61
CR003349	2.57	0.81	0.90	0.35	1.70	0.46
CR003350	26.63	4.25	16.33	2.45	10.33	1.75
CR003351	26.50	1.61	10.20	0.92	16.37	0.97
CR003352	16.80	5.03	11.73	3.86	5.07	1.14
CR003353	53.73	6.01	49.50	5.82	4.43	0.75
CR003354	2.97	0.95	2.87	0.85	0.13	0.12
CR003355	12.07	2.61	10.47	2.08	1.63	0.59
CR003356	7.27	0.72	4.70	0.53	2.67	0.21
CR003357	25.93	4.55	25.30	4.22	0.63	0.35
CR003358	3.90	0.79	2.73	0.45	1.17	0.51
CR003359	32.93	4.34	25.67	3.25	7.33	1.24
CR003360	14.90	4.85	14.13	4.66	0.90	0.52
CR003361	3.53	0.60	2.73	0.55	0.87	0.15
CR003362	6.60	1.47	6.17	1.45	0.47	0.21
CR003363	16.70	1.08	11.80	0.79	4.93	0.60
CR003364	15.63	2.45	6.73	0.81	8.93	1.70
CR003365	26.90	3.05	20.23	2.02	6.67	1.16
CR003366	24.53	1.26	20.47	1.45	4.07	0.23
CR003367	37.33	1.40	14.03	0.40	23.37	1.25
CR003368	11.10	1.91	10.53	1.90	0.60	0.10
CR003369	1.60	0.46	0.90	0.20	0.70	0.36
CR003370	2.83	0.57	2.33	0.40	0.50	0.17
CR003371	3.40	0.80	2.67	0.75	0.73	0.15
CR003372	1.77	0.75	1.13	0.57	0.63	0.23
CR003373	1.40	0.36	1.00	0.35	0.37	0.12
CR003374	0.27	0.21	0.27	0.21	0.03	0.06
CR003375	1.27	0.64	1.23	0.58	0.03	0.06
CR003376	2.83	0.81	2.73	0.81	0.13	0.06
CR003377	17.53	6.35	16.97	6.11	0.57	0.25
CR003378	9.80	1.37	8.50	1.21	1.37	0.15
CR003379	13.20	1.18	12.00	1.05	1.27	0.15
CR003380	2.93	0.58	2.47	0.57	0.47	0.15

CR003381	4.07	1.21	3.33	0.96	0.73	0.25
CR003382	0.97	0.25	0.97	0.25	0.00	0.00
CR003383	15.70	3.22	2.07	0.35	13.70	2.82
CR003384	1.70	0.62	1.50	0.56	0.20	0.10
CR003385	36.77	0.70	33.23	0.74	3.60	0.26
CR003386	8.27	1.63	8.20	1.57	0.13	0.06
CR003387	7.87	1.58	7.80	1.64	0.03	0.06
CR003388	12.97	1.30	11.87	1.21	1.17	0.25
CR003389	44.27	1.72	41.47	1.59	2.83	0.15
CR003390	20.23	2.08	18.73	1.92	1.60	0.17
CR003391	15.47	5.87	15.20	5.72	0.30	0.10
CR003392	2.43	0.55	2.37	0.59	0.07	0.06
CR005298	15.70	2.79	4.13	0.87	11.60	2.00
CR005299	9.43	0.68	8.93	0.68	0.60	0.00
CR005300	31.53	3.44	27.60	2.77	3.97	0.76
CR005301	6.77	1.44	5.47	0.96	1.40	0.61
CR005302	34.80	7.17	33.67	7.01	1.13	0.21
CR005303	35.50	5.90	35.00	5.81	0.50	0.10
CR005304	45.27	4.71	0.83	0.15	44.47	4.57
CR005305	7.53	1.06	5.93	1.10	1.60	0.10
CR005306	9.97	0.38	7.13	0.23	2.87	0.12
CR005307	12.90	2.43	3.67	0.61	9.30	1.80

[00716] Table 12 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* dgRNAs electroporated with Spy Cas9 protein (RNP) in primary cyno hepatocytes.

Table 12: TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003336	8.18	1.93	8.10	1.94	0.07	0.01
CR003337	24.94	5.80	24.10	4.71	0.84	1.10
CR003338	44.94	9.99	44.89	9.97	0.05	0.01
CR003339	8.95	0.89	4.93	0.64	4.02	0.25
CR003340	12.53	2.22	7.72	0.13	4.80	2.09
CR003341	8.43	10.53	7.66	9.91	0.77	0.63
CR003344	35.72	4.67	33.81	5.29	1.91	0.61
CR003345	52.92	3.26	30.74	0.78	22.19	2.48
CR003346	1.91	0.86	1.82	0.82	0.09	0.04
CR003347	72.41	0.38	72.15	0.73	0.25	0.34
CR003352	1.25	0.20	1.16	0.21	0.09	0.01
CR003353	4.75	0.43	4.67	0.47	0.08	0.04
CR003358	20.47	0.30	19.01	0.51	1.46	0.21

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CR003359	46.17	1.14	40.66	2.00	5.51	0.86
CR003360	29.47	0.63	29.05	1.00	0.42	0.37
CR003361	4.53	0.14	4.46	0.18	0.08	0.04
CR003362	4.59	0.80	4.36	0.77	0.22	0.03
CR003363	15.64	1.92	13.24	2.65	2.39	0.73
CR003364	19.62	2.54	14.27	2.72	5.35	0.17
CR003365	10.31	1.81	9.33	1.80	0.97	0.01
CR003366	18.52	0.71	17.62	0.33	0.90	0.39
CR003368	18.56	3.89	18.30	3.77	0.26	0.11
CR003369	1.53	0.25	1.28	0.40	0.25	0.15
CR003370	2.52	0.64	2.40	0.63	0.12	0.01
CR003371	1.83	0.38	1.69	0.41	0.14	0.03
CR003372	2.15	0.30	1.83	0.33	0.32	0.04
CR003382	10.86	2.04	8.54	1.93	2.33	0.11
CR003383	8.86	2.30	4.31	0.69	4.55	1.61
CR003384	3.75	0.35	2.50	0.37	1.25	0.02
CR003385	30.96	1.61	26.84	2.20	4.12	0.59
CR003386	5.54	1.42	3.51	1.26	2.03	0.15
CR003387	4.72	0.03	4.55	0.08	0.17	0.11
CR003388	6.81	0.17	6.59	0.28	0.22	0.11
CR003389	18.83	4.99	18.05	4.92	0.78	0.07
CR003390	16.87	3.88	16.49	3.48	0.39	0.39
CR003391	36.44	1.09	35.73	1.37	0.71	0.28
CR003392	7.02	0.97	6.63	0.59	0.38	0.37
CR005299	13.48	2.96	13.23	2.74	0.26	0.22
CR005301	46.76	1.75	46.34	2.19	0.42	0.44
CR005302	1.34	0.19	1.26	0.19	0.08	0.00
CR005303	59.28	1.05	58.72	1.06	0.56	0.00
CR005305	11.28	0.39	11.13	0.39	0.15	0.00
CR005307	4.56	0.71	2.01	0.49	2.55	0.21

[00717] Table 13 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific *TTR* dgRNAs electroporated with Spy Cas9 protein (RNP) on primary cyno hepatocytes.

Table 13: TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and cyno specific dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR000689	24.41	1.67	18.11	2.41	6.30	0.93
CR005364	27.70	0.74	0.58	0.29	27.11	0.60
CR005365	64.94	2.03	0.10	0.04	64.85	2.05
CR005366	77.00	1.17	0.33	0.27	76.67	0.99
CR005367	50.79	0.53	0.53	0.25	50.26	0.36

CR005368	27.60	2.07	0.33	0.45	27.27	2.32
CR005369	42.01	0.33	8.09	0.55	33.92	0.31
CR005370	63.52	3.21	0.59	0.33	62.93	2.88
CR005371	8.42	0.69	0.31	0.12	8.10	0.57
CR005372	17.98	1.39	0.83	0.77	17.16	0.71

Example 3. Screening of sgRNA sequences

Cross screening of TTR sgRNAs in multiple cell types

[00718] Guides in modified sgRNA format targeting human and/or cyno *TTR* were delivered to primary human hepatocytes and primary cyno hepatocytes as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % edit. The screening data for the guide sequences in Table 2 in both cell lines are listed below (Table 14 through 16).

[00719] Table 14 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs transfected with Spy Cas9 protein (RNP) in primary human hepatocytes.

Table 14: TTR editing data in primary human hepatocytes transfected with Spy Cas9 protein and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000480	81.80	1.98	77.15	2.19	4.70	0.28
G000481	46.90	1.71	27.77	3.88	19.43	4.76
G000482	66.67	2.35	56.57	4.14	10.10	1.85
G000483	47.90	6.56	19.57	3.37	28.50	3.25
G000484	62.97	0.90	29.23	0.21	33.83	0.95
G000485	56.07	3.37	53.07	2.84	3.13	0.60
G000486	69.73	6.86	9.83	1.93	59.93	5.63
G000487	67.30	2.75	65.27	3.41	2.07	1.06
G000488	61.27	1.95	26.30	1.55	35.00	1.30
G000489	60.17	2.75	51.07	3.18	9.43	0.45
G000490	55.90	7.88	46.13	7.55	9.80	0.69
G000491	74.30	1.55	70.27	2.37	4.33	0.72
G000492	60.97	5.81	57.90	4.64	3.13	1.35
G000493	41.40	3.08	38.90	3.29	2.67	0.35
G000494	62.23	3.30	61.47	3.25	0.77	0.31
G000495	50.80	1.85	45.80	1.25	5.37	0.64
G000496	72.33	1.63	44.73	2.14	27.67	1.46
G000497	59.67	1.40	51.10	1.14	8.73	0.71

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G000498	72.80	3.75	60.17	3.12	12.70	0.72
G000499	66.40	3.55	65.23	3.72	1.17	0.38
G000500	65.53	1.21	62.00	1.11	3.83	0.40
G000501	60.93	1.91	55.13	1.43	6.00	0.56

[00720] Table 15 shows the average and standard deviation at 12.5 nM for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in primary human hepatocytes.

Table 15: TTR editing data in primary human hepatocytes transfected with Spy Cas9 mRNA and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000480	73.28	0.61	59.85	0.13	13.47	0.51
G000481	34.30	5.26	14.62	2.59	19.77	2.72
G000482	40.93	3.95	27.70	2.92	13.25	0.97
G000483	27.82	2.93	4.05	0.51	23.85	2.43
G000484	43.37	6.79	13.98	2.61	29.48	4.15
G000485	30.82	5.76	28.87	5.50	1.97	0.28
G000486	59.13	5.62	2.82	0.86	56.37	4.92
G000487	49.57	0.99	47.38	0.89	2.27	0.24
G000488	49.40	5.05	11.98	1.40	37.48	3.68
G000489	24.25	2.82	14.17	2.01	10.28	1.38
G000490	24.72	2.35	19.38	2.04	5.38	0.41
G000491	45.93	1.22	42.42	1.06	3.60	0.33
G000492	34.65	2.21	32.45	2.01	2.22	0.25
G000493	11.55	1.35	10.65	1.58	0.97	0.30
G000494	26.22	4.03	25.17	3.89	1.07	0.15
G000495	47.77	1.88	43.40	1.91	4.45	0.17
G000496	63.30	2.60	11.08	2.10	52.25	0.67
G000497	40.33	3.32	34.48	2.71	5.85	0.61
G000498	60.02	5.42	45.20	4.34	14.90	1.08
G000499	39.30	6.04	38.58	5.86	0.77	0.12
G000500	35.50	0.61	32.47	0.49	3.10	0.18
G000501	40.32	1.50	33.82	2.04	6.62	0.55
G000567	27.28	7.59	17.35	4.72	10.02	2.94
G000568	43.75	5.83	43.00	5.81	0.80	0.18
G000570	68.42	3.64	68.08	3.61	0.35	0.00
G000571	20.47	3.41	14.47	2.72	6.13	0.78
G000572	55.42	8.13	41.62	6.48	13.85	1.60

[00721] Table 16 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs electroporated with Spy Cas9 protein

(RNP) on primary cyno hepatocytes. Note that guides G000480 and G000488 have one mismatch to cyno, which may compromise their editing efficiency in cyno cells.

Table 16: TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000480	10.20	0.56	9.83	0.81	0.37	0.25
G000481	69.13	8.62	33.73	2.67	35.50	11.23
G000482	75.17	2.34	55.23	2.00	20.03	0.85
G000485	22.93	0.95	22.00	0.82	1.07	0.21
G000486	79.90	0.79	11.90	0.85	68.07	0.35
G000488	9.63	0.50	5.37	0.38	4.27	0.35
G000489	67.53	1.15	53.53	1.56	14.17	0.64
G000490	61.67	0.72	54.47	1.10	7.27	1.23
G000491	66.20	1.11	64.37	0.47	1.90	0.70
G000493	50.13	0.74	48.07	1.69	2.10	0.98
G000494	81.53	0.71	79.57	0.49	2.07	0.67
G000498	91.37	1.48	68.50	1.64	22.87	1.50
G000499	83.40	0.36	82.00	0.20	1.43	0.55
G000500	45.20	3.66	42.60	3.80	2.63	0.25

[00722] Table 17 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific *TTR* sgRNAs electroporated with Spy Cas9 protein (RNP) on primary cyno hepatocytes.

Table 17: TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and cyno specific sgRNAs (e.g., those having an analogous human gRNA, See Table 3)

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000502	95.10	0.96	13.97	1.69	81.27	2.60
G000503	58.53	2.40	52.07	1.68	6.50	2.46
G000504	77.17	0.96	69.73	1.29	7.53	0.57
G000505	95.53	1.06	95.50	1.01	0.10	0.10
G000506	89.43	1.36	86.90	1.64	3.07	0.42
G000507	71.17	3.22	67.03	2.39	4.60	1.65
G000508	45.63	3.01	41.57	2.95	4.17	0.91
G000509	93.03	0.81	43.60	1.30	49.73	1.76
G000510	90.80	0.53	89.13	0.40	1.77	0.12
G000511	62.77	1.63	60.87	1.55	2.00	0.35

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Example 4. Screening of lipid nanoparticle (LNP) formulations containing Spy Ca9 mRNA and sgRNA

[00723] Cross screening of LNP formulated TTR sgRNAs with Spy Cas9 mRNA in primary human hepatocytes and primary cyno hepatocytes.

[00724] Lipid nanoparticle formulations of modified sgRNAs targeting human TTR and the cyno matched sgRNA sequences were tested on primary human hepatocytes and primary cyno hepatocytes in a dose response curve. Primary human and cyno hepatocytes were plated as described in Example 1. Both cell lines were incubated at 37°C, 5% CO_2 for 24 hours prior to treatment with LNPs. The LNPs used in the experiments detailed in Tables 18-21 were prepared using the Nanoassemblr™ procedure, each containing the specified sgRNA and Cas9 mRNA (SEQ ID NO:2), each having Lipid. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 45:44:9:2 molar ratio, respectively, and had a N:P ratio of 4.5. LNPs were incubated in hepatocyte maintenance media containing 6% cyno serum at 37°C for 5 minutes. Post incubation the LNPs were added onto the primary human or cyno hepatocytes in an 8 point 2-fold dose response curve starting at 100 ng mRNA. The cells were lysed 72 hours post treatment for NGS analysis as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % editing at 12.5 ng mRNA input and 3.9 nM guide concentration. The dose response curve data for the guide sequences in both cell lines is shown in Figs. 4 through 7. The % editing at 12.5 ng mRNA input and 3.9 nM guide concentration are listed below (Table 16 through 18).

[00725] Table 18 shows the average and standard deviation at 12.5 ng of cas9 mRNA for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary human hepatocytes as dose response curves. G000570 exhibited an uncharacteristic dose response curve compared to the other sgRNAs which may be an artifact of the experiment. The data are shown graphically in FIG.4.

Table 18: TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO:2) and sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA Avg % Edit	Std Dev % Edit
G000480	59.33	0.73

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G000481	24.37	0.37
G000482	19.10	2.64
G000483	7.37	0.67
G000484	16.67	1.23
G000485	14.23	2.36
G000486	61.33	2.59
G000487	17.37	0.95
G000488	44.80	3.00
G000489	16.85	0.06
G000490	10.53	1.90
G000491	31.60	2.33
G000492	15.87	0.44
G000493	7.33	0.73
G000494	6.37	1.07
G000495	23.97	1.66
G000496	30.73	3.76
G000497	15.10	3.30
G000498	24.43	1.30
G000499	16.07	1.67
G000500	23.57	2.44
G000501	32.30	2.49
G000567	48.95	1.06
G000568	54.60	3.68
G000570	88.30	1.84
G000572	55.45	1.20

[00726] Table 19 shows the average and standard deviation at 12.5 ng of mNRA and 3.9 nM guide concentration for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested *TTR* sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG.5.

Table 19: TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO: 2) and sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA, Avg % Edit	Std Dev % Edit
G000480	0.73	0.15
G000481	49.20	1.39
G000482	26.13	5.33
G000483	0.73	0.60
G000484	0.10	0.00
G000485	1.43	1.02
G000489	31.87	2.40

G000490	15.23	1.08
G000491	6.37	0.38
G000492	0.70	0.28
G000493	7.63	1.14
G000494	14.30	1.06
G000495	0.73	0.06
G000497	0.23	0.06
G000498	37.90	1.42
G000499	14.63	0.70
G000500	10.47	0.32
G000501	1.37	0.31
G000567	0.10	0.00
G000568	9.25	0.21
G000570	17.30	0.85
G000571	20.20	2.26
G000572	30.60	0.42

[00727] Table 20 shows the average and standard deviation at 12.5 ng of mRNA and 3.9 nM guide concentration for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific *TTR* sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG.6.

Table 20: TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO: 2) and cyno matched sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA % Edit	Std Dev % Edit
G000502	80.70	0.14
G000506	60.13	0.70
G000509	74.47	7.28
G000510	61.87	2.54

Cross screening of LNP formulated TTR sgRNAs with Spy Cas9 mRNA in primary human hepatocytes and primary cyno hepatocytes

[00728] Lipid nanoparticle formulations of modified sgRNAs targeting human TTR and the cyno matched sgRNA sequences were tested on primary human hepatocytes and primary cyno hepatocytes in a dose response curve. Primary human and cyno hepatocytes were plated as described in Example 1. Both cell lines were incubated at 37°C, 5% CO_2 for 24 hours prior to treatment with LNPs. The LNPs used in the experiments detailed in Tables 20-

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22 were prepared using the cross-flow procedure described above but purified using PD-10 columns (GE Healthcare Life Sciences) and concentrated using Amicon centrifugal filter units (Millipore Sigma), each containing the specificed sgRNA and Cas9 mRNA (SEQ ID NO:1). The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had a N:P ratio of 6.0. LNPs were incubated in hepatocyte maintenance media containing 6% cyno serum at 37°C, 5% CO_2 for 5 minutes. Post incubation the LNPs were added onto the primary human or cyno hepatocytes in an 8 point 3-fold dose response curve starting at 300 ng mRNA. The cells were lysed 72 hours post treatment for NGS analysis as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on EC50 values and maximum editing percent. The dose response curve data for the guide sequences in both cell lines is shown in Figs. 4 through 7. The EC 50 values and maximum editing percent are listed below (Table 19 through 22).

[00729] Table 21 shows the EC50 and maximum editing the tested human specific *TTR* sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary human hepatocytes as dose response curves. The data are shown graphically in FIG.4.

Table 21: TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA and human specific sgRNAs

GUIDE ID	EC50	Max Editing
G000480	0.10	98.69
G000481	1.43	87.05
G000482	0.65	97.02
G000483	1.88	77.39
G000484	0.95	94.14
G000488	0.72	95.83
G000489	1.38	86.33
G000490	1.52	94.16
G000493	2.42	63.95
G000494	1.28	75.70
G000499	0.63	96.31
G000500	0.39	88.70

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G000568	0.78	95.72
G000570	0.23	98.22
G000571	2.21	71.28
G000572	0.42	97.94

[00730] Table 22 shows the EC50 and maximum editing the tested human specific *TTR* sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG. 16.

Table 22: TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA and human specific sgRNAs

GUIDE ID	EC50	Max Editing
G000480	5.28	20.32
G000481	0.93	95.07
G000482	0.89	97.47
G000483	4.40	56.52
G000484	3.47	0.22
G000488	11.56	21.63
G000489	1.79	89.21
G000490	3.09	90.76
G000493	4.97	61.15
G000494	2.77	60.84
G000499	2.00	74.94
G000500	4.42	58.04
G000567	1.76	97.06
G000568	1.87	87.93
G000570	2.00	96.73
G000571	1.55	97.03
G000572	0.79	100.31

[00731] Table 23 shows the EC50 and maximum editing the tested cyno matched *TTR* sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary human hepatocytes as dose response curves. The data are shown graphically in FIG.17.

Table 23: TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA and cyno specific sgRNAs

GUIDE ID	EC50	Max Editing
G000502	0.70	91.50

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G000504	5.16	7.16
G000505	3.57	13.48
G000506	1.26	89.49

[00732] Table 24 shows the EC50 and maximum editing the tested cyno matched *TTR* sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG. 18.

Table 24: TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA and cyno specific sgRNAs

GUIDE ID	EC50	Max Editing
G000502	0.26	100.05
G000503	2.26	83.41
G000504	1.42	98.04
G000505	1.10	99.97
G000506	0.66	99.18

Example 5. Off-Target analysis of TTR dgRNAs and sgRNAs

Off-target analysis of TTR guides

[00733] An oligo insertion based assay (See, e.g., Tsai et al., Nature Biotechnology 33, 187–197; 2015) was used to determine potential off-target genomic sites cleaved by Cas9 targeting *TTR*. Forty-five dgRNAs from Table 1 (and two control guides with known off-target profiles) were screened in the HEK293_Cas9 cells. The human embryonic kidney adenocarcinoma cell line HEK293 constitutively expressing Spy Cas9 ("HEK293_Cas9") was cultured in DMEM media supplemented with 10% fetal bovine serum and 500 µg/ml G418. Cells were plated at a density of 30,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with a lipoplex containing individual crRNA (15 nM), trRNA (15 nM), and donor oligo with (10 nM) Lipofectamine RNAiMAX (0.3 µL/well) and OptiMem. Cells were lysed 24 hours post transfection and genomic DNA was extracting using Zymo's Quick gDNA 96 Extraction kit (catalog # D3012) following the manufacturer's recommended protocol. The gDNA was quantified using the Qubit High Sensitivity dsDNA kit (Life Technologies). Libraries were prepared per the previously described method in Tsai et al, 2015 with minor modifications. Sequencing

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was performed on Illumina's MiSeq and HiSeq 2500. The assay identified potential off-target sites for some of the crRNAs which are plotted in FIG.2.

[00734] Table 25 shows the number of off-target integration sites detected in HekCas9 cells transfected with *TTR* dgRNAs along with a double stranded DNA oligo donor sequence.

Table 25: Number of off-target integration sites detected for TTR dgRNAs via an oligo insertion based assay

GUIDE ID	# Sites
CR003335	0
CR003336	2
CR003337	10
CR003338	2
CR003339	3
CR003340	0
CR003342	0
CR003343	2
CR003344	0
CR003345	0
CR003346	0
CR003347	1
CR003348	3
CR003351	1
CR003352	2
CR003353	2
CR003355	1
CR003356	4
CR003357	3
CR003359	6
CR003360	0
CR003363	4
CR003365	3
CR003366	1
CR003367	1
CR003368	2
CR003369	2
CR003377	0
CR003380	0
CR003382	34
CR003383	1
CR003385	3
CR003386	1
CR003387	6
CR003388	2

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CR003389	2
CR003390	1
CR003391	0
CR003392	0
CR005298	0
CR005300	0
CR005301	0
CR005302	1
CR005303	1
CR005304	0

[00735] Additionally, a subset of the guides was assessed for off-target potential as modified sgRNAs in the Hek_Cas9 cells via the oligo based insertion method described above. The off-target results were plotted in FIG.4.

[00736] Table 26 shows the number of off-target integration sites detected in HekCas9 cells transfected with *TTR* sgRNAs along with a double stranded DNA oligo donor sequence.

Table 26: Number of off-target integration sites detected for TTR sgRNAs via an insertion detection method

GUIDE ID	# Sites
G000480	11
G000481	3
G000482	13
G000483	5
G000484	7
G000485	22
G000486	12
G000487	14
G000488	0
G000489	19
G000490	12
G000491	28
G000492	97
G000493	7
G000494	4
G000495	13
G000496	1
G000497	26
G000498	82
G000499	4
G000500	46
G000501	4

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G000567	9
G000568	937
G000570	19
G000571	16
G000572	15

Example 6. Targeted sequencing for validating potential off-target sites

[00737] The HEK293_Cas9 cells used in Example 5 for detecting potential off-targets constitutively overexpress Cas9, leading to a higher number of potential off-target "hits" as compared to a transient delivery paradigm in various cell types. Further, when delivering sgRNAs (as opposed to dgRNAs), the number of potential off-target hits may be further inflated as sgRNA molecules are more stable than dgRNAs (especially when chemically modified). Accordingly, potential off-target sites identified by an oligo insertion method as used in Example 5 may be validated using targeted sequencing of the identified potential off-target sites.

[00738] In one approach, primary hepatocytes are treated with LNPs comprising Cas9 mRNA and a sgRNA of interest (e.g., a sgRNA having potential off-target sites for evaluation). The primary hepatocytes are then lysed and primers flanking the potential off-target site(s) are used to generate an amplicon for NGS analysis. Identification of indels at a certain level may validate potential off-target site, whereas the lack of indels found at the potential off-target site may indicate a false positive in the HEK293_Cas9 cell assay.

Example 7. Phenotypic Analysis

Western blot analysis of secreted TTR

[00739] The hepatocellular carcinoma cell line, HepG2, was transfected as described in Example 1 with select guides from Table 1 in triplicate. Two days post-transfection, one replicate was harvested for genomic DNA and analysis by NGS sequencing for editing efficiency. Five days post-transfection, media without serum was replaced on one replicate. After 4hrs the media was harvested for analysis of secreted TTR by WB as previously described. The data for % edit for each guide and reduction of extracellular *TTR* is provided in FIG.7.

Western blot analysis of intracellular TTR

[00740] The hepatocellular carcinoma cell line, HUH7, was transfected as described in Example 1 with crRNA comprising the guides from Table 1. The transfected pools of cells were retained in tissue culture and passaged for further analysis. At seven days post-

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transfection, cells were harvested and whole cell extracts (WCEs) were prepared and subjected to analysis by Western Blot as previously described.

[00741] WCEs were analyzed by Western Blot for reduction of *TTR* protein. Full length *TTR* protein has a predicted molecular weight of ~16 kD. A band at this molecular weight was observed in the control lanes in the Western Blot.

[00742] Percent reduction of *TTR* protein was calculated using the Licor Odyssey Image Studio Ver 5.2 software. GAPDH was used as a loading control and probed simultaneously with *TTR*. A ratio was calculated for the densitometry values for GAPDH within each sample compared to the total region encompassing the *TTR* band. Percent reduction of *TTR* protein was determined after the ratios were normalized to control lanes. Results are shown in FIG.8.

Example 8. LNP delivery to humanized TTR mice and mice having wt (murine) TTR.

[00743] Mice humanized with respect to the *TTR* gene were dosed with LNP formulations 701-704 containing the guides indicated in Table 27 (5 mice per formulation). These humanized *TTR* mice were engineered such that a region of the endogenous murine *TTR* locus was deleted and replaced with an orthologous human *TTR* sequence so that the locus encodes a human TTR protein. For comparison, 6 mice with murine *TTR* were dosed with LNP700, containing a guide (G000282) targeting murine *TTR*. LNPs with Formulation Numbers 1-5 in Table 27 were prepared using the Nanoassemblr™ procedure as desctibed above while LNPs with Formulation Numbers 6-16 were prepared using the cross-flow procedure described above but purified using PD-10 columns (GE Healthcare Life Sciences) and concentrated using Amicon centrifugal filter units (Millipore Sigma). As negative controls, mice of the corresponding genotype were dosed with vehicle alone (Tris-saline-sucrose buffer (TSS)). The background of the humanized *TTR* mice administered LNPs with Formulation Numers 2-5 in Table 27 was 50% 129S6/SvEvTac 50% C57BL/6NTac; the background of the humanized *TTR* mice administered LNPs having Formulation Numbers 6-16 in Table 25 as well as the mice with murine *TTR* (administered LNP700, Formulation Number 1) was 75% C57BL/6NTac 25% 129S6/SvEvTac.

Table 27. LNP formulations for dosing humanized *TTR* mice.

Formulation Number	LNP	Guide	RNA concentration (mg/ml)	N:P Ratio	Molar Ratios (Lipid A, Cholesterol, DSPC, and PEG2k-DMG, respectively)
1	LNP700	G000282	0.53	4.5	45:44:9:2
2	LNP701	G000481	0.46	4.5	45:44:9:2

3	LNP702	G000489	0.61	4.5	45:44:9:2
4	LNP703	G000494	0.57	4.5	45:44:9:2
5	LNP704	G000499	0.59	4.5	45:44:9:2
6	LNP1148	G000481	0.73	4.5	45:44:9:2
7	LNP1152	G000499	0.45	6.0	50:38:9:3
8	LNP1153	G000482	0.53	6.0	50:38:9:3
9	LNP1155	G000571	0.70	6.0	50:38:9:3
10	LNP1156	G000572	0.58	6.0	50:38:9:3
11	LNP1157	G000480	0.84	6.0	50:38:9:3
12	LNP1159	G000488	0.79	6.0	50:38:9:3
13	LNP1160	G000493	0.71	6.0	50:38:9:3
14	LNP1161	G000500	0.66	6.0	50:38:9:3
15	LNP1162	G000567	0.69	6.0	50:38:9:3
16	LNP1163	G000570	0.66	6.0	50:38:9:3

[00744] LNPs having Formulation numbers 1-5 contained Cas9 mRNA of SEQ ID NO:2 and LNPs having Formulation Numbers 6-16 contained Cas9 mRNA of SEQ ID NO: 1, all in a 1:1 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in the molar ratios recited in Table 27, respectively. Dosing with LNPs having Formulation Numbers 1-5 was at 2 mg/kg (total RNA content) and dosing with LNPs having Formulation Numbers 6-16 was at 1 mg/kg (total RNA content). Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis. Liver editing results for Formulation Numbers 1-5 are shown in FIG.9 and indicate editing of the human *TTR* sequence with each of the four guides tested at a level >35% editing (mean values) with G000494 and G000499 providing values near 60%. Liver editing results for formulation numbers 6-8, 10-13, and 15-16 are shown in FIG.13 and Table 28, which show efficient editing of the human *TTR* sequence with each of the formulations tested. Greater than 38% editing was seen for all formulations, with several formulations providing editing values greater than 60%. Formulations 9 and 14 are not shown due to the design of the PCR amplicon and a resulting low number of sequencing reads.

[00745] The level of human TTR in serum was measured in the mice provided formulation numbers 6-8, 10-13, and 15-16. *See* FIG.14B. FIG.14A is a repeat of FIG.13 provided for comparison purposes. Knockdown of serum human TTR was detected for each formulation

tested, which correlated with the amount of editing detected in liver (See FIG.14A vs 14B, Table 28).

Table 28

GUIDE ID	% Editing	Serum TTR(%TSS)
TSS (vehicle)	0.06	100
G481	61.28	10.52
G499	65.66	8.39
G482	70.86	4.65
G572	73.52	2.11
G480	77.34	3.48
G488	59.125	27.78
G493	38.55	49.73
G567	47.525	44.24
G570	45.5	41.73
G571	33.88	11.39
G500	44.44	34.28

[00746] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000480, G000488, G000489 and G000502. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight

to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and having a N:P ratio of 6. Dosing was at 1, 0.3, 0.1, or 0.03 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG.26A and serum human TTR levels in FIG.26B-C. A dose response for both editing and serum TTR levels was evident.

[00747] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000481, G000482, G000486 and G000499. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had an N:P ratio of 6. Dosing was at 1, 0.3, or 0.1 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG.27A and serum human TTR levels in FIG.27B-C. A dose response for both editing and serum TTR levels was evident.

[00748] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000480, G000481, G000486, G000499 and G000502. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:2 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had an N:P ratio of 6. Dosing was at 1, 0.3, or 0.1 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG.28A and serum human TTR levels in FIG.28B-C. A dose response for both editing and serum TTR levels was evident.

[00749] In separate experiments using wild type CD-1 mice, an LNP formulation comprising guide G000502, which is cross homologous between mouse and cyno, was tested in a dose response study. The formulation contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide. The LNP contained Lipid A, Cholesterol, DSPC, and PEG2k-

DMG in a 45:44:9:2 molar ratio, respectively, and having a N:P ratio of 6. Dosing was at 1, 0.3, 0.1, 0.03, or 0.01 mg/kg (n=5/group). Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis. Results for liver editing are shown in FIG.15A and serum mouse TTR levels in FIG.15B. A dose response for both editing and serum TTR levels was evident.

Example 9. LNP delivery to mice in multiple doses

[00750] Mice (females from Charles River Laboratory, aged approximately 6-7 weeks) were dosed with an LNP formulation LNP705, prepared using cross-flow and TFF procedures as described above containing G000282 ("G282") and Cas9 mRNA (SEQ ID NO: 2) in a 1:1 ratio by weight and a total RNA concentration of 0.5 mg/ml. The LNP had an N:P ratio of 4.5 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 45:44:9:2 molar ratio, respectively. Groups were dosed either once weekly up to one, two, three, or four weeks (QWx1-4) or once monthly up to two or three months (QMx2-3). Dosages were 0.5 mg/kg or 1 mg/kg (total RNA content). Control groups received a single dose on day 1 of 0.5, 1, or 2 mg/kg. Each group contained 5 mice. Serum TTR was analyzed by ELISA and at necropsy the liver, spleen and muscle were each collected for NGS editing analysis. Groups are shown in Table 29. X = sacrifice and necropsy. MPK = mg/kg.

Table 29. Study Groups

Group	Duration/ Dose Regimen	Dose (MPK)	Total Dose (MPK) Given	Dose Day 1	Dose Day 8	Dose Day 15	Dose Day 22	NX Day 28	Dose Day 43	NX Day 49
1	4 Week Multi Dose/ QWx4	0 (TSS control)	0	X	X	X	X	X		
2	2 Month Multi Dose/ QMx3	1	3	X			X		X	X
3		0.5	1.5	X			X		X	X
4	1 Month Multi Dose/ QMx2	1	2	X			X	X		
5		0.5	1	X			X	X		
6	4 Week Multi Dose/ QWx4	1	4	X	X	X	X	X		
7		0.5	2	X	X	X	X	X		
8	3 Week	1	3		X	X	X	X		

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9	Multi Dose/ QWx3	0.5	1.5		X	X	X	X		
10	2 Week Multi Dose/ QWx2	1	2			X	X	X		
11		0.5	1			X	X	X		
12	Single Dose/ QWx1	1	1				X	X		
13		0.5	0.5				X	X		
14		2	2				Day 26	Day 32		

[00751] Table 30 and FIGS. 10A-11B show serum TTR level results (% KD = % knockdown). Table 30 and FIGS. 12A-C show liver editing results.

Table 30. Serum TTR Results.

Time Regimen	Dose	Serum TTR (µg/mL)	Serum TTR (% KD)
QWx4	TSS	1190.7	-
QMx3	0.5	245.01	79.42
QMx2	0.5	776.73	34.77
QWx4	0.5	347.43	70.82
QWx3	0.5	405.70	65.93
QWx2	0.5	432.25	63.70
QWx1	0.5	804.06	32.47
QMx3	1	91.95	92.28
QMx2	1	176.81	85.15
QWx4	1	119.52	89.96
QWx3	1	167.15	85.96
QWx2	1	130.98	89.00
QWx1	1	573.02	51.88
QWx1	2	219.07	81.60

Table 31. Liver Editing Results.

Time Regimen	Dose	Liver Editing (%)
QWx4	TSS	0.38
QMx3	0.5	48.18

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QMx2	0.5	36.66
QWx4	0.5	56.03
QWx3	0.5	51.35
QWx2	0.5	34.77
QWx1	0.5	24.16
QMx3	1	63.40
QMx2	1	57.37
QWx4	1	62.89
QWx3	1	59.22
QWx2	1	60.12
QWx1	1	35.16
QWx1	2	60.57

[00752] The results show that it is possible to build up a cumulative dose and effect with multiple administrations over time, including at weekly or monthly intervals, to achieve increasing editing levels and % KD of TTR.

Example 10. RNA Cargo: varying mRNA and gRNA ratios

[00753] This study evaluated in vivo efficacy in mice of different ratios of gRNA to mRNA. CleanCap™ capped Cas9 mRNAs with the ORF of SEQ ID NO: 4, HSD 5' UTR, human albumin 3' UTR, a Kozak sequence, and a poly-A tail were made by IVT synthesis as indicated in Example 1 with N1-methylpseudouridine triphosphate in place of uridine triphosphate.

[00754] LNP formulations prepared from the mRNA described and G282 (SEQ ID NO: 124) as described in Example 1 with Lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio and with an N:P ratio of 6. The gRNA:Cas9 mRNA weight ratios of the formulations were as shown in FIG.19A and 19B.

[00755] For in vivo characterization, the LNPs were administered to mice at 0.1 mg total RNA (mg guide RNA + mg mRNA) per kg (n=5 per group). At 7-9 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured as described in Example 1. Serum TTR and liver editing results are shown in FIG.19A and 19B. Negative control mice were dosed with TSS vehicle.

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[00756] In addition, the above LNPs were administered to mice at a constant mRNA dose of 0.05 mg mRNA per kg (n=5 per group), while varying the gRNA dose from 0.06 mg per kg to 0.4 mg per kg. At 7-9 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured. Serum TTR and liver editing results are shown in FIG.19C and FIG.19D. Negative control mice were dosed with TSS vehicle.

Example 11. Off-Target analysis of TTR sgRNAs in Primary Human Hepatocyes

[00757] Off-target analysis of sgRNAs targeting TTR was performed in primary human hepatocytes (PHH) as described in Example 5, with the following modifications. PHH were plated at a density of 33,000 cells per well on collagen-coated 96-well plates as described in Example 1. Twenty-four hours post plating, cells were washed with media and transfected using Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) as described in Example 1. Cells were transfected with a lipoplex containing 100 ng Cas9 mRNA, immediately followed by the addition of another lipoplex containing 25 nM of the sgRNA and 12.5 nM of the donor oligo (0.3 µL/well). Cells were lysed 48 hours post-transfection and gDNA was extracted and analyzed as further described in Example 5. The data is graphically represented in FIG.20.

[00758] Table 32 shows the number of off-target integration sites detected in PHH, and compares to the the number of sites that were detected in the HekCas9 cells used in Example 5. Fewer sites were detected in PHH for every guide tested as compared to the HekCas9 cell line, with no unique sites detected in PHH alone.

Table 32. Number of off-target integration sites detected for TTR sgRNAs in PHH via an oligo insertion based assay

GUIDE ID	# Sites in PHH	# Sites in HekCas9 cells (Example 5)
G000480	2	11
G000481	0	3
G000482	2	13
G000483	0	5
G000484	0	7
G000485	3	22
G000486	0	12
G000487	0	14
G000488	0	0

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G000489	2	19
G000490	0	12
G000491	7	28
G000492	5	97
G000493	1	7
G000494	0	4
G000495	1	13
G000496	0	1
G000497	3	26
G000498	19	82
G000499	1	4
G000500	12	46
G000501	0	4
G000567	0	9
G000568	11	936
G000570	1	19
G000571	1	16
G000572	2	15

[00759] Following the identification of potential off-target sites in PHH via the oligo insertion assay, certain potential sites were further evaluated by targeted amplicon sequencing, e.g., as described in Example 6. In addition to the potential off-target sites identified by the oligo insertion strategy, additional potential off-target sites identified by *in silico* prediction were included in the analysis.

[00760] To this end, PHH were treated with LNPs comprising 100 ng of Cas9 mRNA (SEQ ID NO:1) and the gRNA of interest at 14.68 nM (in a 1:1 ratio by weight), as described in Example 4. The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. Following LNP treatment, isolated genomic DNA was analyzed by NGS (e.g., as described in Examples 1 and 6) to determine whether indels could be detected at the potential off-target site, which would be indicative of a Cas9-mediated cleavage event. Tables 33 and 34 show the potential off-target sites that were evaluated for the gRNAs G000480 and G000486, respectively.

[00761] As shown in FIG.21A-B and 22A-B and Table 35 below, indels were detected at low levels for only two of the potential off-target sites identified by the oligo insertion assay for G000480, and only one for G000486. No indels were detected at any of the *in silico* predicted sites for either guide. Further, indels were only detected at these sites using a near-

saturating dose of LNP, as the indel rates observed at the on-target sites for G000480 and G000486 were ~97% and ~91%, respectively (See Table 35). The genomic coordinates of these sites are also reported in Tables 33 and 34, and each correspond to sequences that do not code for any protein.

[00762] A dose response assay was then performed in order to determine the highest dose of LNP in which no off-targets were detected. PHH were treated with LNPs comprising either G000480 or G000486 as described in Example 4. The doses ranged across 11 points with respect to gRNA concentration (0.001 nM, 0.002 nM, 0.007 nM, 0.02 nM, 0.06 nM, 0.19 nM, 0.57 nM, 1.72 nM, 5.17 nM, 15.51 nM, and 46.55 nM). As represented by the dashed vertical line in FIG.21A-B and 22A-B, the highest concentrations (with respect to the concentration of gRNA) at which the potential off-target sites were no longer detected for G000480 and G000486 were 0.57 nM and 15.51 nM, respectively, which resulted in on-target indel rates of 84.60% and 89.50%, respectively.

Table 33. Identified potential off target sites via insertion detection and in silico prediction for G000480 evaluated via targeted amplicon sequencing

GUIDE ID	Off-target (OT) Site ID	Assay Used	Chromosomal Coordinates (hg38)	Strand
G000480	INS-OT.1	Insertion Detection	chr7:94767406-94767426	+
G000480	INS-OT.2	Insertion Detection	chr2:192658562-192658582	+
G000480	INS-OT.3	Insertion Detection	chr7:4834390-4834410	+
G000480	INS-OT.4	Insertion Detection	chr20:9216118-9216138	-
G000480	INS-OT.5	Insertion Detection	chr10:12547071-12547091	+
G000480	INS-OT.6	Insertion Detection	chr6:168377978-168377998	-
G000480	INS-OT.7	Insertion Detection	chr12:114144669-114144689	-
G000480	INS-OT.8	Insertion Detection	chr10:7376755-7376775	+
G000480	INS-OT.9	Insertion Detection	chr2:52950299-52950319	+
G000480	INS-OT.10	Insertion Detection	chr8:56579165-56579185	-
G000480	INS-OT.11	Insertion Detection	chr1:189992255-189992275	+
G000480	PRED-OT.1	*in silico* prediction	chr10:12547071-12547091	+
G000480	PRE-DOT.2	*in silico* prediction	chrX:119702782-119702802	+
G000480	PRED-OT.3	*in silico* prediction	chr1:116544586-116544606	+
G000480	PRED-OT.4	*in silico* prediction	chr6:88282884-88282904	+
G000480	PRED-OT.6	*in silico* prediction	chr5:121891868-121891888	+
G000480	PRED-OT.7	*in silico* prediction	chr3:52544945-52544965	+
G000480	PRED-OT.8	*in silico* prediction	chr15:36949639-36949659	+
G000480	PRED-OT.9	*in silico* prediction	chr5:33866486-33866506	+
G000480	PRED-OT.10	*in silico* prediction	chr5:159755754-159755774	+

G000480	PRED-OT.11	*in silico* prediction	chr5:31349859-31349879	+
G000480	PRED-OT.12	*in silico* prediction	chr11:79485652-79485672	+
G000480	PRED-OT.13	*in silico* prediction	chr15:29448864-29448884	+
G000480	PRED-OT.14	*in silico* prediction	chr5:171153565-171153585	+
G000480	PRED-OT.15	*in silico* prediction	chr9:84855273-84855293	+
G000480	PRED-OT.16	*in silico* prediction	chr6:159953060-159953080	+
G000480	PRED-OT.17	*in silico* prediction	chr16:51849024-51849044	+
G000480	PRED-OT.18	*in silico* prediction	chr3:24108809-24108829	+
G000480	PRED-OT.19	*in silico* prediction	chr18:41118310-41118330	+
G000480	PRED-OT.20	*in silico* prediction	chr10:108975241-108975261	+
G000480	PREDO-T.21	*in silico* prediction	chr1:44683633-44683653	+
G000480	PRED-OT.22	*in silico* prediction	chr2:196214849-196214869	+
G000480	PRED-OT.23	*in silico* prediction	chr9:117353544-117353564	+
G000480	PRED-OT.24	*in silico* prediction	chr1:55583322-55583342	+
G000480	PRED-OT.25	*in silico* prediction	chr12:28246827-28246847	+
G000480	PRED-OT.26	*in silico* prediction	chr4:54545361-54545381	+
G000480	PRED-OT.27	*in silico* prediction	chr13:22364836-22364856	+
G000480	PRED-OT.28	*in silico* prediction	chr13:80816049-80816069	+
G000480	PRED-OT.29	*in silico* prediction	chr7:39078622-39078642	+
G000480	PRED-OT.30	*in silico* prediction	chr2:59944386-59944406	+

[00763] "INS-OT.N" refers to an off-target site ID detected by oligo insertion, where N is an integer specified above; "PRED-OT.N refers to an off-target site ID predicted via *in silico* methods, where N is an integer specified above.

Table 34. Identified potential off target sites via insertion detection and in silico prediction for G000486 evaluated via targeted amplicon sequencing

GUIDE ID	**Off-target (OT) Site ID**	**Assay Used**	**Chromosomal Coordinates (hg38)**	**Strand**
G000486	INS-OT.1	Insertion Detection	chr14:77332157-77332177	+
G000486	INS-OT.2	Insertion Detection	chr14:54672059-54672079	-
G000486	INS-OT.3	Insertion Detection	chr4:108513169-108513189	-
G000486	INS-OT.4	Insertion Detection	chr5:91397023-91397043	-
G000486	INS-OT.5	Insertion Detection	chr9:116626135-116626155	-
G000486	INS-OT.6	Insertion Detection	chr6:73201226-73201246	+
G000486	INS-OT.7	Insertion Detection	chr16:89368352-89368372	-
G000486	INS-OT.8	Insertion Detection	chr7:56308371-56308391	-
G000486	INS-OT.9	Insertion Detection	chr21:43605667-43605687	+
G000486	INS-OT.10	Insertion Detection	chr5:26758030-26758050	+
G000486	INS-OT.11	Insertion Detection	chr17:30656428-30656448	+
G000486	INS-OT.12	Insertion Detection	chr8:130486452-A130486472	+
G000486	PRED-OT.1	*in silico* prediction	chr11:44707064-44707084	+
G000486	PRED-OT.2	*in silico* prediction	chr5:50775396-50775416	+
G000486	PRED-OT.3	*in silico* prediction	chr4:141623949-141623969	+

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G000486	PRED-OT.4	*in silico* prediction	chr1:223481186-223481206	+
G000486	PRED-OT.5	*in silico* prediction	chr6:39951487-39951507	+
G000486	PRED-OT.6	*in silico* prediction	chrY:5456047-5456067	+
G000486	PRED-OT.8	*in silico* prediction	chr6:129868719-129868739	+
G000486	PRED-OT.9	*in silico* prediction	chrX:80450312-80450332	+
G000486	PRED-OT.10	*in silico* prediction	chr7:27256771-27256791	+
G000486	PRED-OT.11	*in silico* prediction	chr3:181416528-181416548	+
G000486	PRED-OT12	*in silico* prediction	chr7:146425020-146425040	+
G000486	PRED-OT.13	*in silico* prediction	chr3:16980977-16980997	+
G000486	PRED-OT.14	*in silico* prediction	chr7:118161002-118161022	+
G000486	PRED-OT.15	*in silico* prediction	chr6:102220539-102220559	+
G000486	PRED-OT.16	*in silico* prediction	chr12:127278991-127279011	+
G000486	PRED-OT.17	*in silico* prediction	chr2:67686631-67686651	+
G000486	PRED-OT.18	*in silico* prediction	chr1:114467665-114467685	+
G000486	PRED-OT.19	*in silico* prediction	chr3:194514436-194514456	+
G000486	PRED-OT.20	*in silico* prediction	chr14:31767581-31767601	+
G000486	PRED-OT.21	*in silico* prediction	chr16:28706209-28706229	+
G000486	PRED-OT.22	*in silico* prediction	chr8:110526279-110526299	+
G000486	PRED-OT.23	*in silico* prediction	chr19:2899814-2899834	+
G000486	PRED-OT.25	*in silico* prediction	chr3:130760261-A130760281	+
G000486	PRED-OT.26	*in silico* prediction	chr11:2506046-2506066	+
G000486	PRED-OT.27	*in silico* prediction	chr2:153918318-153918338	+
G000486	PRED-OT.28	*in silico* prediction	chr14:40590226-40590246	+
G000486	PRED-OT.29	*in silico* prediction	chr18:806650-806670	+
G000486	PRED-OT.30	*in silico* prediction	chr2:117707480-117707500	+

[00764] "INS-OT.N" refers to an off-target site ID detected by oligo insertion, where N is an integer specified above; "PRED-OT.N" refers to an off-target site ID predicted via *in silico* methods, where N is an integer specified.

Table 35. Detected Off Target sites in PHH treated with LNP containing 100 ng mRNA and 31.03 nM gRNA

GUIDE ID	**Off-target (OT) Site ID**	**Site Type**	**Indel Frequency (using LNP with 100 ng Cas9 mRNA and 14.68 nM gRNA)**	**Indel Frequency std. dev.**
G000480	n/a	On-Target	97.33%	1.10%
G000480	INS-OT.2	Off-Target	1.43%	0.40%
G000480	INS-OT.4	Off-Target	0.97%	0.25%
G000486	n/a	On-Target	91.33%	1.97%
G000486	INS-OT.4	Off-Target	0.47%	0.06%

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Example 12. LNP delivery to humanized mouse model of ATTR

[00765] A well-established humanized transgenic mouse model of hereditary ATTR amyloidosis that expresses the V30M pathogenic mutant form of human TTR protein was used in this Example. This mouse model recapitulates the TTR deposition phenotype in tissues observed in ATTR patients, including within the peripheral nervous system and gastrointestinal (GI) tract (See Santos et al., Neurobiol Aging. 2010 Feb;31(2):280-9).

[00766] Mice (aged approximately 4-5 months) were dosed with LNP formulations prepared using the cross-flow and TFF procedures as described in Example 1. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. The LNPs contained Cas9 mRNA (SEQ ID NO: 1) and either G000481 ("G481") or a non-targeting control guide G000395 ("G395"; SEQ ID NO: 273), in a 1:1 ratio of gRNA:mRNA by weight.

[00767] Mice were injected via the lateral tail vein as described in Example 1 with a single 1mg/kg (of total RNA content) dose of LNP with an n=10/group. At 8 weeks post treatment, the mice were euthanized for sample collection. Human TTR protein levels were measured in serum and cerebrospinal fluid (CSF) by ELISA as previously described by Butler et al., Amyloid. 2016 Jun;23(2):109-18. Liver tissue was assayed for editing levels as described in Example 1. Other tissues (stomach, colon, sciatic nerve, dorsal root ganglion (DRG)) were collected and processed for semi-quantitative immunohistochemistry as previously described by Gonçalves et al., Amyloid. 2014 Sep; 21(3): 175–184. Statistical analysis for the immunohistochemistry data was performed using Mann Whitney test with a p-value<0.0001.

[00768] As shown in FIG.23A-B, robust editing (49.4%) of TTR was observed in livers of the humanized mice following the single dose of LNP comprising G481, with no editing detected in the control group. Analysis of the editing events demonstrated that 96.8% of the events were insertions, with the remainder deletions.

[00769] As shown in FIG.24A-B, TTR protein levels were decreased in plasma but not in CSF from the treated mice, with greater than 99% knockdown of TTR plasma levels observed ($p<0.001$).

[00770] The near complete knockdown of TTR observed in the plasma of treated animals correlated with the clearance of TTR protein amyloid deposition in the assayed tissues. As shown in FIG.25, control mice exhibited amyloid staining in tissues which resembles the pathophysiology observed in human subjects with ATTR. Decreasing circulating TTR by

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editing the HuTTR V30M locus resulted in a dramatic decrease of amyloid deposition in tissues. Approximately 85% or better reduction in TTR staining was observed across the treated tissues 8 weeks post-treatment (FIG.25).

Example 13. *TTR* mRNA knockdown in Primary Human Hepatocytes (PHH)

[00771] In one experiment, PHH were cultured and treated with LNPs comprising Cas9 mRNA (SEQ ID NO:1) and a gRNA of interest (See FIG.29, Table 36), as described in Example 4. The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. The LNPs comprised a gRNA:mRNA ratio of 1:2, and the cells were treated at a dose of 300 ng (with respect to the amount of mRNA cargo delivered).

[00772] Ninety-six (96) hours following LNP treatment (with biological triplicates for each condition), mRNA was purified from PHH cells using the Dynabeads mRNA DIRECT Kit (ThermoFisher Scientific) according to the manufacturer's protocol. Reverse Transcription (RT) was performed with Maxima reverse transcriptase (ThermoFisher Scientific) and a poly-dT primer. The resulting cDNA was purified with Ampure XP Beads (Agencourt). For Quantitative PCR, 2% of the purified cDNA was amplified with Taqman Fast Advanced Mastermix and 3 Taqman probe sets, TTR (Assay ID: Hs00174914_m1), GAPDH (Assay ID: Hs02786624_g1), and PPIB (Assay ID: Hs00168719_m1). The assays were run on the QuantStudio 7 Flex Real Time PCR System according to the manufacturer's instructions (Life Technologies). Relative expression of *TTR* mRNA was calculated by normalizing to the endogenous controls (GAPDH and PPIB) individually, and then averaged.

[00773] As shown in FIG.29 and reproduced numerically in Table 36 below, each of the LNP formulations tested resulted in knockdown of *TTR* mRNA, as compared to the negative (untreated) control. The groups in FIG.29 and Table 36 are identified by the gRNA ID used in each LNP preparation. Relative expression of *TTR* mRNA is plotted in FIG.29, whereas the percent knockdown of *TTR* mRNA is provided in Table 36.

Table 36.

GUIDE ID	Avg % Knockdown	Std Dev
G000480	95.19	1.68
G000481	91.39	2.39
G000482	82.31	4.51

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G000483	68.78	13.45
G000484	75.22	9.05
G000488	92.77	3.76
G000489	91.85	2.77
G000490	78.34	5.76
G000493	87.53	4.54
G000494	91.15	3.63
G000499	91.38	1.71
G000500	92.90	3.15
G000567	90.89	5.39
G000568	53.44	20.20
G000570	93.38	2.66
G000571	96.17	2.07
G000572	55.92	24.53

[00774] In a separate experiment, *TTR* mRNA knockdown was evaluated following treatment with LNPs comprising G000480, G000486, and G000502. The LNPs were formulated and PHH were cultured and treated with the LNPs, each as described in the experiment above in this Example with the exception that the cells were treated at a dose of 100 ng (with respect to the amount of mRNA cargo delivered).

[00775] Ninety-six (96) hours following LNP treatment (single treatment for each condition), mRNA was purified from PHH cells using the Dynabeads mRNA DIRECT Kit (ThermoFisher Scientific) according to the manufacturer's protocol. Reverse Transcription (RT) was performed with the High Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific) according to the manufacturer's instructions. For Quantitative PCR, 2% of the cDNA was amplified with Taqman Fast Advanced Mastermix and 3 Taqman probe sets, TTR (Assay ID: Hs00174914_m1), GAPDH (Assay ID: Hs02786624_g1), and PPIB (Assay ID: Hs00168719_m1). The assays were run on the QuantStudio 7 Flex Real Time PCR System according to the manufacturer's instructions (Life Technologies). Relative expression of *TTR* mRNA was calculated by normalizing to the endogenous controls (GAPDH and PPIB) individually, and then averaged.

[00776] As shown in FIG.30 and reproduced numerically in Table 37 below, each of the LNP formulations tested resulted in knockdown of *TTR* mRNA, as compared to the negative (untreated) control. The groups in FIG.30 and Table 37 are identified by the gRNA ID used in each LNP preparation. Relative expression of *TTR* mRNA is plotted in FIG.30, whereas the percent knockdown of *TTR* mRNA is provided in Table 37.

Table 37.

GUIDE ID	Avg % Knockdown	Std Dev
G000480	95.61	0.92
G000486	97.36	0.63
G000502	90.94	2.63

Example 14. Corticosteroid pre-treatment and LNP delivery to non-human primates

[00777] Male cynomologus monkeys in cohorts of n=3 were treated with dexamethasone and varying doses of LNP to provide 1 mg/kg, 3 mg/kg, or 6 mg/kg (RNA) per NHP. Each formulation contained Cas9 mRNA000042 (SEQ ID No. 377) and guide RNA (gRNA) G000502 (SEQ ID No. 114) in a gRNA:mRNA ratio of 1:2 by weight. Except for animals

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treated with vehicle control, all animals received dexamethasone (Dex) pre-treatment at 2 mg/kg by IV bolus injection 1-2 hours prior to LNP administration. Doses of LNP (in mg/kg, total RNA content), were administered by 30 minute IV infusion.

[00778] At day 15 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. A minimum of 1.5 cm^3 of total liver biopsy were collected per animal. Each biopsy specimen was flash frozen in liquid nitrogen and stored at -86 to -60 °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 with all doses well tolerated. Corticosteroid pre-treatment with the described LNP treatment was well tolerated.

[00779] *Materials and Methods for Example 14.* 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.

[00780] 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).

[00781] LNP Formulation

[00782] 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.

Cas9 mRNA and gRNA Cargos

[00783] Capped and polyadenylated Cas9 mRNA was generated by *in vitro* transcription using a linearized plasmid DNA template and T7 RNA polymerase using the method

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described in Example 1.

Genomic DNA isolation

[00784] 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.

NGS Sequencing

[00785] 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.

[00786] 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.

[00787] 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 15: Multiple Dose LNP Study Administered via 30 Minute and 2 Hour IV Infusion in Cynomolgus Monkeys

[00788] 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 38. Two cohorts received an LNP dose of 3 mg/kg in order to compare infusion time. Formulations contained Cas9 mRNA and guide RNA were prepared as described below and in Example 14. The LNP formulations were prepared as described below and in Example 14. 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.

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Table 38: Infusion Study Dosing Groups

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

Table 39: % Editing and Serum TTR

Group Number	Liver Editing (%)	TTR % Reduction
1	0.0 (0.0, 0.0, 0.0)	-28 (-34, -23, -27)
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)

[00789] 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 and is shown in FIG. 31B. Results for the liver editing demonstrated up to about 70% editing. Serum TTR levels are depicted in FIG. 31A. Corticosteroid pre-treatment with the described LNP treatment was well tolerated.

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Table 40: Alanine Transaminase (ALT) Levels

Group	Pre- Bleed		6 Hour		24 Hour		48 Hour		Day 7		Day 29	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Group 1: 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
Group 2: 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
Group 3: 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
Group 4: 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

[00790] Samples were analyzed for percent editing data, serum TTR data, and alanine transaminase (ALT) levels as shown in Table 39 and FIGS. 31A-B, and Table 40 and FIG. 31C, respectively. Results for the liver editing and serum TTR data demonstrate that there is no significant difference in potency 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.

[00791] **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.

[00792] 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).

[00793] LNP Formulation

[00794] 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

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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.

Cas9 mRNA and gRNA Cargos

[00795] 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.

Genomic DNA isolation

[00796] 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.

NGS Sequencing

[00797] 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.

[00798] 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.

[00799] 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 16: Additional Numbered Embodiments

[00800] The following additional embodiments are provided.

[00801] Embodiment A1 is a composition comprising:

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(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.

[00802] Embodiment A2 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: 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.

[00803] Embodiment A3 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.

[00804] Embodiment A4 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.

[00805] Embodiment A5 is the composition of embodiment A1 or A4, for use in inducing a double-stranded break (DSB) within the *TTR* gene in a cell or subject.

[00806] Embodiment A6 is the composition of embodiment A1, A4, or A5 for use in

modifying the *TTR* gene in a cell or subject.

[00807] Embodiment A7 is the composition of embodiment A1, A4, A5, or A6 for use in treating amyloidosis associated with TTR (ATTR) in a subject.

[00808] Embodiment A8 is the composition of embodiment A1, A4, A5, A6, or A7 for use in reducing TTR serum concentration in a subject.

[00809] Embodiment A9 is the composition of embodiment A1, A4, A5, A6, A7, or A8, for use in reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

[00810] Embodiment A10 is the composition for use or method of any one of embodiments A2-A9, wherein the method comprises administering the composition by infusion for more than 30 minutes.

[00811] Embodiment A11 is the method or composition for use of embodiment A10, 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.

[00812] Embodiment A12 is the method or composition for use of embodiment A10 or 11, wherein the composition is administered by infusion for about 1.5-6 hours.

[00813] Embodiment A13 is the method or composition for use of embodiment A10, 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.

[00814] Embodiment A14 is the method or composition for use of embodiment A10, wherein the composition is administered by infusion for about 120 minutes.

[00815] Embodiment A15 is the method or composition for use of any one of embodiments A2-A14, wherein the composition reduces serum TTR levels.

[00816] Embodiment A16 is the method or composition for use of embodiment A15, wherein the serum TTR levels are reduced by at least 50% as compared to serum TTR levels before administration of the composition.

[00817] Embodiment A17 is the method or composition for use of embodiment A151, 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.

[00818] Embodiment A18 is the method or composition for use of any one of embodiments A2-17, wherein the composition results in editing of the TTR gene.

[00819] Embodiment A19 is the method or composition for use of embodiment A18, wherein the editing is calculated as a percentage of the population that is edited (percent

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editing).

[00820] Embodiment A20 is the method or composition for use of embodiment A19, wherein the percent editing is between 30 and 99% of the population.

[00821] Embodiment A21 is the method or composition for use of embodiment A19, 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.

[00822] Embodiment A22 is the method or the composition for use of any one of embodiments A2-A21, wherein the composition reduces amyloid deposition in at least one tissue.

[00823] Embodiment A23 is the method or composition for use of embodiment A22, wherein the at least one tissue comprises one or more of stomach, colon, sciatic nerve, or dorsal root ganglion.

[00824] Embodiment A24 is the method or composition for use of embodiment A22 or 23, wherein amyloid deposition is measured 8 weeks after administration of the composition.

[00825] Embodiment A25 is the method or composition for use of any one of embodiments A22-A24, wherein amyloid deposition is compared to a negative control or a level measured before administration of the composition.

[00826] Embodiment A26 is the method or composition for use of any one of embodiments A22-A25, wherein amyloid deposition is measured in a biopsy sample and/or by immunostaining.

[00827] Embodiment A27 is the method or composition for use of any one of embodiments A22-A26, 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.

[00828] Embodiment A28 is the method or composition for use of any one of embodiments A22-A27, 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.

[00829] Embodiment A29 is the method or composition for use of any one of embodiments A2-A28, wherein the composition is administered or delivered at least two times.

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[00830] Embodiment A30 is the method or composition for use of embodiment A29, wherein the composition is administered or delivered at least three times.

[00831] Embodiment A31 is the method or composition for use of embodiment A29, wherein the composition is administered or delivered at least four times.

[00832] Embodiment A32 is the method or composition for use of embodiment A29, wherein the composition is administered or delivered up to five, six, seven, eight, nine, or ten times.

[00833] Embodiment A33 is the method or composition for use of any one of embodiments A29-A32, 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.

[00834] Embodiment A34 is the method or composition for use of any one of embodiments A29-A32, 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.

[00835] Embodiment A35 is the method or composition for use of any one of embodiments A29-A32, 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.

[00836] Embodiment A36 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.

[00837] Embodiment A37 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is a dual guide (dgRNA).

[00838] Embodiment A38 is the method or composition of embodiment A37, 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.

[00839] Embodiment A39 is the method or composition of any one of embodiments A1-A36, wherein the guide RNA is a single guide (sgRNA).

[00840] Embodiment A40 is the method or composition of embodiment A39, wherein the sgRNA comprises a guide sequence that has the pattern of SEQ ID NO: 3.

[00841] Embodiment A41 is the method or composition of embodiment A39, wherein the sgRNA comprises the sequence of SEQ ID NO: 3.

[00842] Embodiment A42 is the method or composition of any one of embodiments A39-A41, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-72,

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74-78, and 80-82 and the nucleotides of SEQ ID NO: 126.

[00843] Embodiment A43 is the method or composition of any one of embodiments A39-A42, 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.

[00844] Embodiment A44 is the method or composition of embodiment A39, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-113, 115-120, and 122-124.

[00845] Embodiment A45 is the method or composition of any one of the preceding embodiments, wherein the guide RNA comprises at least one modification.

[00846] Embodiment A46 is the method or composition of embodiment A45, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

[00847] Embodiment A47 is the method or composition of embodiment A45 or 46, wherein the at least one modification includes a phosphorothioate (PS) bond between nucleotides.

[00848] Embodiment A48 is the method or composition of any one of embodiments A45-A47, wherein the at least one modification includes a 2'-fluoro (2'-F) modified nucleotide.

[00849] Embodiment A49 is the method or composition of any one of embodiments A45-A48, wherein the at least one modification includes a modification at one or more of the first five nucleotides at the 5' end.

[00850] Embodiment A50 is the method or composition of any one of embodiments A45-A49, wherein the at least one modification includes a modification at one or more of the last five nucleotides at the 3' end.

[00851] Embodiment A51 is the method or composition of any one of embodiments A45-A50, wherein the at least one modification includes PS bonds between the first four nucleotides.

[00852] Embodiment A52 is the method or composition of any one of embodiments A45-A51, wherein the at least one modification includes PS bonds between the last four nucleotides.

[00853] Embodiment A53 is the method or composition of any one of embodiments A45-A52, wherein the at least one modification includes 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

[00854] Embodiment A54 is The method or composition of any one of embodiments A45-A53, wherein the at least one modification includes 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

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[00855] Embodiment A55 is the method or composition of any one of embodiments A45-A54, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

[00856] Embodiment A56 is the method or composition of any one of embodiments A1-A55, wherein the composition further comprises a pharmaceutically acceptable excipient.

[00857] Embodiment A57 is the method or composition of any one of embodiments A1-A56, 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).

[00858] Embodiment A58 is the method or composition of embodiment A57, wherein the LNP comprises a CCD lipid.

[00859] Embodiment A59 is the method or composition of embodiment A58, wherein the CCD lipid is Lipid A or Lipid B, optionally wherein the CCD lipid is lipid A.

[00860] Embodiment A60 is the method or composition of any one of embodiments A57-A59, wherein the LNP comprises a helper lipid.

[00861] Embodiment A61 is the method or composition of embodiment A60, wherein the helper lipid is cholesterol.

[00862] Embodiment A62 is the method or composition of any one of embodiments A57-A61, wherein the LNP comprises a stealth lipid (e.g., a PEG lipid).

[00863] Embodiment A63 is the method or composition of embodiment A62, wherein the stealth lipid is PEG2k-DMG.

[00864] Embodiment A64 is the method or composition of any one of embodiments A57-A63, 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-%

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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.

[00865] Embodiment A64a is the method or composition of embodiment A64, wherein the mol-% PEG lipid is about 3.

[00866] Embodiment A64b is the method or composition of embodiment A64 or A64a, wherein the mol-% amine lipid is about 50.

[00867] Embodiment A64c is the method or composition of any one of embodiments A64-A64b, wherein the mol-% amine lipid is about 55.

[00868] Embodiment A64d is the method or composition of any one of embodiments A64-A64c, wherein the mol-% amine lipid is ± 3 mol-%.

[00869] Embodiment A64e is the method or composition of any one of embodiments A64-A64d, wherein the mol-% amine lipid is ± 2 mol-%.

[00870] Embodiment A64f is the method or composition of any one of embodiments A64-

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A64e, wherein the mol-% amine lipid is 47-53 mol-%.

[00871] Embodiment A64g is the method or composition of any one of embodiments A64-A64f, wherein the mol-% amine lipid is 48-53 mol-%.

[00872] Embodiment A64h is the method or composition of any one of embodiments A64-A64g, wherein the mol-% amine lipid is 53-57 mol-%.

[00873] Embodiment A64i is the method or composition of any one of embodiments A64-A64h, wherein the N/P ratio is 6 ± 1.

[00874] Embodiment A64j is the method or composition of any one of embodiments A64-A64i, wherein the N/P ratio is 6 ± 0.5.

[00875] Embodiment A64k is the method or composition of any one of embodiments A64-A64j, wherein the amine lipid is Lipid A.

[00876] Embodiment A64l is the method or composition of any one of embodiments A64-A64l, wherein the amine lipid is an analog of Lipid A.

[00877] Embodiment A64m is the method or composition of embodiment A64l, wherein the analog is an acetal analog.

[00878] Embodiment A64n is the method or composition of embodiment A64m, wherein the acetal analog is a C4-C12 acetal analog.

[00879] Embodiment A64o is the method or composition of embodiment A64m, wherein the acetal analog is a C5-C12 acetal analog.

[00880] Embodiment A64p is the method or composition of embodiment A64m, wherein the acetal analog is a C5-C10 acetal analog.

[00881] Embodiment A64q is the method or composition of embodiment A64m, wherein the acetal analog is chosen from a C4, C5, C6, C7, C9, C10, C11, and C12 analog.

[00882] Embodiment A64r is the method or composition of any one of embodiments A64-A64q, wherein the helper lipid is cholesterol.

[00883] Embodiment A64s is the method or composition of any one of embodiments A64-A64r, wherein the neutral lipid is DSPC.

[00884] Embodiment A64t is the method or composition of any one of embodiments A64-A64s, wherein the neutral lipid is DPPC.

[00885] Embodiment A64u is the method or composition of any one of embodiments A64-A64t, wherein the PEG lipid comprises dimyristoylglycerol (DMG).

[00886] Embodiment A64v is the method or composition of any one of embodiments A64-A64u, wherein the PEG lipid comprises a PEG-2k.

[00887] Embodiment A64w is the method or composition of any one of embodiments

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A64-A64v, wherein the PEG lipid is a PEG-DMG.

[00888] Embodiment A64x is the method or composition of embodiment A64w, wherein the PEG-DMG is a PEG2k-DMG.

[00889] Embodiment A64y is the method or composition of any one of embodiments A64-A64x, wherein the LNP composition is essentially free of neutral lipid.

[00890] Embodiment A64z is the method or composition of embodiment A64y, wherein the neutral lipid is a phospholipid.

[00891] Embodiment A65 is the method or composition of any one of embodiments A57-A64z, wherein the LNP comprises a neutral lipid, optionally wherein the neutral lipid is DSPC.

[00892] Embodiment A66 is the method or composition of any one of embodiments A64-A65, wherein the amine lipid is present at about 50 mol-%.

[00893] Embodiment A67 is the method or composition of any one of embodiments A64-A66, wherein the neutral lipid is present at about 9 mol-%.

[00894] Embodiment A68 is the method or composition of any one of embodiments A62-A67, wherein the stealth lipid is present at about 3 mol-%.

[00895] Embodiment A69 is the method or composition of any one of embodiments A60-A68, wherein the helper lipid is present at about 38 mol-%.

[00896] Embodiment A70 is the method or composition of any one of the preceding embodiments, wherein the LNP has an N/P ratio of about 6.

[00897] Embodiment A71 is the method or composition of embodiment A70, 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 lipid 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.

[00898] Embodiment A72 is the method or composition of any one of embodiments A64-A71, wherein the amine lipid is Lipid A.

[00899] Embodiment A73 is the method or composition of any one of embodiments A64-A72, wherein the neutral lipid is DSPC.

[00900] Embodiment A74 is the method or composition of any one of embodiments A62-A73, wherein the stealth lipid is PEG2k-DMG.

[00901] Embodiment A75 is the method or composition of any one of embodiments A60-A74, wherein the helper lipid is cholesterol.

[00902] Embodiment A76 is the method or composition of any one of embodiments A70,

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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.

[00903] Embodiment A77 is the method or composition of any one of the preceding embodiments, wherein the RNA-guided DNA binding agent is a Cas cleavase.

[00904] Embodiment A78 is the method or composition of embodiment A77, wherein the RNA-guided DNA binding agent is Cas9.

[00905] Embodiment A79 is the method or composition of any one of the preceding embodiments, wherein the RNA-guided DNA binding agent is modified.

[00906] Embodiment A80 is the method or composition of embodiment A79, wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

[00907] Embodiment A81 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.

[00908] Embodiment A82 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.

[00909] Embodiment A83 is the method or composition for use of any one of embodiments A2-A82, wherein the composition reduces or prevents amyloids or amyloid fibrils comprising TTR.

[00910] Embodiment A84 is the method or composition for use of embodiment A83, wherein the amyloids or amyloid fibrils are in the nerves, heart, or gastrointestinal track.

[00911] Embodiment A85 is the method or composition for use of any one of embodiments A2-A84, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the *TTR* gene.

[00912] Embodiment A86 is the method or composition for use of embodiment A85, wherein NHEJ leads to a deletion or insertion of a nucleotide(s) during repair of a DSB in the *TTR* gene.

[00913] Embodiment A87 is the method or composition for use of embodiment A86, wherein the deletion or insertion of a nucleotide(s) induces a frame shift or nonsense mutation in the *TTR* gene.

[00914] Embodiment A88 is the method or composition for use of embodiment A86, wherein a frame shift or nonsense mutation is induced in the *TTR* gene of at least 50% of liver cells.

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[00915] Embodiment A89 is the method or composition for use of embodiment A88, 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.

[00916] Embodiment A90 is the method or composition for use of any one of embodiments A86-A89, 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.

[00917] Embodiment A91 is the method or composition for use of embodiment A90, 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.

[00918] Embodiment A92 is the method or composition for use of any one of embodiments A86-A91, 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.

[00919] Embodiment A93 is the method or composition for use of embodiment A92, 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.

[00920] Embodiment A94 is the method or composition for use of embodiment A93, wherein the Cas9-overexpressing cells are HEK293 cells stably expressing Cas9.

[00921] Embodiment A95 is the method or composition for use of any one of embodiments A92-A94, 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.

[00922] Embodiment A96 is the method or composition for use of any one of embodiments A92-A94, 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

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protein coding region in the genome of the primary human hepatocytes.

[00923] Embodiment A97 is the method or composition of any one of embodiments A1-A36 or A39-A96, 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.

[00924] Embodiment A98 is the method or composition of embodiment A97, 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.

[00925] Embodiment A99 is the method or composition for use of any one of embodiments A2-98, wherein administering the composition reduces levels of TTR in the subject.

[00926] Embodiment A100 is the method or composition for use of embodiment A99, wherein the levels of TTR are reduced by at least 50%.

[00927] Embodiment A101 is the method or composition for use of embodiment A100, wherein the levels of TTR are reduced by 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100%.

[00928] Embodiment A102 is the method or composition for use of embodiment A100 or A101, wherein the levels of TTR are measured in serum, plasma, blood, cerebral spinal fluid, or sputum.

[00929] Embodiment A103 is the method or composition for use of embodiment A100 or A101, wherein the levels of TTR are measured in liver, choroid plexus, and/or retina.

[00930] Embodiment A104 is the method or composition for use of any one of embodiments A99-A103, wherein the levels of TTR are measured via enzyme-linked immunosorbent assay (ELISA).

[00931] Embodiment A105 is the method or composition for use of any one of embodiments A2-A104, wherein the subject has ATTR.

[00932] Embodiment A106 is the method or composition for use of any one of embodiments A2-A105, wherein the subject is human.

[00933] Embodiment A107 is the method or composition for use of embodiment A105 or 106, wherein the subject has ATTRwt.

[00934] Embodiment A108 is the method or composition for use of embodiment A105 or 106, wherein the subject has hereditary ATTR.

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[00935] Embodiment A109 is the method or composition for use of any one of embodiments A2-A106 or A108, wherein the subject has a family history of ATTR.

[00936] Embodiment A110 is the method or composition for use of any one of embodiments A2-A106 or A108-A109, wherein the subject has familial amyloid polyneuropathy.

[00937] Embodiment A111 is the method or composition for use of any one of embodiments A2-A110, wherein the subject has only or predominantly nerve symptoms of ATTR.

[00938] Embodiment A112 is the method or composition for use of any one of embodiments A2-A111, wherein the subject has familial amyloid cardiomyopathy.

[00939] Embodiment A113 is the method or composition for use of any one of embodiments A2-A110 or 112, wherein the subject has only or predominantly cardiac symptoms of ATTR.

[00940] Embodiment A114 is the method or composition for use of any one of embodiments A2-A113, wherein the subject expresses TTR having a V30 mutation.

[00941] Embodiment A115 is the method or composition for use of embodiment A114, wherein the V30 mutation is V30A, V30G, V30L, or V30M.

[00942] Embodiment A116 is the method or composition for use of embodiment Aany one of embodiments A2-A113, wherein the subject expresses TTR having a T60 mutation.

[00943] Embodiment A117 is the method or composition for use of embodiment A116, wherein the T60 mutation is T60A.

[00944] Embodiment A118 is the method or composition for use of embodiment Aany one of embodiments A2-A113, wherein the subject expresses TTR having a V122 mutation.

[00945] Embodiment A119 is the method or composition for use of embodiment A118, wherein the V122 mutation is V122A, V122I, or V122(-).

[00946] Embodiment A120 is the method or composition for use of any one of embodiments A2-A113, wherein the subject expresses wild-type TTR.

[00947] Embodiment A121 is the method or composition for use of any one of embodiments A2-A107, or A120, wherein the subject does not express TTR having a V30, T60, or V122 mutation.

[00948] Embodiment A122 is the method or composition for use of any one of embodiments A2-A107, or A120-A121, wherein the subject does not express TTR having a pathological mutation.

[00949] Embodiment A123 is the method or composition for use of embodiment A122,

wherein the subject is homozygous for wild-type *TTR*.

[00950] Embodiment A124 is the method or composition for use of any one of embodiments A2-A123, wherein after administration the subject has an improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy.

[00951] Embodiment A125 is the method or composition for use of embodiment A124, wherein the improvement, stabilization, or slowing of change in sensory neuropathy is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

[00952] Embodiment A126 is the method or composition for use of any one of embodiments A2-A125, wherein the subject has an improvement, stabilization, or slowing of change in symptoms of congestive heart failure.

[00953] Embodiment A127 is the method or composition for use of embodiment A126, 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.

[00954] Embodiment A128 is the method or composition for use of any one of embodiments A2-A127, wherein the composition or pharmaceutical formulation is administered via a viral vector.

[00955] Embodiment A129 is the method or composition for use of any one of embodiments A2-A127, wherein the composition or pharmaceutical formulation is administered via lipid nanoparticles.

[00956] Embodiment A130 is the method or composition for use of any one of embodiments A2-A129, wherein the subject is tested for specific mutations in the *TTR* gene before administering the composition or formulation.

[00957] Embodiment A131 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.

[00958] Embodiment A132 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 5. Embodiment A133 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 6. Embodiment A134 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 7. Embodiment A135 is the method or composition of any one of embodiments A1-

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A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 8. Embodiment A136 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 9. Embodiment A137 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 10. Embodiment A138 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 11. Embodiment A139 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 12. Embodiment A140 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 13. Embodiment A141 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 14. Embodiment A142 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 15. Embodiment A143 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 16. Embodiment A144 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 17. Embodiment A145 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 18. Embodiment A146 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 19. Embodiment A147 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 20. Embodiment A148 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 21. Embodiment A149 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 22. Embodiment A150 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 23. Embodiment A151 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 24. Embodiment A152 is the method or composition of any one of embodiments A1-

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A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 25. Embodiment A153 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 26. Embodiment A154 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 27. Embodiment A155 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 28. Embodiment A156 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 29. Embodiment A157 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 30. Embodiment A158 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 31. Embodiment A159 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 32. Embodiment A160 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 33. Embodiment A161 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 34. Embodiment A162 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 35. Embodiment A163 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 36. Embodiment A164 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 37. Embodiment A165 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 38. Embodiment A166 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 39. Embodiment A167 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 40. Embodiment A168 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 41. Embodiment A169 is the method or composition of any one of embodiments A1-

A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 42. Embodiment A170 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 43. Embodiment A171 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 44. Embodiment A172 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 45. Embodiment A173 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 46. Embodiment A174 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 47. Embodiment A175 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 48. Embodiment A176 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 49. Embodiment A177 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 50. Embodiment A178 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 51. Embodiment A179 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 52. Embodiment A180 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 53. Embodiment A181 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 54. Embodiment A182 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 55. Embodiment A183 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 56. Embodiment A184 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 57. Embodiment A185 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 58. Embodiment A186 is the method or composition of any one of embodiments A1-

A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 59. Embodiment A187 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 60. Embodiment A188 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 61. Embodiment A189 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 62. Embodiment A190 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 63. Embodiment A191 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 64. Embodiment A192 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 65. Embodiment A193 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 66. Embodiment A194 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 67. Embodiment A195 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 68. Embodiment A196 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 69. Embodiment A197 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 70. Embodiment A198 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 71. Embodiment A199 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 72. Embodiment A200 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 74. Embodiment A201 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 75. Embodiment A202 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 76. Embodiment A203 is the method or composition of any one of embodiments A1-

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A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 77. Embodiment A204 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 78. Embodiment A205 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 80. Embodiment A206 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 81. Embodiment A207 is the method or composition of any one of embodiments A1-A130, wherein the sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 82. Embodiment A208 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.

[00959] Embodiment A209 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.

[00960] Embodiment A210 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.

[00961] Embodiment A211 is the composition or method of embodiment A210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons listed in Table 4.

[00962] Embodiment A212 is the composition or method of embodiment A210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons of the Low U codon set of Table 5.

[00963] Embodiment A213 is the composition or method of embodiment A210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons of the Low A codon set of Table 5.

[00964] Embodiment A214 is the composition or method of embodiment A210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons of the Low A/U codon set of Table 5.

[00965] Embodiment A215 is the composition or method of embodiment A210, wherein the codons listed in Table 4, Table 5, or Table 7 are codons listed in Table 7.

[00966] Embodiment A216 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.

[00967] Embodiment A217 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.

[00968] Embodiment A218 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.

[00969] Embodiment A219 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.

[00970] Embodiment A220 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.

[00971] Embodiment A221 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.

[00972] Embodiment A222 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.

[00973] Embodiment A223 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.

[00974] Embodiment A224 is the composition or method of embodiment A223, wherein the modified uridine is one or more of N1-methyl-pseudouridine, pseudouridine, 5-methoxyuridine, or 5-iodouridine.

[00975] Embodiment A225 is the composition or method of embodiment A223, wherein the modified uridine is one or both of N1-methyl-pseudouridine or 5-methoxyuridine.

[00976] Embodiment A226 is the composition or method of embodiment A223, wherein the modified uridine is N1-methyl-pseudouridine.

[00977] Embodiment A227 is the composition or method of embodiment A223, wherein the modified uridine is 5-methoxyuridine.

[00978] Embodiment A228 is the composition or method of any one of embodiments

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A223-A227, wherein 15% to 45% of the uridine in the mRNA is substituted with the modified uridine.

[00979] Embodiment A229 is the composition or method of any one of embodiments A223-A228, wherein at least 20% or at least 30% of the uridine in the mRNA is substituted with the modified uridine.

[00980] Embodiment A230 is the composition or method of embodiment A229, wherein at least 80% or at least 90% of the uridine in the mRNA is substituted with the modified uridine.

[00981] Embodiment A231 is the composition or method of embodiment A229, wherein 100% of the uridine in the mRNA is substituted with the modified uridine.

[00982] Embodiment A232 is a use of a composition or formulation of any of embodiments A1 or A4-A231 for the preparation of a medicament for treating a human subject having ATTR.

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Sequence Table

[00983] 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	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGA AGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTC AAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGC AACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAA TGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATC TTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCCCACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACC CGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGGGAGAAAA GAAGAACGGACTGTTCGGAAACCTGATTGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACG AAGGCTGCAAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTC CTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAGTACA AGGAAATCTTCTTCGACCAGAGCAAGAACGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATT CGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGA AGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTC GCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTT CATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAG TCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCGAATTCCTGAGCGGAGAACAGAAGAAGGGCAATC GTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAG CGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCTCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTT TCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCTCACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAG CAGAAAGCTGATCAACGGAATCAGAGACAAGCAGTCCGGAAAGACAATCCTGGATTCCTGAAGTCCGACGATTCGCAAACAGAA GAGAAAGAATGAAGAGGAATCGAAGAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCCGGTCGAAAACACACAGCTG CAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGA	1

CTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGATGAAGAACTACTGGAGACACGCTGCTGAACGCAAAGCTGATC ACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGGAGGACTGAGCGAACTGGACAAGGCGGATTCATCAAGAGACAGCT GGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAGTACGACGAAAACGACAAGCTGA TCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATC AACAACTACCACACGCACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGA ATTCGTCTACGGAGACTACAAGGTCTACGACGTCAAGGAGATGATCGCAAAGAGCGAACAGATCGGAAAGGCAACAGCAAAGT ACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAA ACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAA CATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAA GAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCTAAAGGTCGAA AAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGAT CGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAA ACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTG TACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTA CCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCAT ACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCCGCCA GCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCT AGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAATGAAGATCAATAGCTTATTCATCTCTTTT TCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTCGTTGCAGGCCTTATTCGGATCCATGCCTAAGAAAAAGC GGAAGGTCGACGGGGATAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATAC AAAGTGCCGTCCAAGAAGTTCAAGGTCCTGGGGAACACCGATAGACACAGCATCAAGAAAATCTCATCGGAGCCCTGCTGTTTGA CTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGC AAGAGATCTTTTCGAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAG AAGCATGAACGGCATCCTATCTTTGGAAACACATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTACCATCTGCGGAA GAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCC TGATCGAAGGCGATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCAACTGGTGCAGACCTACAACCAACTGTTCGAA GAAAACCCAATCAATGCTAGCGGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAACCTGAT CGCACAGCTGCCCGGGAGAGAAAAGAACGGACTTTTCGGCAACTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCA ATTTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGACGACGACCTTGGACAATTTGCTGGCACAAATTGGC GATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTGCGCGTGAACACCGAAAT AACCAAAGCGCCGCTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGAC AGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAATGGGTACGCAGGGTACATCGATGGAGGCGCTAGCCAG GAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTCAAGCTGAACAGGGAGGATCT GCTCCGGAAACAGAGAACCTTTGACAACGGATCCATTCCCCACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGG	2

AGGACTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAAAATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTG GCGCGCGGCAATTCGCGCTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAA GGGAGCTTCGGCACAAAGCTTCATCGAACGAATGACCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAGCACAGCC TCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGTGAAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCC GGAGAACAGAAGAAAGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAA GAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCATGATCTCCTGA AGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAACGAGGACATCCTGGAAGATATCGTCCTGACCTTGACCTTTTCGAG GATCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAGGTCATGAAACAACTCAAGCGCCGCCGGTA CACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATTCGCGATAAACAGAGCGGTAAAACTATCCTGGATTTCCTCAAAT CGGATGGCTTCGCTAATCGTAACTTCATGCAATTGATCCACGACGACAGCCTGACCTTTAAGGAGGACATCCAAAAAGCACAAGTG TCCGGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAAATTCTGCAAACTGTGAA GGTGGTCGACGAGCTGGTGAAGGTCATGGGACGCACAAACCGGAGAATATCGTGATTGAAATGGCCCGAGAAAACCAGACTACCC AGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCAC CCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTGCAAAATGGACGGGACATGTACGTGGACCAAGAGCT GGACATCAATCGGTTGTCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGACTCGATCGATAACAAGGTGT TGACTCGCCAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGGCAG CTCCTGAATGCGAAGCTGATTACCCAGAAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGACTCTCAGAGCTGGATAAGGC TGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCTTGGACTCCCGCATGAACACTAAAT ACGACGAGAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGGACTTTCAG TTTTACAAAGTGAGAGAAATCAACAACTACCATGCGCATGACGCATACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAA GTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAGATGATAGCCAAGTCCGAACAGGAAA TCGGGAAAGCAACTGCGAAATACTTCTTTTACTCAAACATCATGAACTTTTTCAAGACTGAAATTACGCTGGCCAATGGAGAAATC AGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGT GCTCTCTATGCCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTCAAGGAATCGATCCTCCCAAAGAGAA ATAGCGACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTCGCATACTCCGTC CTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAAAGCTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATC CTCGTTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATCAAACTCCCCAAGT ACTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGCTGGCCTTGCCT AGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGAAAAACTCAAGGGTCACCGGAAGATAACGAAGCAGAAGCAGCTTTT CGTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCTCGCCGACGCCAACC TCGACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTATCCACTTGTTCACCCTG ACTAACCTGGGAGCCCCAGCCGCCTTCAAGTACTTCGATACTACTATCGATCGCAAAGATACACGTCCACCAAGGAAGTTCTGGA CGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGTGGCGATTGATAGTCTAGCC ATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTC GTTGGTGTAAAGCCAACACCCTGTCTTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAA ATGGAAAGAACCTCGAG		
modified sgRNA	mN*mN*mN*NNNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmGmCmU*mU*mU*mU	3

sequence ("N" may be any natural or non-natural nucleotide)		
30/30/39 poly-A sequence	AAAA

targeting Human TTR (Exon 1)		
CR003341 gRNA targeting Human TTR (Exon 1)	CUGGUAUUUGUGUCUGAGGC	11
CR003342 gRNA targeting Human TTR (Exon 2)	CUUCUCUACACCCAGGGCAC	12
CR003343 gRNA targeting Human TTR (Exon 2)	CAGAGGACACUUGGAUUCAC	13
CR003344 gRNA targeting Human TTR (Exon 2)	UUUGACCAUCAGAGGACACU	14
CR003345 gRNA targeting Human TTR (Exon 2)	UCUAGAACUUUGACCAUCAG	15
CR003346 gRNA targeting Human TTR (Exon 2)	AAAGUUCUAGAUGCUGUCCG	16
CR003347 gRNA	CAUUGAUGGCAGGACUGCCU	17

targeting Human TTR (Exon 2)		
CR003348 gRNA targeting Human TTR (Exon 2)	AGGCAGUCCUGCCAUCAAUG	18
CR003349 gRNA targeting Human TTR (Exon 2)	UGCACGGCCACAUUGAUGGC	19
CR003350 gRNA targeting Human TTR (Exon 2)	CACAUGCACGGCCACAUUGA	20
CR003351 gRNA targeting Human TTR (Exon 2)	AGCCUUUCUGAACACAUGCA	21
CR003352 gRNA targeting Human TTR (Exon 2)	GAAAGGCUGCUGAUGACACC	22
CR003353 gRNA targeting Human TTR (Exon 2)	AAAGGCUGCUGAUGACACCU	23
CR003354 gRNA	ACCUGGGAGCCAUUUGCCUC	24

targeting Human TTR (Exon 2)		
CR003355 gRNA targeting Human TTR (Exon 2)	CCCAGAGGCAAAUGGCUCCC	25
CR003356 gRNA targeting Human TTR (Exon 2)	GCAACUUACCCAGAGGCAAA	26
CR003357 gRNA targeting Human TTR (Exon 2)	UUCUUUGGCAACUUACCCAG	27
CR003358 gRNA targeting Human TTR (Exon 3)	AUGCAGCUCUCCAGACUCAC	28
CR003359 gRNA targeting Human TTR (Exon 3)	AGUGAGUCUGGAGAGCUGCA	29
CR003360 gRNA targeting Human TTR (Exon 3)	GUGAGUCUGGAGAGCUGCAU	30
CR003361 gRNA	GCUGCAUGGGCUCACAACUG	31

targeting Human TTR (Exon 3)		
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 (Exon 3)	AAAUAGACACCAAAUCUUAC	36
CR003367 gRNA targeting Human TTR (Exon 3)	AGACACCAAAUCUUACUGGA	37
CR003368 gRNA	AAGUGCCUUCCAGUAAGAUU	38

targeting Human TTR (Exon 3)		
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 Human TTR (Exon 4)	GCGGCGGGGGCCGGAGUCGU	44
CR003375 gRNA	AAUGGUGUAGCGGCGGGGGC	45

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

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

targeting Human TTR (Exon 4)		
CR003390 gRNA targeting Human TTR (Exon 4)	GGGAUUGGUGACGACAGCCG	60
CR003391 gRNA targeting Human TTR (Exon 4)	GGCUGUCGUCACCAAUCCCA	61
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	GUCACAGAAACACUCACCGU	66

targeting Human TTR (Exon 1)		
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	ACACAAAUACCAGUCCAGCG	73

targeting Cyno TTR		
CR005364 gRNA targeting Cyno TTR	AAAGGCUGCUGAUGAGACCU	74
CR005365 gRNA targeting Cyno TTR	CAUUGACAGCAGGACUGCCU	75
CR005366 gRNA targeting Cyno TTR	AUACCAGUCCAGCGAGGCAG	76
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	AAAAGGCUGCUGAUGAGACC	82

gRNA targeting Cyno TTR		
	Not Used	83
	Not Used	84
	Not Used	85
	Not Used	86
G000480 sgRNA modified sequence targeting Human TTR	mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	87
G000481 sgRNA modified sequence targeting Human TTR	mU*mC*mU*AGAACUUUGACCAUCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	88
G000482 sgRNA modified sequence targeting Human TTR	mU*mG*mU*AGAAGGGAUAUACAAAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	89
G000483 sgRNA modified sequence targeting Human TTR	mU*mC*mC*ACUCAUUCUUGGCAGGAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	90
G000484 sgRNA modified sequence	mA*mG*mA*CACCAAAUCUUACUGGAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	91

targeting Human TTR		
G000485 sgRNA modified sequence targeting Human TTR	mC*mC*mU*CCUCUGCCUUGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmCmGmGmUmGmCmU*mU*mU*mU	92
G000486 sgRNA modified sequence targeting Human TTR	mA*mC*mA*CAAAUACCAGUCCAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmCmGmGmUmGmCmU*mU*mU*mU	93
G000487 sgRNA modified sequence targeting Human TTR	mU*mU*mC*UUUGGCAACUUACCCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmCmGmGmUmGmCmU*mU*mU*mU	94
G000488 sgRNA modified sequence targeting Human TTR	mA*mA*mA*GUUCUAGAUGCUGUCCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmCmGmGmUmGmCmU*mU*mU*mU	95
G000489 sgRNA modified sequence targeting Human TTR	mU*mU*mU*GACCAUCAGAGGACACUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmCmGmGmUmGmCmU*mU*mU*mU	96
G000490 sgRNA modified sequence targeting Human TTR	mA*mA*mA*UAGACACCAAAUCUUACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmCmGmGmUmGmCmU*mU*mU*mU	97
G000491	mA*mU*mA*CCAGUCCAGCAAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA	98

sgRNA modified sequence targeting Human TTR	mAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	
G000492 sgRNA modified sequence targeting Human TTR	mC*mU*mU*CUCUACACCCAGGGCACGUUUUAGAmGmCmUmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	99
G000493 sgRNA modified sequence targeting Human TTR	mA*mA*mG*UGCCUUCCCAGUAAGAUUGUUUUAGAmGmCmUmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	100
G000494 sgRNA modified sequence targeting Human TTR	mG*mU*mG*AGUCUGGAGAGCUGCAUGUUUUAGAmGmCmUmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	101
G000495 sgRNA modified sequence targeting Human TTR	mC*mA*mG*AGGACACUUGGAUUCACGUUUUAGAmGmCmUmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	102
G000496 sgRNA modified sequence targeting Human TTR	mG*mG*mC*CGUGCAUGUGUUCAGAAGUUUUAGAmGmCmUmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	103
G000497 sgRNA modified sequence	mC*mU*mG*CUCCUCCUCUGCCUUGCGUUUUAGAmGmCmUmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	104

targeting Human TTR		
G000498 sgRNA modified sequence targeting Human TTR	mA*mG*mU*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	105
G000499 sgRNA modified sequence targeting Human TTR	mU*mG*mA*AUCCAAGUGUCCUCUGAGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	106
G000500 sgRNA modified sequence targeting Human TTR	mC*mC*mA*GUCCAGCAAGGCAGAGGGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	107
G000501 sgRNA modified sequence targeting Human TTR	mU*mC*mA*CAGAAACACUCACCGUAGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	108
G000567 sgRNA modified sequence targeting Human TTR	mG*mA*mA*AGGCUGCUGAUGACACCGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	109
G000568 sgRNA modified sequence targeting Human TTR	mG*mG*mC*UGUCGUCACCAAUCCCAGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	110
G000570	mC*mA*mU*UGAUGGCAGGACUGCCUGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA	111

sgRNA modified sequence targeting Human TTR	mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	
G000571 sgRNA modified sequence targeting Human TTR	mG*mU*mC*ACAGAAACACUCACCGUGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	112
G000572 sgRNA modified sequence targeting Human TTR	mC*mC*mC*CUACUCCCUAUUCCACCAGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	113
G000502 sgRNA modified sequence targeting Cyno TTR	mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	114
G000503 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*AGGCUGCUGAUGAGACCGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	115
G000504 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*GGCUGCUGAUGAGACCUGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	116
G000505 sgRNA modified sequence	mC*mA*mU*UGACAGCAGGACUGCCUGUUUUAGAmGmCmUmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	117

targeting Cyno TTR		
G000506 sgRNA modified sequence targeting Cyno TTR	mA*mU*mA*CCAGUCCAGCGAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	118
G000507 sgRNA modified sequence targeting Cyno TTR	mC*mC*mA*GUCCAGCGAGGCAGAGGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	119
G000508 sgRNA modified sequence targeting Cyno TTR	mC*mC*mU*CCUCUGCCUCGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	120
G000509 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*GUUCUAGAUGCCGUCCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	121
G000510 sgRNA modified sequence targeting Cyno TTR	mA*mC*mU*UGUCUUCUCUAUACCCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	122
G000511 sgRNA modified sequence targeting Cyno TTR	mA*mA*mG*UGACUUCCAGUAAGAUUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmGmUmUmCmGmGmUmUmGmCmU*mU*mU*mU	123
G000282	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA	124

sgRNA modified sequence targeting Mouse TTR	mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	
exemplary nucleotide sequence following the 3′ end of the Guide Sequence to form a sgRNA	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	125
exemplary nucleotide sequence following the 3′ end of the Guide Sequence to form a sgRNA	GUUUUAGAGCUAUGCUGUUUUG	126
	Not used	127 to 200
Cas9 DNA coding sequence 2	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTC AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA GACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGC CGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGC AGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCAC CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCG GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTA CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTAC	**201**

		202
Cas9 DNA coding sequence 1	ATGGATAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATACAAAGTGCCGTC CAAGAAGTTCAAGGTCCTGGGGAACACCGATAGACACAGCATCAAGAAAATCTCATCGGAGCCCTGTTTGACTCCGGCGAAA CCGCAGAAGCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAGATCTTT TCGAACGAAATGGCAAGGTCGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACG GCATCCTATCTTTGGAAACATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTG	

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Cas9 amino acid sequence	203 MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKK RKV
Cas9 mRNA open reading frame (ORF) 2	204 AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCACGUCAUCACACAGACGAAUACAAGGUCCCGAG CAAGAAGUUCAAGGUCCUGGGAAACACAGACACGCAUCAAGAAGAACCUGAUCGGAGCCACUGCUGUUCGACAGCGGAGAAA CAGCAGAAGCAACAAGAAGACUGAAGAGAACAGCAAGAAGAAGAUACACAAGAAGAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAG ACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCG ACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAGGGA GACCUGAACCCGGACAACAGCGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGAGUCGACAAGAAGCAGAAAGCCGAUGGAAAACACCUGAUCGCACAGCUGC CGGGAGAAAAGAAGAACGGACUGUUUCGGAAACCUGAUCGCACUGAGCCUGGGAGUCACUGACACCGAACUUCAAGAGCAACUUCGACCUG GCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCC AGACCUGUUCCUGGCAGCAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCAC CGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCG GAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCGGACUGGAUUACACUCGACGGAGGAGCAAGCCAGGAAGAUUCUA CAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCCAUCCUGAGAAGACAGGAAGACUUCUAC

Cas9 mRNA ORF 1	205

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UGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAAGAAAGAUGAUCGCAAAGAGCGAAAGUACAAGGAAAGGCA
ACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACC
GACAAGCUGAUCAGAGAAAAGGAGAAAGAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGC
CAGAGAAAUCAACAAGAUCGCACACCAGCGACAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGC
CGCAGGUCAACAUCGUCAAGAAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAG
CUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGC
AAAGGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAGCAGCUUCGAAA
AGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUC
GAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGU
CAACUUCCUGUACCUGGCCAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUUGUCGAACAGC
ACAAGCACUACCUGGACGAAAUCAUCGAACAGAUUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUC
CUGAGCGCCUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGG
AGCACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAGGAAGUCCUGGACGCAACACUGA
UCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAG
AGAAAGGUCUAG

AUGGAUAAGAAGUACUCAAUCGGGCUGGAUAUCGGAACUAAUUCCGUGGGUUGGGCAGUGAUCACGGAUGAAUACAAAGUGCCGUC
CAAGAAGUUCAAGGUCCUGGGGAACACCGAUAGACACAGCAUCAAGAAAAAUCUCAUCGGAGCCCUGCUGUUUGACUCCGGCGAAA
CCGCAGAAGCGACCCGGCUCAAACGUACCGCGAGGCGACGCUACACACCGGCGGAAGAAUCGCAUCUGCUAUCUGCAAGAGAUCUUU
UCGAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACCGCCUGGAAGAAUCUUUCCUGGUGGAGGAGGACAAGAAGCAUGAACG

GCAUCCUAUCUUUGGAAACAUCGUCGACGAAGUGGCGUACCACGAAAAGUACCCGACCAUCUACCAUCUGCGGAAGAAGUUGGUUG
ACUCAACUGACAAGGCCGACCUCAGAUUGAUCUACUUGGCCCUCGCCCAUAUGAUCAAAUUCCGCGGACACUUCCUGAUCGAAGGC
GAUCUGAACCCUGAUAACUCCGACGUGGAUAAGCUUUUCAUUCAACUGGUGCAGACCUACAACCAACUGUUCGAAGAAAACCCAAU
CAAUGCUAGCGGCGUCGAUGCCAAGGCCAUCCUGUCCGCCGGCUGUCGAAGUCGCGGCGCCUCGAAAACCUGAUCGCACAGCUGC
CGGGAGAGAAAAAGAACGGACUUUUUCGGCAACUUGAUCGCUCUCUCACUGGGACUCACACUCCCAAUUUCAAGUCCAAUUUUGACCUG
GCCGAGGACGCGAAGCUGCAACUCUCAAGGACACCUACGACGACGACUUGGACAACAAUUGGCUGGCACAAUUGGCGAUCAGUACGC
GGAUCUGUUCCUUGCCGCUAAGAACCUUUCGGACGCAAUCUUGCUGUCCGAUAUCCUGCGCGUGAACACCGAAAUAACCAAAGCGC
CGCUUAGCGCCUCGAUGAUUAAGCGUACGACGAGCAUCACCAGGAUCUCCACGCUGCUCAAAGCGCUCGUGAGACAGCAACUGCCU
GAAAAGUACAAGGAGAUCUUCUUCGACCAGUCCAAGAAUGGGUACGCAGGGUACAUCGAUGGAGAGGCGCUAGCCAGGAAGAGUUCUA
UAAGUUCAUCAAGCCAAUCCUGGAAAAGAUGGACGGAACCGAAGAACUGCUGGUCAAGCUGAACAGGGAGGAUCUGCUCCGGAAAC
AGAGAACCUUUGACAACGGAUCCAUCCCCACCAGAUCCAUCUGGGUGAGCUGCACGCCAUCUUGCGGCGCCAGGAGGACUUUUAC
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UUCGCGCUUCGCGUGGAUGACUAGAAAAUCAGAGGAAACCAUCACUCCUUGGAAUUUCGAGGAAGUUGUGGAUAAGGGAGCUUCGG
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UACUUCACUGUCUACAACGAACUGACUAAAGUGAAAUACGUUACUGAAGGAAUGAGAAGCCGGCCUUUCUGUCCGGAGAACAGAA
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GAUCGAGGAGAGGCUUAAGACCUACGCUCACUUCGACGAUAAGGUCAUGAAACAACUCAAGCGCCGCCGGUACACUGGUUGGG
GCCGCCUCUCCCGCAAGCUGAUCAACGGUAUUCGCGAUAAACAGAGCGGUAAAACUAUCCUGGAUUUCCUCAAAUCGGAUGGCUUC
GCUAAUCGUAACUUCAUGCAAUUGAUCCACGACAGCCUGACCUUUAAGGAGGACAUCCAAAAGCACAAGUGUCCGGACAGGG
AGACUCACUCCCAUGAACACAUCGCGAAUCUGGCCGGUUCGCCGGCGAUUAAGAAGGGAAUUCUGCAAACUGUGAAGGUGGUCGACG
AGCUGGUGAAGGUCAUGGGACGGCACAACCGGAGAAUAUCGUGAUUGAAAUGGCCCGAGAAAACCAGACUACCCAGAAGGGCCAG
AAAAACUCCCGCGAAAGGAUGAAGCGGAUCGAAGAGGAAUCAAGGAGCUGGGCAGCCAGAUCCUGAAAGAGCACCCGGUGGAAAA
CACGCAGCUGCAGAACGAGAAGCUCUACCUGUACUAUUUGCAAAAUGGACGGGACAUGUACGUGGACCAAGAGCUGGACAUCAAUC
GGUUGUCUGAUUACGACGUGGACCACAUCGUUCCACAGUCCUUUCUGAAGGAUGACUCGAUCGACAUUAACAAGGUGUUGACUCGCAGC
GACAAGAACAGAGGGAAGUCAGAUUAAUGUGCCAUCGGAGGAGGUCGUGAAGAAGAUGAAGAACUACUGGCGGCAGCUCCUGAAUGC
GAAGCUGAUUACCCAGAGAAAGUUUGACAAUCUCACUAAAGCCGAGCGCGGCGGACUCUCAGAGCUGGAUAAGGCUGGAUUUCAUCA
AACGGCAGCUGGUCGAGACUCGGCAGAUUACCAAGCACGUGGCGCAGAUCUUGGACUCCCGCAUGAACACUAAAUACGACGAGAAC
GAUAAGCUCAUCCGGGAAGUGAAGGUGACCUGAAAAGCAAACUUGUGUCGGAAGGACUUUCAGUUUUACAAAGU
GAGAGAAAUCAACAACUACCAUCACGCGCAUGACGCAUACCUCAACGCUGUGGUCGGUACCGCCCUGAUCAAAAAGUACCCUAAAC
UUGAAUCGGAGUUUGUGUACGGAGACUACAAGGUCUACGACGUGAGGAAGAUGAUAGCCAAGUCCGAACAGGAAAUCGGGAAAGCA
ACUGCGAAAUACUUCUUUUACUCAAACAUCAUGAACUUUUUCAAGACUGAAAUUACGCUGGCCAAUGGAGAAAUCAGGAAGAGGCC
ACUGAUCGAAACUAACGGAGAAACGGGCGAAAUCGUGUGGGACAAGGGCAGGGACUUCGCAACUGUUCGCAAAGUGCUCUCUAUGC
CGCAAGUCAAUAUUGUGAAGAAACCGAAAGUGCAAACCGGCGGAUUUUCAAAGGAAUCGAUCCUCCCAAAGAGAAUAGCGACAAG
CUCAUUGCACGCAAGAAAGACUGGGACCCGAAGAAGUACGGAGGAUUCGAUUCGCCGACUGUCGCAUACUCCGUCCUCGUGGUGGC
CAAGGUGGAGAAGGGAAAGAGCAAAAAGCUCAAAUCCGUCAAAGAGCUGCUGGGGAUUACCAUCAUGGAACGAUCCUCGUUCGAGA
AGAACCCGAUUGAUUUCCUCGAGGCGAAGGGUUACAAGGAGGUGAAGAAGGAUCUGAUCAUCAAACUCCCCAAGUACUCACUGUUC
GAACUGGAAAAUGGUCGAAGCGCAUGCUGGCUUCGGCCGGAGAACUCCAAAAAGGAAAUGAGCUGGCCUUGCCUAGCAAGUACGU

	CAACUUCCUCUAUCUUGCUUCGCACUACGAAAAACUCAAAGGGUCACCGGAAGAUAACGAACAGAAGCAGCUUUUCGUGGAGCAGC ACAAGCAUUAUCUGGAUGAAAUCAUCGAACAAAUCUCCGAGUUUUCAAAGCGCGUGAUCCUCGCCGACGCCAACCUCGACAAAGUC CUGUCGGCCUACAAUAAGCAUAGAGAUAAGCCGAUCAGAGAACAGGCCGAGAACACAUUAUCCACACUUGUUCACCCUGACUAACCUGGG AGCCCCAGCCGCCUUCAAGUACUUCGAUACUACUCGAUCGCAAAAGAUACACGUCCACCAAGGAAGUUCUGGACGCGACCCUGA UCCACCAAAGCAUCACUGGACUCUACGAAACUAGGAUCGAUCUGUCGCAGCUGGGUGGCGAUGGCGGUGGAUCUCCGAAAAAGAAG AGAAAGGUGUAAUGA
Cas9 nickase (D10A) amino acid sequence	206 MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGSPKKK RKV
Cas9 nickase (D10A) mRNA ORF	207 AUGGACAAGAAGUACAGCAUCGGACUGGCCAUCGGAACAAACAGCCGUCGGAUGGGCCAGUCAUCACGACGAAUACAAGGUCCCGAG CAAGAAGUUCAAGGUCCUGGGAAACACAGACACGCAUCAAGAAGAACCUGAUCGGAGCCACUGUCGUUCGACAGCGGAGAAA CAGCAGAAGCAACAAGACUGAAGAGAACACGCAAGAAGAAGAUACACAAGAAGAAGAACACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCCAAAGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAG ACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCG ACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCCCACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAGGGA GACCUGAACCCGGACAACAGCGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGAGCAAGAGACUGGAAAACCUGAUCGCCACAGCUGC CGGGAGAAAAGAAGAACGGACUGUUUCGGAAACCUGAUCGCCCUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUG GCAGAAGACGCCAAAGCUGCAGCUGAGCAAGGACACCAUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCGGCGACCAGUACGCC AGACCUGUUCCUGGCCGCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUCCUGAGAGUCAACACCGAAAUCACCAAAGGCAC CGCUGAGCGCCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCCAUCGUCAGACAGCAGCUGCCG GAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCGGAUUACAUCGACGGAGGAGCCAAGCAGGAAGAGAUUCUA CAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGC

dCas9 (D10A H840A) amino acid sequence	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP	208
	AGAGAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUAC CCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAA CAGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCG CACAGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAA UACUUCACAGUCUACAACGAACUGACAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAA GAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAGGACUACUUCAAGAAGAUCGAAU GCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAAAU GAUCGAAGAAAGACUGAAGACACAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAUACACAGGAUGGG GAAGACUGAGAGCAGAAAGCUGAUCAAACGGAAUCAGAGACAAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUC GCAAACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGG AGACAGCCUGCACGAACACAUCGCCAACCUGGCGAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACG AACUGGUCAAGGUCAUGGGACACAAGCCGGAAACAUCGUCAUCGAAAUGGCAAGAGAAAACCAGACACAGAAGGGACAG AAGAACAGCAGAGAAGAAUGAAGAGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACA GACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAUCGACAACAAGGUCCUGACAAGAGC GACAAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGC AAAGCUGAUCACACAGAAGUUCGACAACCUGACAAAGGCAGAGAGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCA AGAGACAGCUGGUCGAAACAAGACACAGAUCACACGUCGCACACGAUGCAGAUGAUCGCAAAGAGCGACAGCUUCGAAA GACAAGCUGAUCAGAGAAGUCAAGGUCACUCACACUGAAGAGCAAGUCGGUCAGCGACUUCAGAAGGACUUCCAGUUCUACAAGGU CAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGC UGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCA ACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACC GCUGAUCGAAACAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGC CGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAG CUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACACCCGACAGUCGCAUACAGCGUCCUGGUCGUCGC AAAGGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACACAUCAUGGAAAGAAGCAGCUUCGAAA AGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAAGCAGCUGUUCGUCGAACAGC ACAAGCACUACCUGGACGAAAUCAUCGAACAGAUUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUC CUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGG AGCACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGGCACAAGGAAGUCCUGGACGCAACACUGA UCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAG AGAAAGGUCUAG	

	EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKK RKV	209
dCas9 (D10A H840A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAG CAAGAAGUUCAAGGUCCUGGGAAACACAGACACGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAA CAGCAGAAGCAACAAGACUGAAGAGAACACAGCAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAG ACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCG ACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCCCACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGA GACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGACGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGC CGGGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUG GCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACGAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGCGACCAGUACGC AGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCAC CGCUGAGCGCAAGCGGAGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAGCGG GAAAAGUACAAGGAAAUCUUCGACCAGAGCAAGAACGGAUACGCGGAUUACAUCGACGGAGGAGCAAGCCAGGAAGAAAUUCUA CAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCCAAUCCUGAGAAGACAGGAAGACUUCUAC CCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAA CAGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCG CACAGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAA UACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAA GAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAU GCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAAAU GAUCGAAGAAAGACUGAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGACGUAUCACAGGAUGGG GAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUC GCAAACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGG	

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Cas9 bare coding sequence	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAA
	GAAGUUCAAGGUCCUGGGAAACACAGACGCACAUCAAGAAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG
	CAGAAGCAACAAGACUGAAGAGAAGAAGAUACACAAGAAGAAAGAACACAGAAUCUGCUACCUGCAGGAAAUCUUCAGC
	AACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA
	CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACACAAUCUACCACCUGAGAAAGAAGCUGGUCGACA
	GCACAGACAAGGCAGACCUGAGACUGAUCUACUUGGCACUGGCCCACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC
	GAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGA
	CCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGC
	UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACACAGCAGCUGCCGGAA
	AAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAUUCUACAA
	GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGA
	GAACAUUCGACAACGGAAGCAUCCCGCCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCG

	UUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGGGAAACAG CAGAUUCGCAUGGAUGACAAGAAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCCAC AGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUAC UUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAA GGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCU UCGACAGCGUCGAAGUCAGCGGAGUCGAAGACAGAUUCAACGCCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGAC AAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCGUCCUGACACUGACGUUGUUCGAAGACAGAGAAAUGAU CGAAGAAAGACUGAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACACACAGGAUGGGGAA GACUGAGCAGAAAGCUGAUCAACGGAUCAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCA AACAGAAACUUCAUGCAGCUGAUCCACGACGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGA CAGCCUGCACGAACACAUCGCCAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAAC UGGUCAAGGUCAUGGGAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG AACAGCAGAGAGAAGAAUGAAGAGGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGGAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGAC UGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACAAGAGCGAC AAGAACAGAGGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCCAAA GCUGAUCACACAGAAGCGAAAGUUCGACAACCUGACAAAGGCAGAGAGGAACUGGAGCGAAGGCAGGAUUCAUCAAGA GACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAACACAAAGUACGACGAAAACGAC AAGCUGAUCAGAGAAGUCAAGGUCACAUCACACUGAAGAGCAAGUCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAAGUCAG AGAAAUCAACAUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGG AAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACA GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCU GAUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGCCGC AGGUCAACAUCGUCAAGAAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUG AUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUUCGAGGAGGAUUCGACAGCCCGACAGUCGCUACAGCGUCCUGGUCGUCGCUCAAA GGUCGAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACACAAUCAUGGAAAGAAGCAGCUUCGAAAAGA ACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAA CUUCCUGUACCUGGCCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACA AGCACUACCUGGACGAAAUCAUCGAGCAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAACACAUCAUCCACCUGUUCACACUGACAACCUGGGAGC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACACAGAAAGAGAUACACACAAGCACAAAGGAAGUCCUGGACGCAACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGUC	211
Cas9 nickase bare coding sequence	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAA GAAGUUCAAGGUCCUGGGAAACACAGACGCACAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG CAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAUACACAAGAAAGAAAUCUGCUACCUGCAGGAAAUCUUCAGC AACGAAAUGGCAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA	

CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCGACA
GCACAGACAAGGCAGACCUGAGACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC
CUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAA
CGCAAGCGGAGUCGACGCAAGGCAAUCCUGAGCGCAAGAGCAGAGACUGGAAAACCUGAUCGCACAGCUGCCGG
GAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCCACUGAGCCUGGACUGACACCGAACUUCAAGAGCAACUUCGACCUGGCA
GAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACAUCCUGGCUCAGAUCGGAGACCAGUACGCAGA
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UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCGGAA
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GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGA
GAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCG
UUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAG
CAGAUUCGCAUGGAUGACAAGAAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCAC
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UCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCCAAGCCUGGGACAUACCACGACCUGCUGAAGAUCAUCAAGGAC
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CGAAGAAAGACUGAAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAUACACAGGAUGGGGAA
GACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAAGAGCGACGGAUUCGCA
AACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGA
CAGCCUGCACGAACACAUCGCAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAAC
UGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACACAUCGUCAUCGACAAAUGGCAAGAGAAAACCAGACACACACAGAAGGGACAGAAG
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GCUGAUCACACAGAAGAAAGUUCGACAACCUGACAAAGGCAGAGAGGAGGACUGAGCGACAAGGCAGGAUUCAUCAAGA
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GACAGCUGGUCGAAACACAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAAAGUACGACGAAAACGAC
AAGCUGAUCAGAGAAGUCAAGGUCACACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAG
AGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUCGGUCCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGG
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GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCU
GAUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGCCGC
AGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUG
AUCGUCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCUACACAGCGUCCUGGUCGUCGCAAA
GGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGA
ACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA
CUGGAAACGGAGAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAA

	CUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACA AGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACACAGGCAGAAAACAUCAUCCACCUGUUCACCCUGACAAACCUGGGAGC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGUC	212
dCas9 bare coding sequence	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAA GAAGUUCAAGGUCCUGGGAAACACAGACGCACAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG CAGAAGCAACAAGACUGAAGAGAAGAAGAUACACAAGAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGC AACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACACAAUCUACCACCUGAGAAAGAAGCUGGUCGACA GCACAGACAAGGCAGACCUGAGACUUGAUCUACUUGGCCACUGGCACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC CUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAA CGCAAGCGGAGUCGACGUCAAGUCCUGAGCGCAAGAGCAAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGG GAGAAAAGAAGAACGGACUGUUCGGAACCUGATTGCCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUGGCA GAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGGAGACCAGUACGCAGA CCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAGGCACCGC UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCGGAA AAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAUUCUACAA GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCG UUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCACGAUCCCGUACUACGUCGGACCGCUGGCCAGAGGAAACAG CAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCCAC AGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUAC UUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCCGGCAUUCCUGAGCGGAGAACAGAAGAA GGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAGGUCACAGUCAAGCAGCUGAAGGAGGACUACUUCAAGAAGAUCGAAUGCU UCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCCAAGCCUGGGGACAUACCACGACCUGCUGAAGAUCAUCAAGGAC AAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAAAUGAU CGAAGAAAGACUGAAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAA GACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCA AACAGAAACUUCAUGCAGCUGAUCCACGACGAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGA CAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAAC UGGUCAAGGUCAUGGGACACAAGCCGGAAAACAUCGUCGAAUGGCAAGAGAAAACCAGACACAACACAGAAGGGACAGAAG AACAGCAGAGAGAAGAUGAAGAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGAC UGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACAAGAGCGAC AAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCAAA GCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAAGGAUUCAUCAAGA GACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAACACAAGUACGACGAAAACGAC	

	AAGCUGAUCAGAGAAGUCAAGGUCACUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAG AGAAAUCAACACAACUACCCACACCGCCACACGACGCAUACCUGAACGCCAGUCGUCGGAACAGCACACUGAUCAAGAAGUACCCGAAGCUGG AAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCACGAAAGAUGAUCGCCAAAGAGCGAACAGGAAAUCGGAAAGGCAACA GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAACUCAGAAAGAGACCGCU GAUCGAAACAAACGGAGAAACAGGAGAAUCGUCUGGGACACAAGGGAGACUUCGCCAACAGUCAGAAAGGUCCUGAGCAUGCCGC AGGUCAACAUCGUCAAGAAGACAGAGGUCCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUG AUCGCAAGAAAGAAGGACUGGGACCCCGAAGAUUCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAA GGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUCAAUCACAAGAAGCAGCUUCGAAAAGA ACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAGAUGCUGGCAAGCGCCAGGAGAACUGCAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAA CUUCCUGUACCUGGCCAAGCCACUACGAAAAGCUGAAGGGAAGCCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACA AGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAAACAUCAUCCACCUGUUCACACUGACAAACCUGGGAGCC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACACAAAGGCACAAAGGAAGUCCUGGACGCCAACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGGACGGAGGAGGCGAAGCCCGAAGAAGAAGAGA AAGGUC	213
Amino acid sequence of Cas9 (without NLS)	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	214
Cas9 mRNA ORF encoding SEQ ID NO: 13 using minimal uridine	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAG CAAGAAGUUCAAGGUCCUGGGAAACACAGACAGAGACAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAA CAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAGAAGAUACACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAG ACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCG ACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGA	

codons as listed in Table 3, with start and stop codons	GACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGAGCUGAGAAGACUGGAAAACCUGAUCGCACAGCUGC CGGGAGAAAAGAAGAACGGACUGUUUCGGAAACCUGAUCGCUGGGACUGACACCGAACUUCAAGAGCAACACUUCGACCUG GCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCC AGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCAC CGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCG GAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUA CAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUAC CCGUUCCUGAAGGACAACAGAGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAA CAGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCG CACAGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAA UACUUCACAGUCUACAACGAACUGACAAGGUCACAGAAGUCGCUACAUGAAGAGAAGAACGGCGGCAUUCCUGAGCGGAGAACAGAA GAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAU GCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAAAU GAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCGUGAAGAGAAGAUACACAGGAUGGG GAAGACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAAAAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUC GCAAACAGAAACUUCAUGCAGCUGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGG AGACAGCCUGCACGAACACAUCGCCAAACCUGGCGCAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACG AACUGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAAAACCAGACACACAGAAGGGACAG AAGAACAGCAGAGAAAGAAUGAAGAGGAAUCAAGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACA GACUGAGCGACUACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACACAAGAAGC GACAAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGC AAAGCUGAUCACACAGAAGUUCGACAACCUGACAAAGCGCAGAGAGGAGCUGAGCGAACUGGACAAGGCAGGAUUCAUCA AGAGACAGCUGGUCGAAACAAGACACAGAUCACACAAAGCACGUCGCACAGAAUGAACACACAAAGUACGACGAAAAC GACAAGCUGAUCAGAGAAGUCAAGGUCACUCACACUGAAGAGCAAGCUGGUCAGCGAAAGGACUUCCCAGUUCUACAAGGU CAGAGAAAUCAACAACUACCACCACGCACACGACCAUACCUGAACGCAGUCGUCGGAACACAGCACUGAUCAAGAAGUACCCGAAGC UGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAAGAAAGAUGAUCGCCAAAGAGCGAACAGGAAAUCGGAAAGGCA ACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACC GCUGAUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGC CGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAG CUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGC AAAGGUCGAAAAGGGAAAGAGCAAGAAGAGCGUCAAGGAACUGCUGGGAAUCACAUCCAUCAAGCUGCCGAAGUACAGCCUGUUC AGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGC ACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUC

	CUGAGCGCAUACACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGG AGCACCGGCAGCAUUCAAGUACUUCGACACAACAUCGACAGAAAGAGAUACACAAGCACACAAGGAAGUCCUGGACGCAACACUGA UCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGACUAG	215
Cas9 coding sequence encoding SEQ ID NO: 13 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAA GAAGUUCAAGGUCCUGGGAAACACAGACGCACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG CAGAAGCAACAAGACUGAAGAGAAGAAGAUACACAAGAAGAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGC AACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACACAAUCUACCACCUGAGAAAGCUGGUCGACA GCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCCCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC CUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAA CGCAAGCGGAGUCGACGCAAAGGCCAAUCCUGAGCGGCAAGAGCAGAAGACUGGAAAACCUGAUCGCCAGCUGCCGG GAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCCCUGGGACUGACCCCGAACUUCAAGAGCAACUUCGACCUGGCA GAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGA CCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCACCGC UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCGGAA AAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGUAUCGACGGAGGAGCAAGCCAGGAAGAUUCUACAA GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAAGGAACAGGAAGACUUCUACCCG UUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCCAGAGGAAACAG CAGAUUCGCAUGGAUGACAAGAAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCCAC AGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUAC UUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCCGGCAUUCCUGGAGCGGAACAGAAGAA GGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCU UCGACAGCGUCGAAGUCGAAGUCAUCAUGCCUCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGAC AAGGACUUCCUGGACAACGAGGAAAACGAGGACAUUCUGGAAGACAUCGUGCUGACCCUGACACUGUUCGAGGACCGAGAU CGAAGAAAGACUGAAAACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAA GACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCA AACAGAAACUUCAUGCAGCUGAUCCACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGA CAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAAC UGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAAAACCAGACACACAGAAGGGACAGAAG AACAGCAGAGAAGAAAUGAAGAGAAGUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGAC UGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACAAGAAGCGAC AAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCAAA GCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGA GACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUUGCUCAGAUCCUGGACAGCAGAAUGAACACAAAGUACGACGAAAACGAC AAGCUGAUCAGAGAAGUCAAGGUCACUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAG AGAAAUCAACAACUACCACCACGACGCACUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGG	

	AAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCCAAAGAGCGAACAGGAAAUCGGAAAGGCAACA GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCU GAUCGAAACAAACGGAGAAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCCAACAGUCAGAAAGGUCCUGAGCAUGCCGC AGGUCAACAUCGUCAAGAAGACAGAAGGAGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUG AUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAA GGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGA ACCCGAUCGACUUCCUGGAAGCAAAGGAUACAAGGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAGAAAGAGAAUGCUGGCAAGCUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAA CUUCCUGUACCUGGCAAGCCACUACGAAAGGGAAGCCCGGAAGACAACGAAGCAGCUGUUCGUCGAACAGCACA AGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAAGAACAUCAUCCACCUGUUCACACUGACAAACCUGGGAGC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAGAGAUACGACGACAAGGAAGUCCUGGACGCAACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGAC	216
Amino acid sequence of Cas9 nickase (without NLS)	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	217
Cas9 nickase mRNA ORF encoding SEQ ID NO: 16 using minimal uridine codons as	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGACAAACACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAG CAAGAAGUUCAAGGUCCUGGGAAACACAGACACGCACAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAA CAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAUACACACAGAGAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAGACACAAGAAGCACGAAAG ACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCG ACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGA GACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAACCUGAUCGCCACAGCUGC	

listed in Table 3, with start and stop codons	CGGGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUG
	GCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCUCAGCUGGGAGACCAGUACGCC
	AGACCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACAAAGGCAC
	CGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCG
	GAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAUUCUA
	CAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGC
	AGAGAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACGUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUAC
	CCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGGCAAGAGGAAA
	CAGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCG
	CACAGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAA
	UACUUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAA
	GAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAU
	GCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAG
	GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCGUCCUGACACUGACGCUGUUCGAAGACAGAGAAAU
	GAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAUACACAGGAUGGG
	GAAGACUGAGCAGAAAGCUGAUCAAGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUC
	GCAAACAGAAACUUCAUGCAGCUGAUCCACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGG
	AGACAGCCUGCACGAACACAUCGCCAACCUGGCGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACG
	AACUGGUCAAGGUCAUGGGACACAAGCCGGAAACACAUCGUCAUCGAAAUGGCAAGAGAAACCAGACACAACACAGAAGGGACAG
	AAGAACAGCAGAGAAAGAAUGAAGAGAAUCAAGGAAGUGGGAAGCCAGAUCCUGAAGUCCACACCCGGUCGAAAA
	CACACAGCUGCAGAACGAAAAGCUGUACCUGUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACA
	GACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACACGAAGC
	GACAAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGC
	AAAGCUGAUCACACAGAAGAAGUUCGACAACCUGACAAAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCA
	AGAGACAGCUGGUCGAAACAAGACACAAAGCACGUCGCACAGAUCCUGGACAGCAGAAUGAACACAAAGUACGACGAAAAC
	GACAAGCUGAUCAGAGAAGUCAAGGUCACUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGU
	CAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUCGGAACAGCACACUGAUCAAGAAGUACCCGAAGC
	UGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAAGAAAGAUGAUCGCCAAAGAGCGAACAGGAAAUCGGAAAGGCA
	ACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACC
	GCUGAUCGAAACAAACGGAGAAACAGGAAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGC
	CGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACACAGGAGGAUUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAG
	CUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAGAAGUACGGAGGGAUUCGACACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGC
	AAAGGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAUCACAUGGAAAGAAGCAGCUUCGAAA
	AGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUC
	GAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGU
	CAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUUCGUCGAACAGC
	ACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUC
	CUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGG
	AGCACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGGAAGUCCUGGACGCCACAACACUGA

Cas9 nickase coding sequence encoding SEQ ID NO: 16 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	UCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGACUAG	218
	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAA	
	GAAGUUCAAGGUCCUGGGAAACACAGACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG	
	CAGAAGCAACAAGACUGAAGAGAAGAAGAUACACAAGAAAGAAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGC	
	AACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA	
	CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACACAAUCUACCACCUGAGAAAGAAGCUGGUCGACA	
	GCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC	
	CUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAA	
	CGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGAGCAGAAGACUGGAAAACCUGAUCGCCACAGCUGCCGG	
	GAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUGGCA	
	GAAGACGCAAAGCUGCAGCUGAGCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCGAGA	
	CCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGAGUCAACACACAGAAAUCACAAGGCACCGC	
	UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCGGAA	
	AAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAUUCUACAA	
	GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGA	
	GAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCG	
	UUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAG	
	CAGAUUCGCAUGGAUGACAAGAAAAGAGCGAAGAAACAAUCACACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCAC	
	AGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUAC	
	UUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAA	
	GGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCU	
	UCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGAC	
	AAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACCUUGUTCGAAGACAGAGAAAUGAU	
	CGAAGAAAGACUGAAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAA	
	GACUGAGCAGAAAGCUGAUCAACGGAUCAGAGACAAGCAGAGCGGAAAGACAAUCCGAGGAAGAGCGACGGAUUCGCA	
	AACAGAAACUUCAUGCAGCUGAUCCCACGACGCUGACAUCCAGGAAGACUGCAUCCAGAGGAGCAAGCGCAC	
	CAGCCUGCACGAACACAUCGCAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCCUGCAGACAGUCAAGGUCGUCGACGAAC	
	UGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG	
	AACAGCAGAGAAAGAAUGAGAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACAC	
	ACAGCUGCAGAACGAAAAGCUGUACCUGUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGAC	
	UGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACAAGAAGCGAC	
	AAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCAAA	
	GCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGAGAGGAGCUGGAGAAGCUGGUUAAGCAGCGUAACGAGA	
	GACAGCUGGUCGAAACAAGAUCACAAAGCGCAGAUUCUGGAUAAGAUCGAGGAAAUCAUCCACUUCCAGCUGAAGUACAAGGAA	
	AAGCUGAUCAGAGAAGUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAG	
	AGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGG	
	AAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAGAGCGAACAGGAAAUCGGAAAGGCAACA	
	GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCU	
	GAUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAAGGUCCUGAGCAUGCCGC	

	AGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAAGCUG AUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCCAUACACGCUCCUGGUCGUCGCAAA GGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGA ACCCGAUCGACUUCCUGGAAGCAAAGGAAUACAAGGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAAACGGAAGAAAGAGAAAGAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAA CUUCCUGUACCUGGCAAGCCACUACGAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACA AGCACUACCUGGACGAAAUCAUCGAACAGAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAAACAUCAUCCACACCUGUUCACACUGACAAACCUGGGAGC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACACGAAAGAGAUACACACAAGGAAGUCCUGGACGCAACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGAC	219
Amino acid sequence of dCas9 (without NLS)	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	220

dCas9 coding sequence encoding SEQ	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGCAUCACAGACGAAUACAAGGUCCCGAGCAA	221
	GAAGUUCAAGGUCCUGGGAAACACAGACAGCACAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG	
	CAGAAGCAACAAGACUGAAGAACAGCAAGAAGAUACACAAGAAAGAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGC	

ID NO: 19 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	AACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCGACA GCACAGACAAGGCAGACCUGAGACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC CUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAA CGCAAGCGGAGUCGACGCAAGGCAAUCCUGAGCGCAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGG GAGAAAAGAAGAACGGACUGUUCGGAAACCUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUGGCA GAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGA CCUGUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGAGUCAACACACAGAAAUCACAAGGCACCGC UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCGGAA AAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAUUCUACAA GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCG UUCCUGAAGGACAACAGAGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGGCAAGAGGAAACAG CAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCAC AGAGCUUCAUCGAAGAAUGACAAACUUCGACAGAACCUGCCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUAC UUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAGCCCGGCAUUCCUGAGCGGAGAACAGAAGAA GGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGUCAAGAAGGAGAACUACAAGGAAUCGAAUGCU UCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGAC AAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAAAUGAU CGAAGAAAGACUGAAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCGUGAAGAGAGAAGAUACACAGGAUGGGGAA GACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCA AACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGA CAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAAC UGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCGAUCGAAAUGGCAAGAGAAAACCAGACACACAGAAGGGACAGAAG AACAGCAGAGAAAGAAUGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGAC UGAGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACAAGAGCGAC AAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCAAA GCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGAGGAGACUGAGCGAACUGGACAAGGCAAGGAUUCAUCAAGA GACAGCUGGUCGAAACACAAGACACGUCGCACAGAUCCUGGACAGCAGACGAAUGAACACACAAAGUACGACGAAAACGAC AAGCUGAUCAGAGAAGUCAAGGUCACUACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAG AGAAAUCAACAACUACCACGCACACGACGCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGG AAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACA GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCU GAUCGAAACAAACGGAGAAACAGGAGAAACGGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGCCGC AGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUG AUCGUCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAA GGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAAGUCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGA ACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA

	CUGGAAAACGGAAGAGAAGAUGCUGGCAAGCGCAGGAGAUCUGCAGAAGGGAAACGAACUGCCGAGCAAGUACGUCAA CUUCCUGUACCUGGCCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACA AGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAAACAUCAUCACCACCUGUUCACACUGACAAACCUGGGAGC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAGAGAUACACACAAGGAAGUCCUGGACGCAACACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGACGGAGGAGGAAGC	222
Amino acid sequence of Cas9 with two nuclear localization signals as the C-terminal amino acids	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD GSGSPKKKRKVDGSPKKKRKVDSG	223
Cas9 mRNA ORF encoding SEQ ID NO: 22 using minimal uridine codons as listed in Table 3, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACACAGACGAAUACAAGGUCCCGAG CAAGAAGUUCAAGGUCCUGGGCAACACAGACAGACACAUCGAAAGAACCUGAUCGGAGCCACUGCUGUUCGACAGCGGAGAAA CAGCAGAAGCAACAAGAGACUGAAGAGAAGAUACACACGAAGAAGAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAGGACAAGAAG ACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCG ACAGCACAGACAAGGCAGACCUGAGAUCUACCUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGA GACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGAGACUGAGAAGACUGGAAAACCUGAUCGCCAGCUGC CGGGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCCUGCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUG GCAGAAGACGCCAAAGCUGCAGCUGAGCAAGGACACCAUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCGGAGACCAGUACGCC AGACCUGUUCCUGGCCAGCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUCCUGAGAGUCAACACCGAAAUCACCAAAGGCAC CGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCCACUGGUCAGACAGCAGCUGCCG GAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCGGACAUCGACGGAGGAGCAAGCCAGGAAGAGAUUCUA CAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUAC	

CCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCCAAGGAAA CAGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCG CACAGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAA UACUUCACAGUCUACAACGAACUGACAAAGGUCACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAA GAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAGGACUACUUCAAGAAGAUCGAAU GCUUCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAAG GACAAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAAAU GAUCGAAGAAAGACUGAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAUACACAGGAUGGG GAAGACUGAGCAGAAAGCUGGAAUCAAGGAGAUCAGAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUC GCAAACAGAAACUUCAUGCAGCUGAUCCACGACGAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGG AGACAGCCUGCACGAACACAUCGCCAACCUGGCGGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACG AACUGGUCAAGGUCAUGGGACACAAGCCGGAAACAUCGUCAUCGAAAUGGCAAGAGAAACCAGACACACAGAAGGGACAG AAGAACAGCAGAGAAAGAAUGAAGAGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAA CACACAGCUGCAGAACGAAAAGCUGUACCUGUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACA GACUGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAUCGACAACAAGGUCCUGACAAAGAGC GACAAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGC AAAGCUGAUCACACAGAAAGUUCGACAACCUGACAAAGGCAGAGAGGAAGUCGAGCUGAAGUCCAUUGGACACAAGGCAGGAUUCAUCA AGAGACAGCUGGUCGAAACGUACGCACAGAUCACACUGGCACAGCGCGAUGAAUGAACACAAAGUACGACGAAAACAAC GACAAGCUGAUCAGAGAAGUCAAGGUCACUCACACUGAAGAGCAAGUCCUGGGACAAGGAAGAGAUCGGAAAGGCUAUCGC CAGAGAAAUCAACAACUACCACCACGCACACGACGCAUACCUGAACGCAGUCGUCGGAACACGCACUGAUCAAGAAGUACCCGAAGC UGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAAGAAAGAUGAUCGCAAAGAGCGAAAGGUACAAGCA ACAGCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACC GCUGAUCGAAACAAACGGAGAAACAGGAGAAGAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGC CGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAG CUGAUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGC AAAGGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAA AGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUC GAACUGGAAAACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGU CAACUUCCUGUACCUGGCCAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGC ACAAGCACUACCUGGACGAAAUCAUCGAACAGAUUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUC CUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGG AGCACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAGGAAGUCCUGGACGCAACACUGA UCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAAG AGAAAGGUCGACGGAAGCCCGAAGAAGAGAAGGUCGACAGCGGAUAG	224
Cas9 coding sequence encoding SEQ ID NO: 23	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAA GAAGUUCAAGGUCCUGGGAAACACAGACAGCACAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG CAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAUACACAAGAAGAAAGAAGUCUGCUACCUGCAGGAAAUCUUCAGC AACGAAAUGGCAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA

using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCGACA GCACAGACAAGGCAGACCUGAGACCUGAUCUACCUGGCACACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC CUGAACCCGGACACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAA CGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGAGCAAGAGACUGGAAAACCUGAUCGCACAGCUGCCGG GAGAAAAGAAGAACGGACUGUUUCGGAAACCUGAUCGCCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUGGCA GAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGA CCUGUUUCCUGGCAGCAAAGAACCUGAUCCUGCUGAGCGACAUCCUGAGAGUCAACACACAGAAAUCACAAGGCACCGC UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCGGAA AAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAA GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCG UUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAG CAGAUUCGCAUGGAUGACAAGAAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCAC AGAGCUUCAUCGAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUAC UUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAGCCGGCAUUCCUGGAGCACAGCUGCUGAACGCAAA GGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGUCGAAGGAAGACUACUUCAAGAAGAUCGAAUGCU UCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGAC AAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACCUUGUUCGAAGACAGAGAAAUGAU CGAAGAAAGACUGAAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAUACACAGGAUGGGGAA GACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCA AACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGA CAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAAC UGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACACAUCGUCAUCGAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG AACAGCAGAGAAGAAUGAGAGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGAC UGAGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACAAGAAGCGAC AAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCAAA GCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGAGGAGACUGAGCGAACUGGACAAGGCAAGAUUCAUCAAGA GACAGCUGGUCGAAACAAGACAGAUCACAAGCACGUCGCACAGAUCCUGGACUCCCGAUGAUAACAGCUUCUACAACGAA AAGCUGAUCAGAGAAGUCAAGGUCAAGUACGUCACCGAGGGAAUGAGAAAGCCCGCCUUCCUGAGCGGAGAACAAAAGAAGGCCAUC AGAAAUCAACAACUACCACCACGACGACGCACUACCUGAACGCCGUCGUUGGAACAGCCCUGAUCAAGAAGUACCCGAAGCUGG AAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCCAAGAGCGAACAGGAAAUCGGAAAGGCAACA GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCU GAUCGAAACAAACGGAGAAACAGGAGAAUCGUCUGGGACAAGGGAAGACUUCGCCACAGUCAGAAAGGUCCUGAGCAUGCCGC AGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUG AUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAA GGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGA ACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAACGGAAGAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAA

	CUUCCUGUACCUGGCAAGCCACUACGAAAGCUGAAGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACA AGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACACAUCAUCCACCUGUUCACACUGACAACAACCUGGGAGC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGAAGGAAGUCCUGGACGCAACACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAGA AAGGUCGACGGAAGCCCGAAGAAGAAAGAGGUCGACAGCGGA	225
Amino acid sequence of Cas9 nickase with two nuclear localization signals as the C-terminal amino acids	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGSPKKK RKVDGSPKKKRKVDSG	226
Cas9 nickase mRNA ORF encoding SEQ ID NO: 25 using minimal uridine codons as listed in Table 3, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGACACAAACAGCCGUCGGAUGGGCCAGUCAUCACACAGACGAAUACAAGGUCCCCAG CAAGAAGUUCAAGGUCCUGGGAAACACAGACCGCCACAGCGCAUCAAGAAGAACCUGAUCGGAGCCCUGCUGUUCGACAGCGGAGAAA CAGCAGAAGCCAACAAGACUGAAGAGAACACAGCAAGAAGAAGAUACACAAGAAGAAGAACACAGAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCCAAAGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAG ACACCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCG ACAGCACAGACAAGGCAGACCUGAGACUGAUCUACCUGGCACUGGCCCACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAGGGA GACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCCAGACUGAGAAGCAGAAGAGCUGGAAAACCUGAUCGCCAGCUGCC CGGGAGAAAAGAAGAACGGACUGUUUCGGAAACCUGAUCGCCCUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUG GCAGAAGACGCCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGCGACCAGUACGCC AGACCUGUUCCUGGCAGCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUCCUGAGAGUCAACACAGAAAUCACCAAAGGCAC CGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCCAUCGUCAGACAGCAGCUGCCG GAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCGGGAUUACAUCGACGGAGGAGCCAAGCCAGGAAGAUUCUA CAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGC	

Cas9 nickase coding sequence encoding SEQ	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAA	227
	GAAGUUCAAGGUCCUGGGAAACACAGACAGGACACAUCAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG	
	CAGAAGCAACAAGACUGAAGAGAACAGCAAGAAGAUACACAAGAAGAAAGAUACAGAAUCUGCUACCUGCAGGAAAUCUUCAGC	
	AACGAAAUGGCAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA	

ID NO: 25 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCGACA GCACAGACAAGGCAGACCUGAGACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC CUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAA CGCAAGCGGAGUCGACGCAAGGCAAUCCUGAGCGCAAGACUGAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGG GAGAAAAGAAGAACGGACUGUUUCGGAAACCUGAUCGCCACUGAGCCUGGACUGACACCGAACUUCAAGAGCAACUUCGACCUGGCA GAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGA CCUGUUUCCUGGCAGCAAAGAACCUGAUCCUGCUGAGCGACAUCCUGAGAGUCAACACACAGAAAUCACAAGGCACCGC UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCGGAA AAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAUUCUACAA GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCG UUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAG CAGAUUCGCAUGGAUGACAAGAAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCAC AGAGCUUCAUCGAAGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUAC UUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAAGCCGGCAUUCCUGAGCGGAGAACAGAAGAA GGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGUCGAAGGAAGACUACUUCAAGAAGAUCGAAUGCU UCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCCAAGCCUGGGAACAUACCACGACCUGCUGAAGAUCAUCAAGGAC AAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACCUUGUUCGAAGACAGAGAAAUGAU CGAAGAAAGACUGAAAGACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAUACACAGGAUGGGGAA GACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCA AACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGA CAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAAC UGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACACAUCGUCAUCGAAAUGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG AACAGCAGAGAAGAAUGAGAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUCGACAUCAACAGAC UGAGCGACUACGUCGCCCGCAGAUCGAAGGACAGCAUCGACGACAUCGACGCAAGAAGCGAC AAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCAAA GCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAGGCAGAGAGAGGAGGACUGAGCGACAAGGCAAGGUUCAUCAAGA GACAGCUGGUCGAAACAAGACAGAUCACAAGCACGUGGCACAGAUCCUGGACUCCCGACUCGACAGCAAGAUGAUCCGCCU AAGCUGAUCAGAGAAGUCAAGGUCAAGUACCUGGAGGAGAAGAACCAGUACGACAUCGACGUCAAGGAGAUGAACCGCCUGAGC AGAAAUCAACAACUACCACGACGCCUACCUGAACGCCGUCGUUGGAACAGCCCUGAUCAAGAAGUACCCGAAGCUGG AAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCCAAGAGCGAACAGGAAAUCGGAAAGGCAACA GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCU GAUCGAAACAAACGGAGAAACAGGAGAAUCGUCUGGGACAAGGGAAGACUUCGCCACAGUCAGAAAGGUCCUGAGCAUGCCGC AGGUCAACAUCGUCAAGAAGACAGGAGGUCCAGACAGGAGGGAUUCAGCATCCUGCCGAAGAGAAACAGCGACAAGCUG AUCGUCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCCUACAGCGUCCUGGUCGUCGCCAAA GGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGA ACCCGAUCGACUUCCUGGAAGCAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAACGGAGAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAA

	CUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACA AGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAACAACCUGGGAGC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACACAAGCACACAAAGGAAGUCCUGGACGCAACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGAC GGAAGCGGAAGCCCGAAGAAGAGAAGGUCGACGGAAGCCCGAAGAAGAGAGAAAGGUCGACAGCGGA	228
Amino acid sequence of dCas9 with two nuclear localization signals as the C-terminal amino acids	MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGSPKKK RKVDGSPKKKRKVDSG	229
dCas9 mRNA ORF encoding SEQ ID NO: 28 using minimal uridine codons as listed in Table 3, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGACAAACACAGCGUCGGAUGGGCACGUCAUCACACAGACGAAUACAAGGUCCCGAG CAAGAAGUUCAAGGUCCUGGGAAACACAGACACGCAUCAAGAAGAACCUGAUCGGAGCCACUGCUGUUCGACAGCGGAGAAA CAGCAGAAGCAACAAGAGUGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAG ACACCCGAUCUUCGGAAACAUCGUCGACGAAGUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCG ACAGCACAGACAAGGCAGACCUGAGAUCUACCUGGCACACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGA GACCUGAACCCGGACAACAGCGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGAGACUGAGAAGCAGAAGAUGGAAAACCUGAUCGCCACAGCUGC CGGGAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCCUGAGCCUGGGAGUCACACCGAACUUCAAGAGCAACUUCGACCUG GCAGAAGACGCCAAAGCUGCAGCUGAGCAAGGACACCAUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCGGAGACCAGUACGCC AGACCUGUUCCUGGCCAGCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUCCUGAGAGUCAACACCGAAAUCACCAAAGGCAC CGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCCACUGGUCAGACAGCAGCUGCCG GAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCGGACGAGGAGCAAGCCAGGAAGAUUCUA CAAGUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGAGAAAGC AGAGAACACUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUAC	

dCas9 coding sequence encoding SEQ ID NO: 28 using	230
GGAAGCGGAAGCCCGAAGAAGAAGAGGAAGGGUCGACGGAAGAGCCCGAAGAAGAAGAGGUCGACAGCGGAUAG	
UCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGAC	
AGCACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACACAGAAAGAGAUACACAAGCACAAGGAAGUCCUGGACGCAACACUGA	
AGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUC	
GAACUGGAAAACGGAAGAAAGAGAAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGU	
CAACUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGAAGACAACGAAGCAGCUGUUCGUCGAACAGC	
ACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUC	
CUGAGCGCCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGG	
AGCACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAGGAAGUCCUGGACGCAACACUGA	
UCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGAC	
GGAAGCGGAAGCCCGAAGAAGAAGAGGAAGGGUCGACGGAAGAGCCCGAAGAAGAAGAGGUCGACAGCGGAUAG	
GACAAGAAGUACAGCAUCGGACUGGCAAUCGGACACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAA	230
GAAGUUCAAGGUCCUGGGAAACACAGACACAGCAUCAAGAAGAAGAACCUGAUCGGAGCACUGCUGUUCGACAGCGGAGAAACAG	
CAGAAGCAACAAGACUGAAGAACAGCAAGAAGAUACACAAGAAGAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGC	
AACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACA	
CCCGAUCUUCGGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCACCUGAGAAAGAAGCUGGUCGACA	

minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GCACAGACAAGGCAGACCUGAGACCUGAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAAGGAGAC CUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAA CGCAAGCGGAGUCGACGCAAGGCAAUCCUGAGCGCAAGACUGAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGG GAGAAAAGAAGAACGGACUGUUCGGAAACCUGAUCGCCUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUCGACCUGGCA GAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGA CCUGUUUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGAGUCAACACACAGAAAUCACAAGGCACCGC UGAGCGCAAGCAUGAUCAAGAGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCUGCUGCCGGAA AAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGAUACGCAGGUAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAA GUUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGCUGCGAAAGCAGA GAACAUUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCG UUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAG CAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCAC AGAGCUUCAUCGAAAGAAUGACAAACUUCGACAAGAACGGAAAAGGUCCUGCCGAAGCACAGCCUGCUGUACGAAUAC UUCACAGUCUACAACGAACUGACAAAGGUCAAGUACGUCACAGAAGGAAUGAGAGUCGAAGCGGCAUUCCUGAGCGGAGAACAGAAGAA GGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCU UCGACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCCAAGCCUGGGACAUACCACGACCUGCUGAAGAUCAUCAAGGAC AAGGACUUCCUGGACAACGAAGAAAACGAAGACAUCCUGGAAGACAUCGUCCUGACACUGACCUUGUUCGAAGACAGAGAAAUGAU CGAAGAAAGACUGAAGACACAUACGCCACACCUGUUCGACGACAAGGUCAUGAAGCAGCGUGAAGAGAAGAAGAAUACACAGGAUGGGGAA GACUGAGCAGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCCUGAAGAGCGACGGAUUCGCA AACAGAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGACAGGGAGA CAGCCUGCACGAACACAUCGCAAACCUGGCAGAAGCCCGGCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAAC UGGUCAAGGUCAUGGGAAGACACAAGCCGGAAAACACAUCGUCAUCGAAAUGGCAAGAGAAAACCAGACACACAGAAGGGACAGAAG AACAGCAGAGAAAGAAUGAAGAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCGGUCGAAAACAC ACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGAC UGAGCGACUACGUCGACCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACAAGAAGCGAC AAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGGUCGUCAAGAAGAUGAAGAACUACUGGAGACAGCUGCUGAACGCAAA GCUGAUCACACAGAAGUUCGACAACCUGACAAGGCAGAGAGAGGAGACUGAGCGACAAGGCAAGGAUUCAUCAAGA GACAGCUGGUCGAAACACAAGACACGUCGCACAGAUCCUGGACAGCAGAAUGAACACAAGUACGACGAAAACGAC AAGCUGAUCAGAGAAGUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUCCAGUUCUACAAGGUCAG AGAAAUCAACAACUACCACCACGACGCACUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGG AAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACA GCAAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGAGAAAUCAGAAAGAGACCGCU GAUCGAAACAAACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUGCCGC AGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUG AUCGCAAGAAAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACACAGUCGCCUACAGCGUCCUGGUCGUCGCAAA GGUCGAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUCGAAAAGA ACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAA CUGGAAACGGAAGAGAAGAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCACUGCCGAGCAAGUACGUCAA CUUCCUGUACCUGGCAAGCCACUACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUCGAACAGCACA

	AGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUCCACCUGUUCACACUGACAAACCUGGGAGC ACCGGCAGCAUUCAAGUACUUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGACGCAACACUGAUCC ACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGACCUGAGCCAGCUGGGAGGAGAC GGAAGCGGAAGCCCGAAGAAGAGAAGGUCGACGGAAGCCCGAAGAAGAGAAAGGUCGACAGCGGA	
T7 promoter	TAATACGACTCACTATA	231
Human beta-globin 5' UTR	ACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACC	232
Human beta-globin 3' UTR	GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCC TTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGC	233
Human alpha-globin 5' UTR	CATAAACCCTGGCGCGCTCGCGGCCCGGCACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACC	234
Human alpha-globin 3' UTR	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCT TTGAATAAAGTCTGAGTGGGCGGC	235
Xenopus laevis beta-globin 5' UTR	AAGCTCAGAATAAACGCTCAACTTTGGCC	236
Xenopus laevis beta-globin 3' UTR	ACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAATACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGC CATTCGTATCTGCTCCCTAATAAAAAGAAAGTTTCTTCACATTCT	237
Bovine Growth Hormone 5' UTR	CAGGGTCCCTGTGGACAGCTCACCAGCT	238
Bovine Growth Hormone 3' UTR	TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATG AGGAAATTGCATCGCA	239
Mus musculus hemoglobin alpha, adult chain 1 (Hba-a1),	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAG TAGGAAG	240

3'UTR		
HSD17B4 5' UTR	TCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTC	241
G282 guide RNA targeting TTR	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmAmGmCAAGUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	242
Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGA AGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTC AAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGC AACAAGACTGAAGAGAACAGCAAGAAGATACACAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAA TGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATC TTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACC CGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGAAGCAGAAGAACTGGAAAACCTGATCGCCCAGCTGCCGGGAGAAAA GAAGAACGGACTGTTCGGAAACCTGATTGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGAC CAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTC CTGGCAGCAAAGAACCTGAGCGACGCAATCCTGAGAGTCAACACAGAAATCACAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACA AGGAAATCTTCTTCGACCAGAGCAAGAATGGATACGCGGGATACATTGACGGAGGAGCCAAGCCAGGAAGATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATT CGACAACGGAAGCATCCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGA AGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTC GCATGGATGACAAGAAAGAGCGAAGAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTT CATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAG TCTACAACGAACTGACAAAGGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATC GTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGATCGAATGCTTCGACAG CGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACT TCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAG CAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAA ACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTG CACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAA GGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAGAAGATACACAGGATGGGGAAGACAGAAGAACAGCA GAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTG CAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGA CTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAAGCTGATC	243

	244
Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 4, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCATGGACAAGAAGTACA GCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTC CTGGGAACACAGACAGACACATCAAGAAGAAGATCAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAA ACTGAAGAGAACACAGCAAGAAGATACACAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAA AGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAGCGCCACCCGATCTTCGGA AACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACTAAGGC AGACCTGAGACTGATCTACCTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACA ACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTTCAACAGTACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTC GACGCAAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGAAAAACCTGATCGCCACAGCTGCCGGGAGAAAAGAAGAAA CGGACTGTTCGGAAACCTGATCGCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAGC TGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCA GCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCAT GATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAA TCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCG ATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAA CGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACA ACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGGGAACAGCAGATTCGCATGG ATGACAAGAAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGA

AAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACA ACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGAC CTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGA AATCAGCGGAGTCGAAGACAGATTCAACGCCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG ACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTG AAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAGCAGAAA GCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCA TGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAA CACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCAT GGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACACAACACAGAAGGACAGAAGAACAGCAGAGAAA GAATGAAGAGAATCGAAGAAGGAATCAAGGAACTCCTGAAGGAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAAC GAAAAGCTGTACCTGTACTACCTGCAGAACGGCGAAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACTACGA CGTCGACCACATCGTCCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACACGAAGCGACAAGAACAGGGAA AGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAG AGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGA AACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAG AAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAAC TACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGT CTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAGCACTACTTCT TCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAAC GGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGT CAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGA AGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGA AAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAGAAGCAGCTTCGAAAAGAACCCGATCGACTT CCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAA GAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTG GCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGA CGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACA AGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTC AAGTACTTCGACACACAACAATCGACAGAAAGAGATACACAAGAAGGAAGTCCTGGACGCAACACACTGATCCACCAGAGCATCAC AGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGACGGAGGAGGAAGCCCGAAGAAGAGAAGGTCTAGCTAG CCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTT TCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAA AAATGGAAAGAACCTCGAG	245-249
Not Used	

		250
Cas9 ORF with splice junctions removed; 12.75% U content	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTC AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACcggCTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA GACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCAGCTGC CGGGAGAAAAGAAATGGACTGTTCGGAAACCTGATTGCCCTGTCGCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCC AGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGAGAGTCAACACAGAAATCACAAAGGCAC CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCTTCCTGAGCAGCTGCCG GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCGGAGGAGCAAGCCAGGAAGAATTCTA CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTAC CCGTTCCTGAAGGACAACAGAGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAA CAGCAGATTCGCATGGATGACAAGAAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG CACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAA TACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAA GAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAAT GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAG GACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAAT GATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGG GAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGTCCGGAAAGACAATCCTGGATTTCCTGAAGTCGGACGGATTC GCAAACAGAAACTTCATGCAGCTGATCCACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGG AGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACG AACTGGTCAAGGTCATGGGACACAAGCCGGAAACATCGTCATCGAAATGGCAAGAGAAACCAGACACAGAAGGGACAG AAGAACAGCAGAAAGAATGAAGAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAA CACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAaAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACA GACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGC GACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGC AAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCA AGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAAC GACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGT CAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGC TGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACC	250

		GCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGC CGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAG CTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGC AAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTC GAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAAGCAGCTGTTCGTCGAACAGC ACAAGCACTACCTGGACGAAATCATCGAGCAAATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTC CTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGG AGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAGGAAGTCCTGGACGCCACAACTGA TCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGAGGAAGCCCGAAGAAGAAG AGAAAGGTCTAG	251
Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 50, Kozak sequence, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGA AGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTC AAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCGAAGC AACAAGACTGAAGAGAACAGCAAGAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAA TGGCAAAGGTCGACGACAGCTTCTTCCACcggCTGGAAGAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATC TTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACC CGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGAAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGGGAGAAAA GAAGAACGGACTGTTCGGAAACCTGATTGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACG CAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTC CTGGCAGCAAAGAACCTGAGCGACGCAATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCCAAGATGATC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAGTACA AGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGAGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATT CGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGA AGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACACAGCAGATTC GCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTT CATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAG TCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATC GTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAG CGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACT TCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAG CAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCCAACAGAA ACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTG	

	CACGAACACATCGCCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAA	252
	GGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCA	
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	GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATC	
	ACACAGAGAAAGTTCGACAACCTGACAAGGCAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGT	
	GGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGA	
	TCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATC	
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	TCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT	
	AATAAAAAATGGAAAGAACCTCGAG	
Cas9 ORF with minimal uridine codons frequently used in humans in general; 12.75% U content	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG	252
	CAAGAAGTTCAAGGTGCTGGGCAACACCGACAGACACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA	
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	GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGC	
	CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCC	
	CCCTGAGCGCCAGCATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGAGACAGCAGCTGCCC	

GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACAGAGAGGACCTGCTGAGAAAGC AGAGAACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGAGAAGACAGGAGGACTTCTAC CCCTTCCTGAAGGACAACAGAGAGAAGATCGAGAAGATCCTGACCTTCAGAATCCCCTACTACGTGGGCCCCCTGGCCAGAGGCAA CAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG CCCAGAGCTTCATCGAGAGAATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAA GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACAGAAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT GCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACAGATTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACAGAGAGAT GATCGAGGAGAGACTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGAGAAGATACACCGGCTGGGG GCAGACTGAGCAGAAAGCTGATCAACGGCATCAGAGACAAGCAGTCCGGCAAGACCATCCTGGATTTCCTGAAGTCCGACGGCTTC GCCAACAGAAACTTCATGCAGCTGATCCACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGG CGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG AGCTGGTGAAGGTGATGGGCAGACACAAGCCCGAGAACATCGTGATCGAGATGGCCAGAGAGAACCAGACCACACCCAGAAGGGCCAG AAGAACAGCAGAGAGAGAATGAAGAGAATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAA CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCAGAGACATGTACGTGGACCAGGAGCTGGACATCAACA GACTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCAGAAGC GACAAGAACAGAGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGC CAAGCTGATCACCCAGAGAAAGTTCGACAACCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCA AGAGACAGCTGGTGGAAGACCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCAGAATGAACACCAAGTACGACGAGAAC GACAAGCTGATCAGAGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGT GAGAGATCAACAACTACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC TGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGAGAAAGATGATCGCCAAGAGCGAGATCGGCAAGGCC ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCAGAAAGAGACC CCTGATCGAGACCAACGGCGAGATCGTGTGGGACAAGGGCAGAGACTTCGCCACCGTGAGAAAGGTGCTGAGCATGC CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGAGAAACAGCGACAAG CTGATCGCCAGAAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGAGAAGCAGCTTCGAGA AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC GAGCTGGAGAACGGCAGAAAGAGAATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGT GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC ACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGAGAGTGATCCTGGCCGACGCCAACCTGGACAAGGTG CTGAGCGCCTACAACAAGCACAGAGACAAGCCCATCAGAGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACAGAAAGAGATACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGA TCCACCAGAGCATCACCGGCCTGTACGAGACCAGAATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCAGCCCCAAGAAGAAG AGAAAGGTGTGA	Cas9	253
GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGA		

transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 52, Kozak sequence, and 3' UTR of ALB	AGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAGTTC AAGGTGCTGGGCAACACCGACAGACACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCGCCGAGGC CACCAGACTGAAGAGAACCGCCAGAAGAATACACCAGAAGAACAGAATCTGCTACCTGCAGGAGATCTTCAGCAACGAGA TGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGAGACACCCCATC TTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAGCTGGTGGACAGCACCGA CAAGGCCGACCTGAGACTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCAGAGGCCACTTCCTGATCGAGGGCGACCTGAACC CCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGC GGCGTGGACGCCAAGGCCATCCTGAGCGCCAGACTGAGCAAGAGCAGAAGACTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAA GAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACG CCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTC CTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGC CAGCATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGAGACAGCAGCTGCCCGAGAAGTACA AGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAAGTTCATC AAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACAGAGAGGACCTGCTGAGAAAGCAGAGAACCTT CGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGAGAAGACAGGAGGACTTCTACCCCTTCCTGA AGGACAACAGAGAGAAGATCGAGAAGATCCTGACCTTCAGAATCCCCTACTACGTGGGCCCCCTGGCCAGAGGCAACAGCAGATTC GCCTGGATGACCAGAAAGAGCGAGAGGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTT CATCGAGAGAATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCG TGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATC GTGGACCTGCTGTTCAAGACCAACAGAAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAG CGTGGAGATCAGCGGCGTGGAGGACAGATTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACT TCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACAGAGAGATGATCGAGGAG AGACTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGAGAAGATACACCGGCTGGGGCAGACTGAG CAGAAAGCTGATCAACGGCATCAGAGACAAGCAGAGCGGCAAGACCATCCTGGATTCCTGAAGAGCGACGGCTTCGCCAACAGAA ACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGACAGCCTG CACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAA GGTGATGGGCAGACACAAGCCCGAGAACATCGTGATCGAGATGGCCAGAGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCA GAGAGAGAATGAAGAGAATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTG CAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCAGAGACATGTACGTGGACCAGGAGCTGGACATCAACAGACTGAGCGA CTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACA GAGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAGCTGATC ACCCAGAAGAAGTTCGACAACCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGAGACAGCT GGTGGAGACCAGACAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCAGAATGAACACCACGAGTACGACGAGAACGACAAGCTGA TCAGAGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTGAGAGAGATC AACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGA GTTCGTGTACGGCGACTACAAGGTGTACGACGTGAGAAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGT ACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCAGAAAGAGACCCCTGATCGAG ACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCAGAGACTTCGCCACCGTGAGAAAGGTGCTGAGCATGCCCCAGGTGAA CATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGAGAAACAGCGACAAGCTGATCGCCA

	GAAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAG AAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGAGGAAGCAGCTTCGAGAAGAACCCCAT CGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAGCTGGAGA ACGGCAGAAAGAGAATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAACTTCCTG TACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTA CCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGAGAGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCT ACAACAAGCACAGAGACAAGCCCATCAGAGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCC GCCTTCAAGTACTTCGACACCACCATCGACAGAAAGAGATACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAG CATCACCGGCCTGTACGAGACCAGAATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGCAGCCCCAAGAAGAAGAGAAAGGTGT GACTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTT TCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	254
Cas9 ORF with minimal uridine codons infrequently used in humans in general; 12.75% U content	ATGGACAAAAAATACAGCATAGGGCTAGACATAGGGACGAACAGCGTAGGGTGGGCGGTAATAACGGACGAATACAAAGTACCGAG CAAAAAATTCAAAGTACTAGGGAACACGGACCGACACAGCATAAAAAAAAACCTAATAGGGGCGCTACTATTCGACAGCGGGGAAA CGGCGGAAGCGACGCGACTAAAACGAACGGCGCGACGACGATACACGCGACGACGAAAAAACCGAATATGCTACCTACAAGAAATATTC AGCAACGAAATGGCGAAAGTAGACGACAGCTTCTTCCACCGACTAGAAGAAAGCTTCCTAGTAGAAGAAGACAAAAAACACGAACG ACACCCGATATTCGGGAACATAGTAGACGAAGTAGCGTACCACGAAAAATACCCGACGATATACCACCTACGAAAAAAACTAGTAG ACAGCACGGACAAAGCGGACCTACGACTAATATACCTAGCGCTAGCGCACATGATAAAATTCCGAGGGCACTTCCTAATAGAGGG GACCTAAACCCGGACAACAGCGACGTAGACAAACTATTCATACAACGTACAAACCAACTATTCGAAGAAAACCCGAT AAACGCGAGCGGGGTAGACGCGAAAGCGATACTAAGCAAAAGCCGACGACTAGAAAACCTAATAGCGCAACTAC CGGGGGAAAAAAAAAAAACGGGCTATTCGGGAACCTAATAGCGCTAAGCCTAGGGCTAACGCCGAACTTCAAAGCAACTTCGACCTA GCGGAAGACGCGAACTACAACTAAGCAAAGACACGTACGACGACCTAGACAACCTACTAGCGCAAATAGGGGACCAATACGC GGACCTATTCCTAGCGGCGAAAAACCTAAGCGACGCGATACTACTAAGCGACATACTACGAGTAAACACGGAAATAACGAAAGCGC CGCTAAGCGCGAGCATGATAAAACGATACGACGAACACCACCAAGACCTAACGCTACTAAAAGCGCTAGTACGACACAACACTACCG GAAAATACAAGAAATATTCTTCGACCAAAGCAAAAACGGGTACATAGACGGGGGGGCGAGCCAAGAAATTCTA CAAATTCATAAAACCGATACTAGAAAAAATGGACGGGAAGAACTACTAGTAAACTAACCGAGAAGACCTACTACGAAAAC AACGAACGTTCGACACGGGAGCATACCGCACCAAATACACCTAGGGGAACTACACGACGCGATACTACGACACGAGAAGACTTCTAC CCGTTCCTAAAAGACAACCGAGAAAAAATAGAAAAATACTAACGTTCCGAATACCGTACTACGTAGGGCCGCTAGCGCGAGGGAA CAGCCGATTCGCGTGGATGACGCGAAAAAGCGAAGAAACGATAACGCCGTGGAACTTCGAAGAAGTAGTAGACAAAGGGGCGAGCG CGCAAAGCTTCATAGAACGAATGACGAACTTCGACAAAAACCTACCGAACGAAAAAGTACTACCGAAACACAGCCTACTATACGAA TACTTCACGGTATACAACGAACTAACGAAAGTAAAATACGTAACGGAAGGGATGCGAAAACCGGCGCGTTCCTAAGCGGGGAACAAAA AAAAGCGATAGTAGACCTACTATTCAAACGAACCGAAAAGTAACGGTAAAACAACTAAAAGAAGACTACTTCAAAAAATAGAAT GCTTCGACAGCGTAGAAATAAGCGGGGTAGAAGACCGATTCAACGCGAGCCTAGGGACGTACCACGACCTACTAAAAATAAATAAA GACAAAGACTTCCTAGACAACGAAGAAACGAAGACATACTAGAAGACATAGTACTAACGCTAACGCTATTCGAAGACCGAGAAAT GATAGAAGAACGACTAAAAACGTACGCGCACCTATTCGACGACAAAGTAATGAAACACACTAAAACGACGACGATACACGGGGGTGGG GGCGACTAAGCCGAAAACTAATAAACGGGATACGAGACAAACAAAGCGGGAAAACGATACTAGACTTCCTAAAAAGCGACGACGGGTTC GCGAACCGAAACTTCATGCAACTAATACACGACGACAGCCTAACGTTCAAAGAAGACATACAAAAAGCGCAAGTAAGCGGGCAAGG GGACAGCCTACACGAACACATAGCGAACCTAGCGGGGAGCCCGGCGATAAAAAAAAGGGATACTACAAACGGTAAAAGTAGTAGACG

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Cas9 transcript with 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 54, Kozak sequence, and 3′ UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAAA AATACAGCATAGGCTAGACATAGGCGACGAACAGCGTAGGTGGCGGTAATACGGACGATACAAAGTACCCGAGCAAAAATTC AAAGTACTAGGGAACACGGACCGACACAGCATAAAAAAAAACCTAATAGGGGCGCTACTATTCGACAGCGGGGAAACGGCGGAAGC GACGCGACTAAAACGAACGGCGCGACGATACACGCGACGAAAAAACCGAATATGCTACCTACAAGAAATATTCAGCAACGAAA TGGCGAAAGTAGACGACACAGCTTCTTCCACCGACTAGAAGAAGCTTCCTAGTAGAAGACAAAAAACACGAACGACACCCGATA TTCGGGAACATAGTAGACGAAGTAGCGTACCACACGAAAAAATACCCGACGATATACCACCTACGAAAAAAACTAGTAGACAGCACGGA CAAAGCGGACCTACGACTAATATATACCTAGCGCTAGCGCACATGATAAATTCCGAGGGCACTTCCTAATAGAAGGGGACCTAAACC CGGACAACAGCGACGTAGACAAACTATTCATACACTAGTACAAACGTACAACCAACTATTCGAAGAAAACCCGATAAACGCGAGC GGGGTAGACGCGAAAGCGATACTAAGCGCGCGACTAAGCAAAGCCGACGACTAGAAAACCTAATAGCGCAACTACCGGGGGAAAA AAAAAACGGGCTATTCGGGAACCTAATAGCGCTAAGCCTAGGGCTAACGCCGAACTTCAAAGCAACTTCGACCTAGCGGAAGACG CGAAACTACAACTAAGCAAAGACACGTACGACGACCTAGACAACCTACTAGCGCAAATAGGGGACCAATACGCGGACCTATTC CTAGCGGCGAAAAACCTAAGCGACGCGATACTACTAAGCGACATACTACGAGTAAACACGGAAATAACGAAAGCGCCGCTAAGCGC GAGCATGATAAAACGATACGACGAACACCACCAAGACCTAACGCTACTAAAAGCGCTAGTACGACACAACTACCGGAAAAATACA AAGAAATATTCTTCGACCAAAGCAAAAACGGGTACGCGGGGTACATAGACGGGGGGGCGAGCCAAGAAGAATTCTACAAATTCATA AAACCGATACTAGAAAAAATGGACGGGACGGAAGAACTACTAGTAAAACTAAACCGAGAAGACCTACTACGAAAACAACGAACGTT

CGACAACGGGAGCATACCGCACCAATACACCTAGGGAACTACACGCGATACTACGACGACAAGAAGACTTCTACCCGTTCCTAA AAGACAACCGAGAAAAAATAGAAAAAATACTAACGTTCCGAATACCGTACTACGTAGGGCCGCTAGCGCGAGGGAACAGCCGATTC GCGTGGATGACGCGAAAAAGCGAAGAAACGATAACGCCGTGGAACTTCGAAGAAGTAGTAGTACAAAGGGGCGAGCGCGCAAAGCTT CATAGAACGAATGACGAACTTCGACAAAAACCTACCGAACGAAAAAGTACTACCGAAACACAGCCTACTATACGAATACTTCACGG TATACAACGAACTAACGAAAGTAAAATACGTAACGGAAGGGATGCGAAAACCGGCGTTCCTAAGCGGGAACAAAAAAAAGCGATA GTAGACCTACTATTCAAAACGAACCGAAAGTAACGGTAAAACAACTAAAGAAGACTACTTCAAAAAAATAGAATGCTTCGACAG CGTAGAAATAAGCGGGGTAGAAGACCGATTCAACGCGAGCCTAGGGACGTACCACGACCTACTAAAAATAATAAAAGACAAAGACT TCCTAGACAACGAAGAAAACGAAGACATACTAGTACTAACGCTAACGCTATTCGAAGACCGAGAAATGATAGAAGAA CGACTAAAAACGTACGCGCACCTATTCGACGACAAGTAATGAAACAACTAAAAACGACGACGGGGTGGGGGCGACTAAG CCGAAACTAATAAACGGGATACGAGACAAACAAGCGGGAAAACGATACTAGACTTCCTAAAAGCGACGGGTTCGCGAACCGAA ACTTCATGCAACTAATACACGACGACAGCCTAACGTTCAAGAAGACATACAAAAAGCGCAAGTAAGCGGGCAAGGGGACAGCCTA CACGAACACATAGCGAACCTAGCGGGAGCCCGGCGATAAAAAAAGGGATACTACAAACGGTAAAAGTAGTAGACGAACTAGTAAA AGTAATGGGGCGACACAAACCGGAAAACATAGTAATAGAAATGGCGCGAGAAAACCAAACGACGCAAAAAGGGCAAAAAAACAGCC GAGAACGAATGAAACGAATAGAAGAAGGGATAAAAGAACTAGGGAGCCAAATACTAAAAGAACACCCGGTAGAAAACACGCAACTA CAAAACGAAAAACTATACCTATACTACCTACAAAACGGGCGAGACATGTACGTAGCAAGAACTAGACATAAACCGACTAAGCGA CTACGACGTAGACCACACATAGTACCGCAAAGCTTCCTAAAAGACGACATAGCAACAAGTACTAACGCGAAGCGACACAAAAACC GAGGGAAAGCGACAACGTACCGAGCGAAGAAGTAGTAAAAAAAATGAAAAACTACTGGCGACACAACTACTAAACGCGAAACTAATA ACGCAACGAAAATTCGACAACCTAACGAAAGCGGAACGAGGGGGCTAAGCGAACTAGACAAAGCGGGTTCATAAAACGACACAACT AGTAGAAACGCGACAAATAACGAAACACGTAGCGCAAATACTAGACAGCCGAATGAACACGAAATACGACGAAAACGACAAACTAA TACGAGAAGTAAAAGTAATAACGCTAAAAAGCAAACTAGTAAGCGACTTCCGAAAAGACTTCCAATTCTACAAAGTACGAGAAATA AACAACTACCACACCACGCGCACGACGCGTACCTAAACGCGGTAGTAGGGACGGCGCTAATAAAAAAATACCCGAAACTAGAAAGCGA ATTCGTATACGGGGACTACAAGTATACGACGTACGAAAAATGATAGCGAAAAGCGAACAAGAAATAGGGAAAGCGACGGCGAAAT ACTTCTTCTACAGCAACATATGAACTTCTTCAAACGGAAATAACGCTAGCGAACGGGGAAATACGAAACGACCGCTAATAGAA ACGAACGGGGAAACGGGGGAAATAGTATGGGACAAGGGCGAGACTTCGCGACGGTACGAAAAGTACTAAGCATGCCGCAAGTAAA CATAGTAAAAAAAAACGGAAGTACAAACGGGGGGGTTCAGCAAAGAAAGCATACTACCGAAACGAAACAGCGACAAACTAATAGCGC GAAAAAAAGACTGGGACCCGAAAAAATACGGGGGGTTCGACAGCCCGACGGTAGCGTACAGCGTACTAGTAGTAGCGAAAGTAGAA AAAGGGAAAAGCAAAAAACTAAAAAGCGTAAAAAGAACTACTAGGGATAACGATAATGGAACGAAGCAGCTTCGAAAAAAACCCGAT AGACTTCCTAGAAGCGAAAGGGTACAAAGAAGTAAAAAAAAGACCTAATAATAAAACTACCGAAATACAGCCTATTCGAACTAGAAA ACGGGCGAAAACGAATGCTAGCGAGCGCGGGGGAACTACAAAAGGGAACGAACTAGCGCTACCGAGCAAATACGTAAACTTCCTA TACCTAGCGAGCCACTACGAAAAACTAAAGGGAGCCCGGAAGACAACGAACAAAACACAACTATTCGTAGAACAACACAAACACTA CCTAGACGAAATAATAGAACAAATAAGCGAATTCAGCAAACGAGTAATACTAGCGGACGCGAACCTAGACAAAGTACTAAGCGCGT ACAACAAACACCGAGACAAACCGATACGAGAACAAGCGGAAAACATAATACACCTATTCACGCTAACGAACCTAGGGGCGCCGGCG GCGTTCAAATACTTCGACACGACGATAGACCGAAAACGATACACGAGCACGAAGAAGTACTAGACGCGACGCTAATACACCAAAG CATAACGGGGCTATACGAAACGCGAATAGACCTAAGCCAACTAGGGGGGGGACGGGGGGGAGCCCGAAAAAAAACGAAAAGTAT GACTAGCCATCACATTTAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAATGAAGATCAATAGCTTATTCATCTCTTTT TCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	Cas9	256
AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGA		

transcript with AGG as first three nucleotides for use with CleanCap™, 5′ UTR of HSD, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3′ UTR of AIB	AGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAGTTC AAGGTCCTGGGAAACACAGACAGCACATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGC AACAAGACTGAAGAGAACAGCAAGAAGATACACAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAA TGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATC TTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACC CGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGGGAGAAAA GAAGAACGGACTGTTCGGAAACCTGATCGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACG CAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTC CTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACA AGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATT CGACAACGGAAGCATCCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGATCTTCTACCCGTTCCTGA AGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCGCATTC GCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCCACAGAGCTT CATCGAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAG TCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAAGCAATC GTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAG CGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACT TCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAGAAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAG CAGAAAGCTGATCAACGGAATACGAGAAGCAAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAA ACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTG CACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAA GGTCATGGGAAGACACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGGGCAGGAAGAACAGCA GAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTG CAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGA CTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATC ACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCT GGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGA TCAGAGAAGTCAAGGTCACCACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATC AACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGA ATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGT ACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAA ACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAA CATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAA

	GAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCCAAAGGTCGAA AAGGGAAAGAGCAAGAAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGAT CGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAA ACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTG TACCTGGCAAGCCACTACGAAAGCTGAAAGGGAAGCCCGGAAGACAACGAAGACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTA CCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCAT ACAACAAGCACAGAGACAAGCCGATCAGAGAACACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCCGGCA GCATTCAAGTACTTCGACACAACAATCGACCGCAAGCGTACCGTGATCTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAAGTCCTGGACAAGATCGCCGAAGAAGAGAAAGGTCT AGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAATGAAGATCAATTAGCTTATTCATCTCTTTT TCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	257
Cas9 transcript with 5' UTR from CMV, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3' UTR of ALB	GGGCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACG GTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACTCACCGTCCTTGACACGGCCACCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACACAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACA CAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGA ACAGCAAGAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAGGTCGACGA CAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCG ACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGA CTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGT CGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGG CAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCCGGGGAGAAAAAGAATGGCCTGTTC GGAAACCTGATCGCCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCAGCTGAG CAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGCGACCAGTACGCCGACCTGTTCCTGGCAGCAAAGAACC TGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACGAAATCACCAAGGCACCCGCTGAGCGCAAGCATGATCAGGA TACGACGAACACCACCACGACCTGACCCTGCTGAAGGCTTCAGACAGCAGCTGCCCGGAAAAGTACAAGGAAATCTTCTTCGA CCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAA AGATGGACGGAACAGAAGAAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATC CCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAA GATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAA AGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACA AACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGAC AAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCA AGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGA GTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGA AACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACG CACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAAC GGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGAT	

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Cas9 transcript with 5' UTR from HBB, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3' UTR of HBB	GGGacatttgcttctgacacaactgtgttcactagcaacctcaaacagacaccggatctgccaccATGGACAAGAAGTACAGCATC GGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGG AAACACAGACACGATCACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCACAAGACTGA AGAGAACAGCAAGAAGAGATACACACAAGAAGAAAGAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTC GACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACAT CGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAACTGGTCGACAGCACAGACAAGGCAGACC AAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGGAAAACCTGATCGCCACAGCTGCCGGGAGAAAGAACCCGATCAACTTCCTG TGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCAG CTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAA

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	gcatctggattctgcctaataaaaaacatttatttcattgcctcgag	259
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	AAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGA CAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAG GATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGA GGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAA GGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAG TCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGACGTAAGAGACGAATGCTGGCAAGCGGCAGGA GAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGG AAGCCCGGAAGACAACGAACAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAAT TCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAA CAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAG AAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACC TGAGCCAGCTGGGAGGAGACGGAGGAAGCCCGAAGAAGAAAGGTCTAGctagcaccagcctcaagaacacccgaatgg agtcctaagctacataataccaacttacacttacaaaaatgttgtcccccaaaatgtagccattcgtatctgctccctaaataaaaa gaaagttcttcacattctctcgag	260
Cas9 transcript with AGG as first three nucleotides for use with CleanCap™, 5′ UTR from XBG, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3′ UTR of XBG	AGGaagctcagaataaacgctcaactttggccggatctgccacCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAAC AGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACGACACAGCAT CAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAAGAAGACTGAAGAGAACGCAAGAAGAGAT ACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGA CTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCA CGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCAC TGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATC CAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAG ACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCCGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCAC TGAGCCTGGGACTGACACCCAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACACATACGAC GACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTGGCAGCCAAGAACCTGTCAGACGCCATCCT GCTGAGCGACATCCTGAGAGTCAACACAGAAATCACCAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACC AGGACCTGACACTGCTGAAGGCCAGCTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGA TACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGA AGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACC TGGGAGAACTGCACGCCATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTG ACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAGAGCGAAGAAACAAT CACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACC TGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTC ACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGT CACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCA ACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTG GAAGACATCGTCCTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGA CAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGC	

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Cas9 transcript with AGG as first three nucleotides for use with CleanCap™, 5′ UTR from HSD, ORF corresponding to SEQ ID	AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGA AGTACAGCATCGGACTGGACATCGGAACACCAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTC AAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGC AACAAGACTGAAGAGAACAGCAAGAAGAGATACACAAGAAGAAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAA TGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATC TTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACC CGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCAGCTGCCCGGGAGAAAA GAAGAACGGACTGTTCGGAAACCTGATTGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGAT CAAAGCTGCAGCTGAGCAAGGACACATACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCGACCTGTTC

NO: 4, Kozak sequence, and 3' UTR of ALB	CTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACA AGGAAATCTTCTTCGACCAGAGCAAGAACGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATT CGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGA AGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTC GCATGGATGACAAGAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTT CATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAG TCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATC GTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAG CGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACT TCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAG CAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAA ACTTCATGCAGCTGATCCACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTG CACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAA GGTCATGGGAAGACACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAAAACCAGACAACACAGAAGGACAGAAGAACAGCA GAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAAATCGGAAAGGCAACAGCAAAGT CAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGA CTACGACGTCGACCACATCGTCCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACA GAGGAAAGAGCGACAACGTCCCCGAAGCGAAGAAGATCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCCAAAGCTGATC ACACAGAAGAAAGTTCGACAACCTGACCAAGGCAGAGAGAAGGCTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGCT GGTCGAACAAGACACGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACACAAGTACGACGAAAACGACAAGCTGA TCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATC AACAACTACCACACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGA ATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGT ACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAA ACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAA CATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAA GAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCTACAAGGTCGAA AAGGGAAAGACAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGAT CGACTTCCTGGAAGCAAAGGGATACAAGGAAGGACCTGATCATCAAGCTGCCGAAGTACAGTCTGTTCGAACTGGAAA ACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTG TACCTGGCAAGCCACTACGAAAAGCTGAAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTA CCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCAT ACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCA GCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAG CATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAGAAGAGAAAGGTCT AGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTT

	TCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT AATAAAAAATGGAAAGAACCTCGAG	
30/30/39 poly-A sequence	AAAAAA

	GGAAAAGCTGAGAACACTGCTGGCAGAAAAGCTGTCGTCGAGACCGGAAGCAGTCCACGAATACGTCACACCGCTGTTCGTCTCGA GAGCACCGAACAGAAAGATGTCGGGACAGGGACACATGGAAACAGTCAAGTCGGCAAAGAGACTGGACGAAGGAGTCTCGGTCCTG AGAGTCCCGCTGACACAGCTGAAGCTGAAGGACCTGGAAAAGATGGTCAACAGAGAAAGAGAACCGAAGCTGTACGAAGCACTGAA GGCAAGACTGGAAGCACACAAGGACGACCCGGCAAAGGCATTCGCAGAACCGTTCTACAAGTACGACAAGGCAGGAAACAGAACAC AGCAGGTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGACAGGAGTCTGGGTCAGAAACCACAACGGAATCGCAGACAACGCAACA ATGGTCAGAGTAGACGTCTTCGAAAAGGGAGACAAGTACTACCTGGTCCCGATCTACTCGTGGCAGGTCGCAAAGGGAATCCTGCC GGACAGAGCAGTCGTCCAGGGAAAGGACGAAGAAGATCGGCAGCTGATCGACGACTCGTTCAACTTCAAGTTCTCGCTGCACCCGA ACGACCTGGTCGAAGTCATCACAAAGAAGGCAAGAATGTTCGGATATACTTCGCATCGTGCCACAGAGGAAACAGGAAACATCAACATC AGAATCCACGACCTGGACCACACAAGATCGGAAAGAACGGAATCCTGGAAGGAATCGGAGTCAAGACAGCACTGTCGTTCCAGAAGTA CCAGATCGACGAACTGGGAAAGGAAATCAGACCGTGCAGACTGAAGAAGAGACCGCCGGTCAGATCCGGAAAGAGAACAGCAGACG GATCGGAATTCGAATCGCCGAAGAAGAGAAAGGTCGAATGA	266
ORF encoding Neisseria meningitidis Cas9 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGGAATCGCATCGGTCGGATGGGCAATGGTCGAAATCGA CGAAGAAGAAAACCCGATCAGACTGATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTCCCGAAGACAGGAGACTCGCTGG CAATGGCAAGAAGACTGGTCAAGATCGGTCAGAAGACTGACAAGAAGAGAGCACACACAGACTGCTGAGAACAAGAAGACTGCTGAAG AGAGAAGGAGTCCTGCAGGCAGCAAACTTCGACGAAAACGGACTGATCAAGTCGCTGCCCGAACACACACCGTGGCAGCTGAGAGCAGC AGCACTGGACACAGAAAGCTGACACCGCTGGAATGGTCGGCAGTCCTGCTGCACCTGATCAAGCACAGAGGATACCTGTCGCAGAGAA AGAACGAAGGAGAAACAGCAGACAAGGAACTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAGACAGGAGAC TTCAGAACACCGGCAGAACTGGCACTGAACAAGTTCGAAAAGGAATCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACAC ATTCTCGAGAAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAAGGAATTCGGAAACCCGCACGTCTCGGGAGGAC TGAAGGAAGGAATCGAAACACTGCTGATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTGGGACACTGCACA TTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATACAGCAGAAAGATTCATCTGGCTGACAAAGCTGAACACAACCTGAGAAT CCTGGAACAGGGATCGGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATGGACGAACCGTACAGAAAGTCGAAGCTGACAT ACGCACAGGCAAGAAAGCTGCTGGGACTGGAAGACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGACAACGCAGAAGCATCG ACACTGATGGAAATGAAGGCATACCACGCAATCTCGAGAGCACTGGAAAGGAAGGACTGAAGTCGCCGCTGAACCT GTCGCCGGAACTGCAGGACGAAATCGGAACAGCATTCTCGCTGTTCAAGACAGACGAAGACATCACAGGAAGACTGAAGGACAGAA TCCAGCCGGAAATCCTGGAAGCACTGCTGTTCGACAATCTCGCTAGTTCGTCCAGATCTCGCTGAGGCACTGAGAAGATC GTCCCGCTGATGGAACAGGGAAAGAGATACGACGAAGCATGCGCAGAAATCTACGGAGACCACTACGGAAGAAGAACACAGAAGA AAAGATCTACCTGCCGCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTCGTCCTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCA ACGGAGTCGTCAGAAGATACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGAAAGTCGTTCAAGGACAGAAAG GAAATCGAAAAGAGACAGGAAGAAAACAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGTCGG AGAACCGAAGTCGAAGGACATCCTGAAGCTGTACGAACAGCAGCACGGAAAGTGCCTGTACTCGGGAAAGGAAATCAACC TGGGAAGACTGAACGAAAAGGGATACGTCGAAATCGACCACGCACTGCCGTTCTCGAGAACATGGGACGACTCGTTCAACAACAAG GTCCTGGTCCTGGGATCGGAAAACCAGAACACAAGGGAAACCAGACACCGTACGAATACTTCAACGGAAAGGACAACTCGAGAGAATG GCAGGAATTCAAGGCAAGAGTCGAAACATCGAGATTCCCGAGATCGAAGAAGCAGAGAATCCTGCTGCTGGCAGAAGTTCGACGAAGACG GATTCAAGGAAAGAACCTGAACGACACAAGATACGTCAACAGATTCCTGTGCCAGTTCGTCGCAGACAGAATGAGACTGACAGGA AAGGGAAAGAGAGTCTTCGCATCGAACGGACAGATCACAACCTGCTGAGAGGATTCTGGGGACTGAGAAAGGTCAGAGCAGA AACGACAGACACCACGCACTGGACGCAGTCGTCGCATGCTCGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGAT ACAAGGAAATGAACGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTCCTGCACCAGAAGACACACTTCCCGCAGCCG TGGGAATTCTTCGCACAGGAAGTCATGATCAGAGTCTTCGGAAAGCCGGACGGAAAGCCGGAATTCGAAGAAGCAGACACACTGGA	

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Transcript comprising SEQ ID NO: 65 (encoding Neisseria meningitidis Cas9)	GGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTGATCCCGCCACCATGGCGCAGCATTCAAGCCGAACTCGATCAACTACATCC TGGGACTGGACATCGGAATCGGCATCGGTCGGATGGGCAATGGTCGACAATCGACGAAGAAGAAACCCGATCAGACTGATCGACCTG GGAGTCAGAGTCTTCGAAAGCAGGAGACAGGAGACTCGCTGGCAATGGCAAGAAGACTGGCAAGATCGGTCAGAAG ACTGACAAGAAGAAGAGCACACAGACTGCTGAGAACAAGAGACTGCTGAAGAGAGAAGGAGTCCTGCAGGCAGCAAACTTCGACG AAAACGGACTGATCAAGTCGCTGCCGAACACACCGTGGCAGCTGAGAGCAGCACTGGACAGAAAGCTGACACCGCTGGAATGG TCGGCAGTCCTGCTGCACCTGATCAAGCACAGAGGATACCTGTCGCCGAGAAGGAGAACAGCAGACAAGGAACTGGG AGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAGACAGGAGACTTCAGAACACCGGCAGAACTGGCACTGAACAAGT TCGAAAAGGAATCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACACATTCTCGAGAAAGGACCTGCAGGCAGAAGCTGCTGGGACTGGAAGA CTGCTGTTCGAAAGCAGAAGGAATTCGGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAACACTGCTGATGACACA GAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTGGGACACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACA CATACACAGCAGAAAGATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTGGAACAGGGATCGGAAAGACCGCTGACAGAC ACAGAAAGAGCAACACACTGATGGACGAACCGTACAGAAAGTCGAAGCTGACATACGCACAGGCAAGAAAGCTGCTGGGACTGGAAGA CACAGCATTCTTCAAGGGACTGAGATACGGAAAGGACAACGCAGAAGCATCGACACTGATGGAAATGAAGGCATACCACGCAATCT CGAGAGCACTGGAAAGGAAGGACTGAAGGACAAGAAGTCGCCGCTGAACCTGTCGCCGGAAGACTGCAGGACGAAATCGGAACAGCA TTCTCGCTGTTCAAGACAGCGAAGGACATCACAGGAAGACTGAAGGCAATCCAGCCCGGAAATCCTGGAAGCACTGCTGTAGCA CATCTCGTTCGACAAGTTCGTCCAGATCTCGCTGAAGGCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATACGACG AAGCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAACACAGAAGAAAAGATCTACCTGCCGCCGATCCCGGCAGACGAA ATCAGAAACCCGGTCGTCCTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGAAGATACGGATCGCCGGCAAG AATCCACATCGAAACAGCAAGAGAAGTCGGAAAGTCGTTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAACAGAAAGG ACAGAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGTCGGAGAAGACCGAAGTCGAAGGACATCCTGAAGCTGAGA CTGTACGAACAGCAGCACGGAAAGTGCCTGTACTCGGGAAAGGAAATCAACCTGGGAAGACTGAACGAAAAGGGATACGTCGAAAT CGACCACGCACTGCCGTTCTCGAGAACATGGGACGACTCGTTCAACAACAAGGTCCTGGTCCTGGGATCGGAAAACCAGAACAAGG GAAACCAGACACCGTACGAATACTTCAACGGAAAGGACAACTCGAGAGAATGGCAGGAATTCAAGGCAAGAGTCGAAACATCGAGA TTCCCGAGATCGAAGAAGCAGAGAATCCTGCTGACGAAGTTCGACGAAGACGGATTCAAGGAAAGAAACCTGAACGACACAAGATA CGTCAACAGATTCCTGTGCCAGTTCGTCGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAGAAGAGTCTTCGCATCGAACGGAC AGATCACAAACCTGCTGAGAGGATTCTGGGGACTGAGAAAGGTCAGAGCAGAAAACGACACAGACACCACGCACTGGACGCAGTCGTC GTCGCATGCTCGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGATACAAGGAAATGAACGCATTCGACGGAAAGAACAAT CGACAAGGAAACAGGAGAAGTCCTGCACCAGAAGACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAAGTCATGATCAGAG

	TCTTCGGAAAGCCGGACGGAAAGCCGGAATTCGAAGAAGCAGACACACTGGAAAAGCTGAGAACACTGCTGGCAGAAAAGCTGTCG TCGAGACCGGAAGCAGTCCACACGAATACGTCACACACCGCTGTTCGTCTCGAGAGCACCGAACAGAAAGATGTCGGGACAGGGACACAT GGAAACAGTCAAGTCGGCAAAGAGACTGGACGAAGGAGTCTCGGTCCTGAGAGTCCCGCTGACACAGCTGAAGGCTGAAGGACCTGG AAAAGATGGTCAAACAGAGAAAGAGAAACCGAAGCTGTACGAAGCACTGGAAGGCAAGACTGGAAGCACACACAAGGACGACCCGGCAAAG GCATTCGCAGAAACCGTTCTACAAGTACGACAAGGCAGGAAACAGAACACAGCAGGTCAAGAGTCGAGTCAGAACAGGTCCAGAA GACAGGAGTCTGGGTCAGAAACCACAACGGAATCGCAGACACAACGCCAACAATGGTCAGAGTAGACGTCTTCGAAAAGGGAGACAAGT ACTACCTGGTCCCGATCTACTCGTGGCAGGTCGCAAAGGGAATCCTGCCGGACAGAGCAGTCGTCCAGGGAAAGGACGAAGAAGAC TGGCAGCTGATCGACGACTCGTTCAACTTCAAGTTCTCGCTGCACACCCGAACGACCTGGTCGAAGTCATCACAAAGAAGGCAAGAAT GTTCGGATACTTCGCATCGTGCCACAGAGGAACACATCAACATCAGAATCCACGACCTGGACCACACAAGATCGGAAAGAATGCG GAATCCTGGAAGGAATCGGAGTCAAGACAGCACTGTCGTTCCAGAAGTACCAGATCGACGAACTGGGAAAGGAAATCAGACCGTGC AGACTGAAGAAGAGACCGCCGGTCAGATCCGGAAAGAACAGCAGACGGATCGGAATTCGAATCGCCGAAGAAGAGAAAGGT CGAATGATAGCTAGCTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTT GTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATG CTGGGGATGCGGTGGGCTCTATGG	268
Amino acid sequence of Neisseria meningitidis Cas9	MAAFKPNSINYILGLDIGIASVGWAMVEIDEENPIRLIDLGVRVFERAEVPKTGDSLAMARRLARSVRRLTRRRAHRLLRTRRLL KREGVLQAANFDENGLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVAGNAHALQTG DFRTPAELALNKFEKESGHIRNQRSDYSHTFSRKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHC TFEPAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEA STLMEMKAYHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLKDRIQPEILEALLKHISFDKFVQISLKALRR IVPLMEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPARIHIETAREVGKSFKDR KEIEKRQEENRKDREKAAAKFREYFPNFVGEPKSKDILKLRLYEQQHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSFNN KVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQRILLQKFDEDGFKERNLNDTRYVNRFLCQFVADRMRLT GKGKKRVFASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGEVLHQKTHFPQ PWEFFAQEVMIRVFGKPDGKPEFEEADTLEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSAKRLDEGVSVL RVPLTQLKLKDLEKMVNRERERPKLYEALKARLEAHKDDPAKAFAEPFYKYDKAGNRTQQVKAVRVEQVQKTGVWVRNHNGIADNAT MVRVDVFEKGDKYYLVPIYSWQVAKGILPDRAVVQGKDEEDWQLIDDSFNFKFSLHPNDLVEVITKKARMFGYFASCHRGTGNINI RIHDLDHKIGKNGILEGIGVKTALSFQKYQIDELGKEIRPCRLKKRPPVRSGKRTADGSEFESPKKKRKVE	269
	Not Used	
G502 guide RNA	mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	270
G509 guide RNA	mA*mA*mA*GUUCUAGAUGCCGUCCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	271
G534 guide RNA	mA*mC*mG*CAAAUAUCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA mAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmCmU*mU*mU*mU	272
DNA coding sequence of	TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGG AGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACT	273

eGFP	GAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGC GCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGT TGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCTAATACGACTCACTATAGGGTCCCGCAGT CGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTTCGTTATTCGGATCCATGGTGAGCAAGGGCGAGGAGCTGTTCA CCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCC ACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTA CGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGG AGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATC GAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATAT CATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACC ACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAA GACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAA GTAATAGGAATTATGCAGTCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAATGAAGATCAATA GCTTATTCATCTCTTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTT TTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAATCTAGACTTAAGCTTGATGAGCTCTAGCTT GGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGT GTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCG TGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTC GCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACG CAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGC CCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCC TGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGC TTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAG CCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGG TAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAG TATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGT AGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTC TGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATC TCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGG CCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGC GCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAAT AGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCA ACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGT TGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACT GGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGC GCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGAT CCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGA

	AGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCAT TTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCC GAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCG	274
	Not Used	274
CMV-1 5′ UTR	CAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCAT	275
CMV-2 5′ UTR	AGAAGACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACGG	276
CMV-3 5′ UTR	TGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACTCACCG	277
SV40 NLS	PKKKRKV	278
Exemplary NLS 1	LAAKRSRTT	279
Exemplary NLS 2	QAAKRSRTT	280
Exemplary NLS 3	PAPAKRERTT	281
Exemplary NLS 4	QAAKRPRTT	282
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	CCGGCACCGGCAAAGAGAGAAAGAACAACA	295
Exemplary coding sequence for NLS4	CAGGCAGCAAAGAGACCGAGAACAACA	296
Exemplary coding sequence for NLS5	AGAGCAGCAAAGAGACCGAGAACAACA	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	GCAGCAGCAAAGAGAAAGTACTTCGGCAGCA	301
Exemplary coding sequence for NLS10	AGAGCAGCAAAGAGAAAGGCATTCGGCAGCA	302
Exemplary coding sequence for NLS11	AGAGCAGCAAAGAGAAAGTACTTCGGCAGTC	303
Exemplary coding sequence for alternate SV40 NLS	CCGAAGAAGAAGAGAAGAGTC	304
exemplary Kozak sequence	gccgccRcccAUGG	305

	Not Used	306
Cas9 ORF using long half life codons of Table 4, with start and stop codons	ATGGACAAGAAGTACTCTATCGGTTTGGACATCGGTACCAACTCTGTCGGTTGGGCCGTCATCACCGACGAATACAAGGTCCCATC	307
	TAAGAAGTTCAAGGTCTTGGGTAACACCGACAGACACTCTATCAAGAAGAACTTGATCGGTGCCTTGTTGTTCGACTCTGGTGAAA	
	CCGCCGAAGCCACCAGATTGAAGAGAACCGCCAGAAGAAGATACACCAGAAGAAAGAGAACAGAATCTGCTACTTGCAAGAAATCTTC	
	TCTAACGAAATGGCCAAGGTCGACGACTCTTTCTTCCACAGATTGGAAGAATCTTTCTTGGTCGAAGAAGACAAGAAGCACGAAAG	
	ACACCCAATCTTCGGTAACATCGTCGACGAAGTCGCCTACCACGAAAAGTACCCAACCATCTACCACTTGAGAAAGAAGTTGGTCG	
	ACTCTACCGACAAGGCCGACTTGAGATTGATCTACTTGGCCTTGGCCCACATGATCAAGTTCAGAGGTCACTTCTTGATCGAAGGT	
	GACTTGAACCCAGACAACTCTGACGTCGACAAGTTGTTCATCCAATTGGTCCAAACCTACAACCAATTGTTCGAAGAAAACCCAAT	
	CAACGCCTCTGGTGTCGACGCCAAGGCCATCTTGTCTGCCAGATTGTCTAAGAGCAGAAGATTGGAAAACTTGATCGCCCAATTGC	
	CAGGTGAAAAGAAGAACGGTTTGTTCGGTAACTTGATCGCCTTGTCTTTGGGTTTGACCCCAAACTTCAAGTCTAACTTCGACTTG	
	GCCGAAGACGCCAAGTTGCAATTGTCTAAGGACACCTACGACGACGACTTGGACAACTTGTTGGCCCAAATCGGTGACCAATACGC	
	CGACTTGTTCTTGGCCGCCAAGAACTTGTCTGACGCCATCTTGTTGTCTGACATCTTGAGAGTCAACACCGAAATCACCAAGGCCC	
	CATTGTCTGCCTCTATGATCAAGAGATACGACGAACACCACCAAGACTTGACCTTGTTGAAGGCCTTGGTCAGACAACAATTGCCA	
	GAAAAGTACAAGGAAATCTTCTTCGACCAATCTAAGAACGGTTACGCCGGTTACATCGACGGTGGTGCCTCTCAAGAAGAATTCTA	
	CAAGTTCATCAAGCCAATCTTGGAAAAGATGGACGGTACCGAAGAATTGTTGGTCAAGTTGAACAGAGAAGACTTGTTGAGAAAGC	
	AAAGAACCTTCGACAACGGTTCTATCCCACACCAAATCCACTTGGGTGAATTGCACGCCATCTTGAGAAGACAAGAAGACTTCTAC	
	CCATTCTTGAAGGACAACAGAGAAAAGATCTTGACCTTCAGAATCCCATACTACGTCGGTCCATTGGCCAGAGGTAA	
	CAGCAGATTCGCCTGGATGACCAGAAAGTCTGAAGAAACCATCACCCCATGGAACTTCGAAGAAGTCGTCGACAAGGGTGCCTCTG	
	CCCAATCTTTCATCGAAAGAATGACCAACTTCGACAAGAACTTGCCAAACGAAAAGGTCTTGCCAAAGCACTCTTTGTTGTACGAA	
	TACTTCACCGTCTACAACGAATTGACCAAGGTCAAGTACGTCACCGAAGGTATGAGAAAGCCAGCCTTCTTGTCTGGTGAACAAAA	
	GAAGGCCATCGTCGACTTGTTGTTCAAGACCAACAGAAAGGTCACCGTCAAGCAATTGAAGGAAGACTACTTCAAGAAGATCGAAT	
	GCTTCGACTCTGTCGAAATCTCTGGTGTCGAAGACAGATTCAACGCCTCTTTGGGTACCTACCACGACTTGTTGAAGATCATCAAG	
	GACAAGGACTTCTTGGACAACGAAGAAAACGAAGACATCTTGGAAGACATCGTCTTGACCTTGACCTTGTTCGAAGACAGAGAAAT	
	GATCGAAGAAAGATTGAAGACCTACGCCCACTTGTTCGACGACAAGGTCATGAAGCAATTGAAGAGAAGAAGATACACCGGTTGGG	
	GTAGATTGAGCAGAAAGTTGATCAACGGTATCAGAGACAAGCAATCTGGTAAGACCATCTTGGACTTCTTGAAGTCTGACGGTTTC	
	GCCAACAGAAACTTCATGCAATTGATCCACGACGACTCTTTGACCTTCAAGGAAGACATCCAAAAGGCCCAAGTCTCTGGTCAAGG	
	TGACTCTTTGCACGAACACATCGCCAACTTGGCCGGTTCTCCAGCCATCAAGAAGGGTATCTTGCAAACCGTCAAGGTCGTCGACG	
	AATTGGTCAAGGTCATGGGTAGACACAAGCCAGAAAACATCGTCATCGAAATGGCCAGAGAAACCAACCACCCAAAGGGTCAA	
	AAGAACAGCAGAGAAGAAATGAAGAGGAATCGAAGAAGGTATCAAGGAATTGGGTTCTCAAATCTTGAAGGAACACCCAGTCGAAAA	
	CACCCAATTGCAAAACGAAAAGTTGTACTTGTACTACTTGCAAAACGGTAGAGACATGTACGTCGACCAAGAATTGGACATCAACA	
	GATTGTCTGACTACGACGTCGACCACATCGTCCCACAATCTTTCTTGAAGGACGACTCTATCGACAACAAGGTCTTGACCAGATCT	
	GACAAGAACAGAGGTAAGTCTGACAACGTCCCATCTGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAATTGTTGAACGC	
	CAAGTTGATCACCCAAAGAAAGTTCGACAACTTGACCAAGGCCGAAAGAGGTGGTTTGTCTGAATTGGACAAGGCCGGTTTCATCA	
	AGAGACAATTGGTCGAAACCAGACAAATCACCAAGCACGTCGCCCAAATCTTGGACAGCAGAATGAACACCAAGTACGACGAAAAC	

	GACAAGTTGATCAGAGAAGTCAAGGTCATCACCTTGAAGTCTAAGTTGGTCTCTGACTTCAGAAAGGACTTCCAATTCTACAAGGT CAGAGAAATCAACAACTACCACCACGCCCACGACGCCTACTTGAACGCCGTCGTCGGTACCGCCTTGATCAAGAAGTACCCAAAGT TGGAATCTGAATTCGTCTACGGTGACTACAAGGTCTACGACGTCAGAAAGATGATCGCCAAGTCTGAACAAGAAATCGGTAAGGCC ACCGCCAAGTACTTCTTCTACTCTAACATCATGAACTTCTTCAAGACCGAAATCACCTTGGCCAACGGTGAAATCAGAAAGAGACC ATTGATCGAAACCAACGGTGAAATCGTCTGGGACAAGGGTAGAGACTTCGCCACCGTCAGAAAGGTCTTGTCTATGC CACAAGTCAACATCGTCAAGAAGACCGAAGTCCAAACCGGTGGTTTCTCTAAGGAATCTATCTTGCCAAAGAGAAACTCTGACAAG TTGATCGCCAGAAGAAGGACTGGGACCCAAAGAAGTACGGTGGTTTCGACTCTCCAACCGTCGCCTACTCTGTCTTGGTCGTCGC CAAGGTCGAAAAGGGTAAGTCTGTAAGAAGTTGTTGGTGGTATCACCATCATGGAAAGATCTTCTTTCGAAA AGAACCCAATCGACTTCTTGGAAGCCAAGGGTTACAAGGAAGTCAAGAAGGACTTGATCATCAAGTTGCCAAAGTACTCTTTGTTC GAATTGGAAAACGGTAGAAAGAGAATGTTGGCCTCTGCCGGTGAATTGCAAAAGGGTAACGAATTGGCCTTGCCATCTAAGTACGT CAACTTCTTGTACTTGGCCTCTCACTACGAAAAGTTGAAGGGTTCTCCAGAAGACAACGAACAAAAGCAAATTGTTCGTCGAACAAC ACAAGCACTACTTGGACGAAATCATCGAACAAATCTCTGAATTCTCTAAGAGAGTCATCTTGGCCGACGCCAACTTGGACAAGGTC TTGTCTGCCTACAACAAGCACAGAGACAAGCCGAAAACATCATCCACTTGTTCACCTTGACCAACTTGGG TGCCCCAGCCGCCTTCAAGTACTTCGACACCACCATCGACAGAAAGAGATACACCTCTACCAAGGAAGTCTTGGACGCCACCTTGA TCCACCAATCTATCACCGGTTTGTACGAAACCAGAATCGACTTGTCTCAATTGGGTGGTGGTTCTCCAAAGAAGAAG AGAAAGGTCTAA	308

	309
Cas9 ORF using low G codons of Table 4, with start and stop codons	ATGGACAAAAAAATACTCCATCGGCCTCGACATCGGCACCAACTCCGTCGGCTGGGCCGTCATCACCGACGAATACAAGTCCCCTC CAAAAAATTCAAAGTCCTCGGCAACACCGACACACTCCATCAAAAAAACCTCATCGGCGCCCTCTTCGACTCCGGCGAAA CCGCCGAAGCCACCAGACTCAAAGAACGCCGCAGAAGAAGATACACCAGAAGAAAAAACAGAATCTGCTACCTCCAAGAAATCTTC TCCAACGAAATGGCCAAAGTCGACGACTCCTTCTTCCACAGACTCGAAGAATCCTTCCTCGTCGAAGAAGACAAAAAACACGAAAG ACACCCCATCTTCGGCAACATCGTCGACGAAGTCGCCTACCACGAAAAATACCCCACCATCTACCACCTCAGAAAAAACTCGTCG ACTCCACCGACAAAGCCGACCTCAGACTCATCTACCTCGCCCTCGCCCACATGATCAAATTCAGAGGCCACTTCCTCATCGAAGGC GACCTCAACCCCGACAACTCCGACGTCGACAAACTCTTCATCCAACTCGTCCAAACCTACAACCAACTCTTCGAAGAAAACCCCAT CAACGCCTCCGGCGTCGACGCCAAAGCCATCCTCTCCGCCAGACTCTCCAAATCCAGAAGACTCGAAAACCTCATCGCCCAACTCC CCGGCGAAAAAAAAAACGGCCTCTTCGGCAACCTCATCGCCCTCTCCCTCGGCCTCACCCCCAACTTCAAATCCAACTTCGACCTC GCCGAAGACGCCAAACTCCAACTCTCCAAAGACACCTACGACGACGACCTCGACAACCTCCTCGCCCAAATCGGCGACCAATACGC CGACCTCTTCCTCGCCGCCAAAAACCTCTCCGACGCCATCCTCCTCTCCGACATCCTCAGAGTCAACACCGAAATCACCAAAGCCC CCCTCTCCGCCTCCATGATCAAAGATACGACGAACACCACCAAGACCTCACCCTCCTCAAAGCCCTCGTCAGACAACAACTCCCC GAAAAATACAAAGAAATCTTCTTCGACCAATCCAAAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAAGAAGAATTCTA

CAAATTCATCAAACCCATCCTCGAAAAAATGGACGGCACCGAAGAACTCCTCGTCAAACTCAACAGAGAAGACCTCCTCAGAAAAC
AAAGAACCTTCGACAACGGCTCCATCCCCCACCAATCCACCTCGGCGAACTCCACGCCATCCTCAGAAGACAAGAAGACTTCTAC
CCCTTCCTCAAAGACAACAGAGAAAAAATCGAAAAAATCCTCACCTTCAGAATCCCCTACTACGTCGGCCCCCTCGCCAGAGGCAA
CTCCAGATTCGCCTGGATGACCAGAAATCCGAAGAAACCATCACCCCCTGGAACTTCGAAGAAGTCGTCGACAAAGGCGCCTCCG
CCCAATCCTTCATCGAAAGAATGACCAACTTCGACAAAAACCTCCCCAACGAAAAAGTCCTCCCCAAACACTCCCTCCTCTACGAA
TACTTCACCGTCTACAACGAACTCACCAAAGTCAATACGTCACCGAAGGCATGAGAAAACCCGCCTTCCTCGACTTCCTCAAATCCGACGGCTTC
AAAAGCCATCGTCGACCTCCTCTTCAAAACCAACAGAAAAGTCACCGTCAAACAACTCAAAGAAGACTACTTCAAAAAATCGAAT
GCTTCGACTCCGTCGAAATCTCCGGCGTCGAAGACAGATTCAACGCCTCCCTCGGCACCTACCACGACCTCCTCAAAATCATCAAA
GACAAAGACTTCCTCGACAACGAAGAAACGAAGACATCGTCCTCACCCTCACCCTCTTCGAAGACAGAGAAAT
GATCGAAGAAAGACTCAAAACCTACGCCCACCTCTTCGACGACAAAGTCATGAAACAACTCAAAGAAGAAGATACACCGGCTGGG
GCAGACTCTCCAGAAAACTCATCAACGGCATCAGAGACAAACAATCCGGCAAAACCATCCTCGACTTCCTCAAATCCGACGGCTTC
GCCAACAGAAACTTCATGCAACTCATCCACGACGACTCCCTCACCTTCAAAGAAGACATCCAAAAAGCCCAAGTCTCCGGCCAAGG
CGACTCCCTCCACGAACACATCGCCAACCTCGCCGGCTCCCCCGCCATCAAAAAAGGCATCCTCCAAACCGTCAAAGTCGTCGACG
AACTCGTCAAAGTCATGGGCAGACACAAACCCGAAAACATCGTCATCGAAATGGCCAGAGAAACCAAACCACCCAAAAAGGCCAA
AAAAACTCCAGAGAAAGAATGAAAAGAAGGCATCAAAGAACTCGGCTCCCAAATCCTCAAAGAACACCCCGTCGAAAA
CACCCAACTCCCAAAACGAAAAACTCTACCTCTACTACCTCCAAAACGGCAGACATGTACGTCGACCAAGAACTCGACATCAACA
GACTCTCCGACTACGACGTCGACCACATCGTCCCCCAATCCTTCCTCAAGACGACTCCCATCGACAACACAAGTCCTCACCAGATCC
GACAAAAACAGAGGCAAATCCGACAACGTCCCCTCCGAAGAAGTCGTCAAAAAAATGAAAAACTACTGGAGACAACTCCTCAACGC
CAAACTCATCACCCAAAGAAATTCGACAACCTCACCAAAGCCGAAAGAGGCGGCCTCTCCGAACTCGACAAAGCCGGCTTCATCA
AAAGACAACTCGTCGAAACCAGACAAATCACCAAACACGTCGCCCAAATCCTCGACTCCCAGAATGAACACCAAATACGACGAAAAC
GACAAACTCATCAGAGAAGTCAAAGTCATCACCCTCAAATCCAAACTCGTCTCCGACTTCAGAAAAGACTTCCAATTCTACAAAGT
CAGAGAAATCAACAACTACCACCCACGCCCACGACGCCTACCTCAACGCCGTCGTCGGCACCGCCCTCATCAAAAAATACCCCAAAC
TCGAATCCGAATTCGTCTACGGCGACTACAAAGTCTACGACGTCAGAAAAATGATCGCCAAATCCGAACAAGAAATCGGCAAAGCC
ACCGCCAAATACTTCTTCTACTCCAACATCATGAACTTCTTCAAAACCGAAATCACCCTCGCCAACGGCGAAATCAGAAAAAGACC
CCTCATCGAAACCAACGGCGAAATCGTCTGGGACAAAGGCAGAGACTTCGCCACCGTCAGAAAAGTCCTCTCCATGCC
CCCAAGTCAACATCGTCAAAAAAACCGAAGTCCAAACCGGCGGCTTCTCCAAAGAATCCATCCTCCCCAAAGAAACTCCGACAAA
CTCATCGCCAGAAAAAAAGACTGGGACCCCAAAAAATACGGCGGCTTCGACTCCCCCACCGTCGCCTACTCCGTCCTCGTCGTCGC
CAAAGTCGAAAAAGGCAAATCCAAAAAACTCAAATCCGTCAAAGAACTCCTCGGCATCACCATCATGGAAAGATCCTCCTTCGAAA
AAAACCCCATCGACTTCCTCGAAGCCAAAGGCTACAAAGAAGTCAAAAAAGACCTCATCATCAAACTCCCCAAATACTCCCTCTTC
GAACTCGAAAACGGCAGAAAAAGAATGCTCGCCTCCGCCGGCGAACTCCAAAAAGGCAACGAACTCGCCCTCCCCTCCAAATACGT
CAACTTCCTCTACCTCGCCTCCCACTACGAAAAACTCAAAGGCTCCCCCGAAGACAACGAACAAAACACAACTCTTCGTCGAACAAC
ACAAACACTACCTCGACGAAATCATCGAACAAATCTCCGAATTCTCCAAAAGAGTCATCCTCGCCGACGCCAACCTCGACAAAGTC
CTCTCCGCCTACAACAAACACAGAGACAAACCCATCAGAGAACAAGCCGAAAACATCATCCACCTCTTCACCCTCACCAACCTCGG
CGCCCCCGCCGCCTTCAAATACTTCGACACCACCATCGACAGAAAAAGATACACCTCCACCAAGAAGTCCTCGACGCCACCCTCA
TCCACCAATCCATCACCGGCCTCTACGAAACCAGAATCGACCTCTCCCAACTCGGCGGCGACGGCGGCTCCCCCAAAAAAAAAA
AGAAAAGTCTGA

Cas9 ORF using low C codons of Table 4, with start and stop codons	ATGGATAAGAAGTATAGTATTGGATTGGATATTGGAACAAATAGTGTGGGATGGGCTGTGATTACAGATGAGTATAAGGTGCCTAG TAAGAAGTTTAAGGTGTTGGGAAATACAGATAGACATAGTATTAAGAAGAATTTGATTGGAGCTTTGTTGTTTGATAGTGGAGAGA CAGCTGAGGCTACAAGATTGAAGAGAACAGCTAGAAGAAGATATACAAGAAGAATAGAATTTGTTATTTGCAGGAGATTTTT AGTAATGAGATGGCTAAGGTGGATGATAGTTTTTTCATAGATTGGAGGAGAGTTTTTGGTGGAGGAGGATAAGAAGCATGAGAG ACATCCTATTTTTGGAAATATTGTGGATGAGGTGGCTTATCATGAGAAGTATCCTACAATTTATCATTGAGAAAGAAGTTGGTGG ATAGTACAGATAAGGCTGATTTGAGATTGATTTATTTGGCTTTGGCTCATATGATTAAGTTTAGAGGACATTTTTTGATTGAGGGA GATTTGAATCCTGATAATAGTGATGTGGATAAGTTGTTTATTCAGTTGGTGCAGACATATAATCAGTTGTTTGAGGAGAATCCTAT TAATGCTAGTGGAGTGGATGCTAAGGCTATTTTTGAGATTGAAGATTGGAGAAATTTGATTGCTCAGTTGC CTGGAGAGAAGAAGAATGGATTGTTTGGAAATTTGATTGCTTTGAGTTTGGGATTGACACCTAATTTTTAAGAGTAATTTTGATTTG GCTGAGGATGCTAAGTTGCAGTTGAGTAAGGATACATATGATGATTTGGATAATTTGTTGGCTCAGATTGGAGATCAGTATGC TGATTTGTTTTTTGGCTGCTAAGAATTTGAGTGATGCTATTTTGTTGAGTGATGAATACAGAGATTACAAAGGCTC CTTTGAGTGCTAGTATGATGATTAAGAGATATGATGCATCATCAGGATTTGACATTGTTGAAGGCTTTGGTGAGACACAGCAGTTGCCT GAGAAGTATAAGGAGATTTTTTTTGATCAGAGTAAGAATGGATATGCTGGATATATTGATGGAGGAGCTAGTCAGGAGGAGTTTTA TAAGTTTATTAAGCCTATTTTGGAGAATGGATGGAACAGAGGAGTTGTTGGTGAATAGAGAGGATTTGTTGAGAAAGC AGAGAACATTTGATAATGGAAGTATTCCTCATCAGATTCATTTGGGAGAGTTGCATGCTATTTTGAGAAGACAGGAGGATTTTTTAT CCTTTTTTGAAGGATAATAGAGAGATTGAGAAGATTTTGACATTTAGAATTCCTTATATGTGGGACCTTTGGCTAGAGGAAA TAGTAGATTTGCTTGGATGACAAGAAAGAGTGAGGAGACAATTACACCTTGGAATTTTGAGGAGGTGGTGGATAAGGGAGCTAGTG CTCAGAGTTTTATTGAGAGAATGAATGACAAATTTTGATAAGAATTTGCCTAATGAGAAGGTGTTGCCTAAGCATAGTTTGTTGTATGAG TATTTTACAGTGTATAATGAGTTGACAAAGGTGAAGTATGTGACAGAGGGAATGAGAAAGCCTGCTTTTTTGAGTGGAGAGCAGAA GAAGGCTATTGTGGATTTGTTGTTAAGACAAATAGAAAGGTGACAGTGAAGCAGTTGAAGGAGGATTATATTTTAAGAAGATTGAGT GTTTTGATAGTGTGGAGATTAGTGGAGTGATAGATTTAATGCTAGTTTGGGAACATATCATGATTTGTTGAAGATTATTAAG GATAAGGATTTTTTTGGATAATGAGGAGAATATTTTGGAGGATATTTGTGTTGACATTGATTTGTTTGAGGATAGAGATGAT GATTGAGGAGAGATTGAAGACATATGCTCATTTGTTTGATGATAAGGTGATGAAGCAGTTGAAGAGAGAAGATATACAGGATGGG GAAGATTGAGTAGAAAGTTGATTAATGGAATTAGAGATAAGCAGAGTGGAAAGACAATTTTGGATTTTTTGAAGAGTGATGGATTT GCTAATAGAAATTTTATGCAGTTGATTCATGATAGTTTGACATTTAAGGAGGATATTCAGAAGGCTCAGGTGAGTGGACAGGG AGATAGTTTGCATGAGCATATTGCTAATTTGGCTGGAAGTCCTGCTATTAAGAAGGGAATTTTGCAGACAGTGAAGGTGGTGGATG AGTTGGTGAAGGTGATGGGAAGACATAAGCCTGAGAATATTGTGATTGAGATGGCTAGAGAGAATCAGATTTGAAGGAGCATCCTGTGGAGAA AAGAATAGTAGAGAGAGAATGAAGAGAATTGAGGAGGGAATTAAGGAGTTGGGAAGTCAGATTTGAAGGAGCATCCTGTGGAGAA TACACAGTTGCAGAATGAGAAGTTGTATTATTTGCAGAATGGAAGAGATATGTATGTGGATCAGGAGTTGGATATTAATA GATTGAGTGATTATGATGTGGATCATATTGTGCCTCAGAGTTTTTTGAAGGATGATATTGATAATAAGGTGTTGACAAGAAGT GATAAGAATAGAGGAAAGAGTGATAATGTGCCTAGTGAGGAGGTGGTGAAGAAGATGAAGAATTATTGGAGACAGTTGTTGAATGC TAAGTTGATTACACAGAGAAAGTTTGATAATTTGACAAAGGCTGAGAGAGGAGGATTGAGTGAGTTGGATAAGGCTGGATTTATTA AGAGACAGTTGGTGGAGACAAGACAGATTACAAAGCATGTGGCTCAGATTTTGGATAGTAGAATGAATACAAAGTATGATGAGAAT GATAAGTTGATTAGAGAGGTGAAGTATTACATTGAAGAGTAAGTTGGTGAGTGATTTTAGAAAGGATTTTCAGTTTTATAAGGT GAGAGATTAATAATTATCATCATGCTCATGATGCTTATTTGAATGCTGTGGTGGGAACAGCTTTGATTAAGAAGTATCCTAAGT TGGAGAGTGAGTTTGTGTATGGAGATTATAAGGTGTATGATGTGAGAAAGATGATTGCTAAGAGTGAGCAGGAGATTGGAAAGGCT ACAGCTAAGTATTTTTTTATAGTAATATTATGAATTTTTTTAAGACAGAGATTACATTGGCTAATGGAGAGATTAGAAAGAGACC TTTGATTGAGACAAATGGAGAGACAGGAGAGATTGTGTGGATAAGGGAAGAGATTTTGCTACAGTGAGAAAGGTGTTGAGTATGC CTCAGGTGAATATTGTGAAGAAGACAGAGGTGCAGACAGGAGGATTTAGTAAGGAGAGTATTTTGCCTAAGAGAAATAGTGATAAG	310

	TTGATTGCTAGAAAGAAGGATTGGGATCCTAAGAAGTATGGAGGATTTGATAGTCCTACAGTGGCTTATAGTGTTGGTGGTGGC TAAGGTGGAGAAGGGAAAGAGTAAGAAGTTGAAGAGTGTTGTTGGGAATTACAATTATGGAGAGAAGTAGTTTTTGAGA AGAATCCTATTGATTTTTTTGGAGGCTAAGGGATATAAGGAGGGTGAAGAAGGATTTGATTATTAAGTTGCCTAAGTATAGTTTGTTT GAGTTGGAGAATGGAAGAAAGAGAATGTTGGCTAGTGCTGGAGAGTTGCAGAAGGGAAATGAGTTGGCTTTGCCTAGTAAGTATGT GAATTTTTTGTATTTGGCTAGTCATTATGAGAAGTTGAAGGGAAGTCCTGAGGATAATGAGCAGAAGCAGTTGTTTGTGGAGCAGC ATAAGCATTATTTGGATGAGATTATTGAGCAGATTAGTGAGTTTAGTAAGAGAGTGATTTTGGCTGATGCTAATTTGGATAAGGTG TTGAGTGCTTATAATAAGCATAGAGATAAGCCTATTAGAGAGCAGGCTGAGAATATTATTCATTTGTTTACATTGACAAATTTGGG AGCTCCTGCTGCTTTTAAGTATTTTTGATACAACAATTGATAGAAAGAGATATACAAGTACAAAGGAGGTGTTGGATGCTACATTGA TTCATCAGAGTATTACAGGATTGTATGAGACAAGAATTGATTTGAGTCAGTTGGGAGGAGATGGAGGAAGTCCTAAGAAGAAG AGAAAGGTGTGA	311
Cas9 ORF using low A codons of Table 4, with start and stop codons	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTC CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC TCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG ACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT CAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTG GCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC CGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC CCCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC GAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC AGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA CTCCCGGTTCGCCTGGATGACCCGGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCG CCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAG TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAA GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT GCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGATGATC GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGG GCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTC GCCAACCGGAACTTCATGCAGCTGATCCACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGG CGACTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG AAGAACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAA CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC	

	312
Cas9 ORF using low A/U codons of Table 4, with start and stop codons	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCGGCGGCGTACACCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG ACAGCACCGACAAGGCCGACCTGCGCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC CCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA CAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG CCCAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAA

	313
Cas9 ORF using low A codons of Table 4, with two C-terminal NLS sequences and start and stop	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTC CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGCGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC TCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGA ACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCAT CAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTG CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTG

codons	
GCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGC	
CGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCC	
CCCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC	
GAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTA	
CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC	
AGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACGCCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC	
CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA	
CTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCG	
CCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAG	
TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAA	
GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT	
GCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG	
GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGAT	
GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG	
GCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTC	
GCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGG	
CGACTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG	
AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG	
AAGAACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAA	
CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC	
GGCTGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCC	
GACAAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGC	
CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCA	
AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAAC	
GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGT	
GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC	
TGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCC	
ACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCC	
CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGC	
CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAG	
CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGC	
CAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGA	
AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTC	
GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGT	
GAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC	
ACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG	
CTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG	
CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGA	
TCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAG	

	CGGAAGGTGGACGGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGCTGA	
Cas9 nickase ORF using low A codons of Table 4, with start and stop codons	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTC CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCGGCGGCGTACACCGGCGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC TCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG ACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT CAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTG GCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC CGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCC CCCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC GAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC AGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA CTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCG CCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAG TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAA GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT GCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGAT GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGTACACCGGCTGGG GCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTC GCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGG CGACTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG AAGAACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAA CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC GGCTGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCC GACAAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGC CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCA AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGATCCCCGGATGAACACCAAGTACGACGAGAAC GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGT	314

	GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC	
	TGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCC	
	ACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCC	
	CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGC	
	CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAG	
	CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGC	
	CAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGA	
	AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTC	
	GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGT	
	GAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC	
	ACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG	
	CTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG	
	CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGA	
	TCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCGGCTCCCCCAAGAAGAAG	
	CGGAAGGTGTGA	315
Cas9 nickase ORF using low A codons of Table 4, with start and stop codons and no NLS	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTC	
	CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGA	
	CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC	
	TCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG	
	GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG	
	ACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC	
	GACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT	
	CAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC	
	CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTG	
	GCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGC	
	CGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCC	
	CCCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC	
	GAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTA	
	CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC	
	AGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC	
	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA	
	CTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCG	
	CCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAG	
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAA	
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT	
	GCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG	
	GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGATGATC	
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG	
	GCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTC	

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Cas9 nickase ORF using low A codons of Table 4, with two C-terminal NLS sequences and start and stop codons	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTC CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGTTCGACTCCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC TCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG ACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT CAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTG GCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC CGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC CCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC GAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC AGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC

dCas9 ORF using low A codons of Table 4, with start	317
ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTC	
CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGA	
CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC	
TCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG	
GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG	

and stop codons	
	ACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC
	GACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT
	CAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC
	CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTG
	GCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC
	CGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC
	CCCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC
	GAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTA
	CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC
	AGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC
	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA
	CTCCCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCG
	CCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAG
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAA
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT
	GCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG
	GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGAT
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCCGGTACACCGGCTGGG
	GCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTC
	GCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGG
	CGACTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG
	AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG
	AAGAACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAA
	CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC
	GGCTGTCCGACTACGACGTGGACGCCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCC
	GACAAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGCCAGCTGCTGAACGC
	CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCA
	AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAAC
	GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGT
	GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC
	TGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCC
	ACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCC
	CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGC
	CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAG
	CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGC
	CAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGA
	AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTC
	GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGT
	GAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC

	ACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG CTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGA TCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAG CGGAAGGTGTGA	318
dCas9 ORF using low A codons of Table 4, with start and stop codons and no NLS	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTC CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGTACACCCGGCGCAAGAACCGGATCTGCTACCTGCAGGAGATCTTC TCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG ACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT CAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGCCTGGAGAACCTGATCGCCCAGCTGC CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTG GCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC CGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC CCCTGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC GAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC AGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA CTCCCGGTTCGCCTGGATGACCCGGAAGAGCCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCG CCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAG TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAA GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT GCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAATCATCAAG GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGAT GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGG GCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTC GCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGG CGACTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG AAGAACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAA CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC GGCTGTCCGACTACGACGTGGACGCCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCC GACAAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGC CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCA AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAAC GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGT	

	GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC	
	TGGAGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCC	
	ACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCC	
	CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGC	
	CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAG	
	CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGC	
	CAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGA	
	AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTC	
	GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGT	
	GAACTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC	
	ACAAGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG	
	CTGTCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG	
	CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGA	
	TCCACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACTGA	
dCas9 ORF using low A codons of Table 4, with two C-terminal NLS sequences and start and stop codons	ATGGACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTC	319
	CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGA	
	CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC	
	TCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG	
	GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG	
	ACTCCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC	
	GACCTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT	
	CAACGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC	
	CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTG	
	GCCGAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGC	
	CGACCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCC	
	CCCTGTCCGCCTCCATGATCAAGCGCTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGCCAGCAGCTGCCC	
	GAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTA	
	CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC	
	AGCGGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC	
	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA	
	CTCCCGGTTCGCCTGGATGACCCGGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCG	
	CCCAGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAG	
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAA	
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT	
	GCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG	
	GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGAT	
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGG	
	GCCGGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTC	
	GCCAACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGG	

	320
Cas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences and start and stop codons	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGTTCGACAGCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCGGCGGCGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC CCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCC GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC

Cas9 ORF using low A/U codons of Table 4, with start	321
	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG
	CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA
	CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC
	AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG
	GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG

and stop codons and no NLS	ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC
	GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT
	CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC
	CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG
	GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC
	CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC
	CCCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC
	GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA
	CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC
	AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC
	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA
	CAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG
	CCCAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAA
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT
	GCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG
	GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGAT
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG
	GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGATTCCTGAAGAGCGACGGCTTC
	GCCAACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGG
	CGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG
	AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG
	AAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAA
	CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC
	GGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGC
	GACAAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGCCAGCTGCTGAACGC
	CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCA
	AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAAC
	GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGATTCCAGTTCTACAAGGT
	GCGGGAGATCAACAACTACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC
	TGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCC
	ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCC
	CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGC
	CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAG
	CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC
	CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGA
	AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC
	GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGT
	GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC

	ACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG	
	CTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG	
	CGCCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGA	
	TCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACTGA	
Cas9 nickase ORF using low A/U codons of Table 4, with start and stop codons	ATGGACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG	322
	CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA	
	CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC	
	AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG	
	GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG	
	ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC	
	GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT	
	CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCTGGAGAACCTGATCGCCCAGCTGCC	
	CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG	
	GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGC	
	CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCC	
	CCCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC	
	GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA	
	CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC	
	AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC	
	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA	
	CAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG	
	CCCAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG	
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAA	
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT	
	GCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG	
	GACAAGGACTTCCTGGACAACGAGGAGAACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAA	
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG	
	GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTC	
	GCCAACCGGAACTTCATGCAGCTGATCCACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGG	
	CGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG	
	AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG	
	AAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAA	
	CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC	
	GGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTGCTGACCCGGAGC	
	GACAAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGC	
	CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCA	
	AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAAC	
	GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGT	
	GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC	

	TGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCC	
	ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCC	
	CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGC	
	CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAG	
	CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC	
	CAAGGTGGAGAAGGGCAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGA	
	AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC	
	GAGCTGGAGAACGGCCGCAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGT	
	GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC	
	ACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG	
	CTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG	
	CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGA	
	TCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAG	
	CGGAAGGTGTGA	
Cas9 nickase ORF using low A/U codons of Table 4, with two C-terminal NLS sequences and start and stop codons	ATGGACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG	323
	CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA	
	CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC	
	AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG	
	GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG	
	ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC	
	GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT	
	CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC	
	CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG	
	GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGC	
	CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCC	
	CCCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC	
	GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA	
	CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC	
	AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC	
	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA	
	CAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG	
	CCCAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG	
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAA	
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT	
	GCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG	
	GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGAT	
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGTACACCGGCTGGG	
	GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTC	
	GCCAACCGGAACTTCATGCAGCTGATCCACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGG	

CGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG AAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAA CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC GGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGC GACAAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGC CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCA AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAAC GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGCAAGGACTTCCAGTTCTACAAGGT GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC TGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCC ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCC CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGC CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAG CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC CAAGGTGGAGAAGGGCAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGA AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGT GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAAGCAGCTGTTCGTGGAGCAGCAC ACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG CTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGG CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACGCACCAGGCACGGCAGCGCCACCCTGA TCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGCAGCCCCCAAGAAGAAG CGGAAGGTGGGACGGCCAGCCCCAAGAAGAAGCGGAAGGTGGACAGCGGCTGA	324
Cas9 nickase ORF using low A/U codons of Table 4, with start and stop codons and no NLS	ATGGACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGTTCGACAGCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCGGCGGCGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC CCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC	

	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA	
	CAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG	
	CCCAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG	
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAA	
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT	
	GCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG	
	GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGAT	
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGG	
	GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGATTTCCTGAAGAGCGACGGCTTC	
	GCCAACCGGAACTTCATGCAGCTGATCCACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGG	
	CGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG	
	AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG	
	AAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAA	
	CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC	
	GGCTGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTGCTGACCCGGAGC	
	GACAAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGCCAGCTGCTGAACGC	
	CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCA	
	AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAAC	
	GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGATTCCAGTTCTACAAGGT	
	GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC	
	TGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGATCGGCAAGGCC	
	ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCC	
	CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGC	
	CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAG	
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	CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGA	
	AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC	
	GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGT	
	GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC	
	ACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG	
	CTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG	
	CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGA	
	TCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACTGA	325
dCas9 ORF using low A/U codons of Table 4, with start and stop	ATGGACAAGAAGTACAGCATCGGCCTGGCCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG	
	CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGTTCGACAGCGGCGAGA	
	CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC	
	AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG	
	GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG	
	ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC	

codons	
	GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT
	CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC
	CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG
	GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC
	CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC
	CCCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC
	GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA
	CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC
	AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC
	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA
	CAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG
	CCCAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAA
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT
	GCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG
	GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGATGATCGAG
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGG
	GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTC
	GCCAACCGGAACTTCATGCAGCTGATCCACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGG
	CGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG
	AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG
	AAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAA
	CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC
	GGCTGAGCGACTACGACGTGGACgcCATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGC
	GACAAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGC
	CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCA
	AGCGGCAGCTGGTGGAGACCCGGCACATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAAC
	GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGT
	GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC
	TGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCC
	ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCC
	CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGC
	CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAG
	CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC
	CAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGA
	AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC
	GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGT
	GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC
	ACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG

	CTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGA TCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGCAGCCCCCAAGAAGAAG CGGAAGGGTGTGA	326
dCas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences and start and stop codons	ATGGACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCTGGAGAACCTGATCGCCCAGCTGCC CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCC CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCC CCCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGCCAGCAGCTGCCC GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA CAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG CCCAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAA GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT GCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG GACAAGGACTTCCTGGACAACGAGGAGAACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAACG GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCCGGTACACCGGCTGGG GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTC GCCAACCGGAACTTCATGCAGCTGATCCACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGG CGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACG AGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAG AAGAACAGCCGCGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAA CACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACC GGCTGAGCGACTACGACGTGGACGCCATCGTGCCCCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTGCTGACCCGGAGC GACAAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGCCAGCTGCTGAACGC CAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCA AGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAAC GACAAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGT GCGGGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGC	

	TGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCC	
	ACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCC	
	CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGC	
	CCCAGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAG	
	CTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGC	
	CAAGGTGGAGAAGGGCAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGA	
	AGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC	
	GAGCTGGAGAACGGCCGCAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGT	
	GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGC	
	ACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTG	
	CTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGG	
	CGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGA	
	TCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGCAGCCCCAAGAAGAAG	
	CGGAAGGTGGGACGGCAGCCCCAAGAAGAAGCGGAAGGTGGACAGCGGCTGA	
dCas9 ORF using low A/U codons of Table 4, with start and stop codons and no NLS	ATGGACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAG	327
	CAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGA	
	CCGCCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC	
	AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCG	
	GCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGG	
	ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGC	
	GACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCAT	
	CAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGC	
	CCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTG	
	GCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGC	
	CGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCC	
	CCCTGAGCGCCAGCATGATCAAGCGCTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCC	
	GAGAAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA	
	CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGC	
	AGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTAC	
	CCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAA	
	CAGCCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCG	
	CCCAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAG	
	TACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAA	
	GAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGT	
	GCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAG	
	GACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGAT	
	GATCGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGG	
	GCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTC	
	GCCAACCGGAACTTCATGCAGCTGATCCACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGG	

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Nme Cas9 ORF using low A codons of Table 4, with start and stop codons	ATGGCCGCCTTCAAGCCCAACTCCATCAACTACATCCTGGGCCTGGACATCGGCATCGCCTCCGTGGGCTGGGCCATGGTGGAGAT CGACGAGGAGAGGAGAACCCCATCCGGCTGATCGACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACTCCCC TGGCCATGGCCCCGGCTGGCCCGTCCGTGCGGCGGCTGACCCCGGCGGCGGCCACCGGCTGCTGCGAGACCCGCCGCTGCTG AAGCGGGAGGGCGTGCTGCAGGCCGCCAACTTCGACGAGAACGGCCTGATCAAGTCCCTGCCCAACACACCCCTGGCAGCTGCGGGC CGCCGCCCTGGACCGGAAGCTGACCCCCCTGGAGTGGTCCGCCGTGCTGCTGCACCTGATCAAGCACCGGGGCTACCTGTCCCAGC GGAAGAACGAGGGCGAGACCGCCGACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAGACCGGC GACTTCCGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGTCCGGCCACATCCGAACCAGCGGTCCGACTACTCCCA CACCTTCTCCCGGAAGGACCTGCAGGCCGAGCTGATCCTGTTCGAGAAGCAGAAGGAGTTCGGCAACACCCCCACGTGTCCGGCG GCCTGAAGGAGGGCATCGAGACCCTGCTGATGACCCAGCGGCCGCTGTCCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGC ACCTTCGAGCCCGCCGAGCCCAAGGCCACCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAAGCTGAACAACCTGCG CCTGTCCCCCGAGCTGCAGGACGAGATCGGCACCGCCTTCTCCCTGTTCAAGACCGACGAGGACATCACCGGCCGGCTGAAGGACC GGATCCAGCCCGAGATCCTGGAGGCCCTGCTGAAGCACATCTCCTTCGACAAGTTCGTGCAGATCTCCCTGAAGGCCCTGCGGCGG ATCGTGCCCCTGATGGAGCAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGACCACTACGGCAAGAAGAACACCGA

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Nme Cas9 ORF using low A/U codons of Table 4, with start and stop codons	ATGGCCGCCTTCAAGCCCAACAGCATCAACTACATCCTGGGCCTGGACATCGGCATCGCCAGCGTGGGCTGGGCCATGGTGGAGAT CGACGAGGAGAGGAGAACCCCATCCGGCTGATCGACCTGGGCGTGCGCGGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACAGCC TGGCCATGGCCCGGGGCTGGCCCGGAGCGTGCGGCGGCTGACCCGGCGGCGGCTGCTGCGGACCCGGCGGCTGCTGCGGGC AAGCGGGAGAGGGCGTGCTGCAGGCCGCCAACTTCGACGAGAACGGCCTGATCAAGAGCCTGCCCAACACCCCCTGGCAGCTGCGGGC CGCCGCCCTGGACCGGAAGCTGATCGCCTGGAGTGGAGCGCCGTGCTGCACCTGATCAAGCACCGGGGCTACCTGAGCCAGC GGAAGAACGAGGGCGAGACCGCCGACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAGACCGGC GACTTCCGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGAGCGGCCACCCTGATGGACGAGCCCTACCGGAAGAGCAAGCTGA CACCTTCAGCCGGAAGGACCTGCAGGCCGAGCTGATCCTGTTCGAGAAGCAGAAGGAGTTCGGCAACCCCCACGTGAGCGGCG GCCTGAAGGAGGGCATCGAGACCCTGCTGATGACCCAGCGGCCCGCCTGAGCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGC ACCTTCGAGCCCGCCGAGGCCCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAAGCTGAACAACCTGCG GATCCTGGAGCAGGGCAGCGAGCGGCCCTGACCGACACCGAGCGGGCCACCCTGATGGACGAGCCCTACCGGAAGAGCAAGCTGA CCTACGCCCAGGCCCGGAAGCTGCTGGGCCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGCAAGGACAACGCCGAGGCC AGCACCCTGATGGAGATGAAGGCCTACCACGCCATCAGCCGGGCCCTGGAGAAGGAGGGCCTGAAGGACAAGAAGAGCCCCCTGAA CCTGAGCCCCGAGCTGCAGGACGAGATCGGCACCGCCTTCAGCCTGTTCAAGACCGACGAGGACATCACCGGCCGCCTGAAGGACC GGATCCAGCCCGAGATCCTGGAGGCCCTGCTGAAGCACATCAGCTTCGACAAGTTCGTGCAGATCAGCCTGAAGGCCCTGCGGCGG ATCGTGCCCCTGATGGAGCAGGGCAAGCGGTACGACGAGGCCTGCGCCGAGATCTACGGCGACCACTACGGCAAGAAGAACACCGA

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Open reading frame for Cas9 with NLS1, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAACACAGAATACACAAGAAGAACAGATCTTCCTGCAGGAAATCTTC AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA GACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCC CGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGC AGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCAC CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCG GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTA CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTAC CCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAA

CAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG
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TACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAA
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GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAG
GACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAGATGAAAT
GATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGG
GAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCGGAAAGACAATCCTGGACTTTCCTGAAGAGCGACGGATTC
GCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGG
AGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAGGGAATCCTGCAGACAGTCAAGGTCGTCGACG
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AAGAACAGCAGAGAAAGAATGAAGAGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAA
CACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACA
GACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTCCTGACAAGAGC
GACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGC
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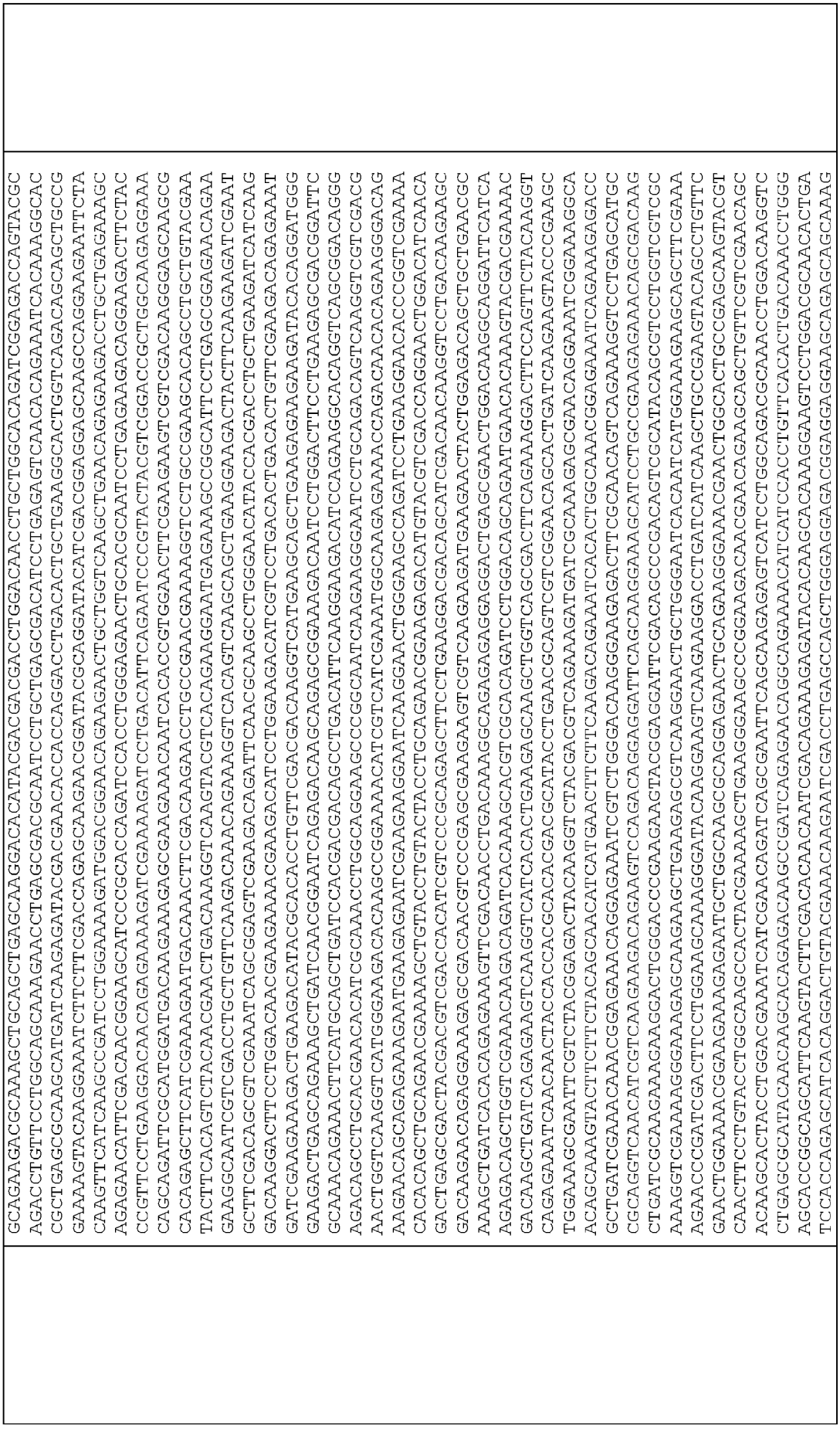
GACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGT
CAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGC
TGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAAACAGGAAATCGGAAAGGCA
ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACC
GCTGATCGAAACAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGC
CGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAG
CTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGC
AAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACATCATGGAAAGAAGCAGCTTCGAAA
AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTC
GAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT
CAACTTCCTGTACCTGGCAAGCCACTACGAAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGC
ACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTC
CTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGG
AGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAGGAAGTCCTGGACGCCACACTGA
TCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCTGGCAGCAAAG
AGAAGCAGAACACATAG

		331
Open reading frame for Cas9 with NLS2, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAACACGCAAGAAGATACACAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTC AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCCGACAAATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA GACCTGAACCCCGGACAACAGCGTCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT CGCTGAGCGCAAGCATGATCTTCCTGGCACTGACCAACCTGACAGTTCAAAGAGGACATCGTCGCAGAAATCGTCAAGCTGACCG CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGAAAACCTGATCGCCACAGCTGC CGGGAGAAAAGAAGAACGGACTGTTTCGGAAACCTGATCGCCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCC AGACCTGTTCCTGGCAGCAAAGAACCTGATCCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCAC CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCG GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTA CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTAC CCGTTCCTGAAGGACAACAGAGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAA CAGCAGATTCGCATGGATGACAAGAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG CACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAA TACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAA GAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAAT GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAG GACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTTCGAAGACAGAGAAAT GATCGAAGAAATCTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGG GAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTC GCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGG AGACAGCCTGCACGAACACATCGCAAACCTGGCAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACG AACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAACCAGACACACAGAAGGGACAG AAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAA CACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACA GACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGC GACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGC AAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCA AGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCCAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAAC GACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGT CAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGC TGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACC GCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGC CGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAG	

CTGATCGCAAGAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGC AAAGGTCGAAAAGGGAAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTC GAACTGGAAAAACGGAAGAAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGC ACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTC CTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGG AGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCCAACACTGA TCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAAGCCAGGCAGCAAAG AGAAGCAGAACAACATAG	332

GACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGC GACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGC AAAGCTGATCACACAGAAAGTTCGACAACCTGACAAAGGCAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCA AGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCCACAGATCCTGGACAGCAGAATGAACACACAAAGTACGACGAAAAC GACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGT CAGAGAAATCAACAACTACCACCACGCACACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGAGC TGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACC GCTGATCGAAACAAACGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGC CGCAGGTCAACATCGTCAAGAAGACAGAGGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAG CTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGC AAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTC GAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAAGCAGCTGTTCGTCGAACAGC ACAAGCACTACCTGGACGAAATCATCGAGCAGATTCAGCGAATTCAGCAAGAGATCATCCTGGCAGACGCAAACCTGGACAAGGTC CTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGG AGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGA TCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAAGGCCCGGCACCGGCA AAGAGAGAAAGAACAACATAG	333

Open reading frame for Cas9 with NLS5, with start and stop codons	334
	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG
	CAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA
	CAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTC
	AGCAACGAAATGGCAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG
	ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCG
	ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA
	GACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT
	CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCACAGCTGC
	CGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG


GCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGC
AGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCAC
CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCG
GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTA
CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC
AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTAC
CCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAA
CAGCAGATTCGCATGGATGACAAGAAAGAGCGCAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG
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TACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAA
GAAGGCAATCGTCGACCTGCTGTTCAAGACAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGATCTACTTCAAGAAGATCGAAT
GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAG
GACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAGGAACGC
GATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGG
GAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGTCGTCGGAACACTGATCAAGAAGTACCCGAAGC
GCAAACAGAAACTTCATGCAGCTGATCCACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGG
AGACAGCCTGCACGAACACATCGCCAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACG
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CACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGACATGTACGTCGACCAGGAACTGGACATCAACA
GACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACCAGAAGC
GACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGC
AAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCA
AGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAAC
GACAAGCTGATCAGAGAAGTCAAGGTCACCCTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGT
CAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACACAGCACTGATCAAGAAGTACCCGAAGC
TGGAAAGCGAATTTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA
ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACC
GCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACACAGTCAGAAAGGTCCTGAGCATGC
CGCAGGTTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAG
CTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAGTACGGAGGATTCGACACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGC
AAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA
AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTC
GAACTGGAAAACGGAAGAAAGAGAAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT
CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGC
ACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTC
CTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGG
AGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCCACACTGA
TCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGCAGAGCAGCAAAG

	AGACCGAGAACAACATAG	
Open reading frame for Cas9 with NLS6, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG	335
	CAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA	
	CAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGATACACACAAGAAGAAACAGAATCTGCTACCTGCAGGAAATCTTC	
	AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG	
	ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCG	
	ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCCCACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA	
	GACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT	
	CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCACAGCTGC	
	CGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG	
	GCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGC	
	AGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCAC	
	CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCG	
	GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGATTCTA	
	CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC	
	AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCCAATCCTGAGAAGACAGGAAGACTTCTAC	
	CCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAA	
	CAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG	
	CACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAA	
	TACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAA	
	GAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAAT	
	GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAG	
	GACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAAT	
	GATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGG	
	GAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTC	
	GCAAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGG	
	AGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACG	
	AACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAACATCGTCATCGAAATGGCAAGAGAAACCAGACACAACACAGAAGGGACAG	
	AAGAACAGCAGAGAAAGAATGAAGAGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAA	
	CACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACA	
	GACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGC	
	GACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGC	
	AAAGCTGATCACACAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGACTGAGCGAACTGGACAAGGCCAGGATTCATCA	

	336
Open reading frame for Cas9 with NLS7, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACAGACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAAACAGCAAGAAGAAGATACACAAGAAGAAACAGAATCTGCTACCTGCAGGAAATCTTC AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA GACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGC CGGGAGAAAAGAAACGGCTGTTTCGGAAACCTGATCGCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGC AGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACCAAGGCAC CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCCACTGGTCAGACAGCAGCTGCCG GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCGACGGAGGAGCAAGCCAGGAAGAATTCTA CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTAC CCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGGAAA CAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG CACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAA TACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAA GAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAAT GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAG GACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAAT

	337
Open reading frame for Cas9 with NLS8, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGATGGCAAGAAGAACAGCAAGATACACACAAGAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTC AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCCCACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA GACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGACATCCTGAGAGTCAACACAGAAATCACCAAAGGCACTGGTCAGATC CGGGAGAAAAGAAGAACGGACTGTTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCC AGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACCAAAGGCAC CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCG	

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CAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG
CACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAA
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GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAG
GACAAGGACTTCCTGGACAACGAAGAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAGAAACT
GATCGAAGAAATGACTGAAGACATACGCCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGG
GAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTC
GCAAACAGAAACTTCATGCAGCTGATCCACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGG
AGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACG
AACTGGTCAAGGTCATGGGACACAAGCCGGAAACATCGTCATCGAAATGGCAAGAGAAACCAGACACAACACAGAAGGGACAG
AAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAA
CACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACA
GACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTCCTGACCAGAAGC
GACAAGGGAAGAGCGGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCC
AAAGCTGATCACACAGAAGTTCGACAACCTGACAAAGGCAGAGAGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCA
AGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCCAGATCCTGGACAGCAGGATGACGAAAAC
GACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAGGACTTCCAGTTCTACAAGGT
CAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGC
TGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAAAGGCA
ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACC
GCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGC
CGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAG
CTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGC
AAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA
AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTC
GAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT
CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGC
ACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTC
CTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGG
AGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGA
TCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGCGCAGCAGCAAAG
AGAAGCTGGAGCATGGCATTCTAG

		338
Open reading frame for Cas9 with NLS9, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAACACGCAAGAAGATACACAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTC AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCCGACAAATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGA GACCTGAACCCGGACAACAGCGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGAAAACCTGATCGCCACAGCTGC CGGGAGAAAAGAAGAACGGACTGTTTCGGAAACCTGATCGCCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGAGACCAGTACGCC AGACCTGTTCCTGGCCGCAAAGAACCTGATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCCCAC CGCTGAGCGCCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCCACTGGTCAGACACAGCAGCTGCCG GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCGGATACGCGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTA CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTAC CCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAA CAGCAGATTCGCATGGATGACAAGAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG CACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAA TACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAA GAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAAT GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAG GACAAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACGTTGTTCGAAGACAGAGAAAT GATCGAAGAAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGG GAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTC GCAAACAGAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGG AGACAGCCTGCACGAACACATCGCAAACCTGGCAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACG AACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAACCAGACACAACACAGAAGGGACAG AAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAA CACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACA GACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGC GACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGC AAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCA AGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCCAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAAC GACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGT CAGAGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGC TGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCA ACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACC GCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGC CGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAG	338

CTGATCGCAAGAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGC AAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTC GAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGC ACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCCAACCTGGACAAGGTC CTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGG AGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCCACAACTGA TCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAAGCGCAGCAGCAAAG AGAAAGTACTTCGCCAGCATAG	339

	340
Open reading frame for Cas9 with NLS11, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAG CAAGAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAA CAGCAGAAGCAACAAGACTGAAGAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTC AGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAG ACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCCGACAATCTACCACCTGAGAAAGAAGCTGGTCG ACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCCCACATGATCAAGTTCAGAGGCCACTTCCTCATCGAGGGA GACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGAT CAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGC CGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG GCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGC AGACCTGTTCCTGGCAGCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACCAAGGCCAC CGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCCACTGGTCAGACAGCAGCTGCCG GAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCGGATACATCGACGGAGGAGCAAGCCAGGAAGATTTCTA CAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGC AGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTAC CCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAA CAGCAGATTCGCATGGATGACAAGAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCG CACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAA TACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAA

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Cas9 ORF using codons with generally high expression in humans (no start or stop codons;	CCTAAGAAAAAGCGGAAGGTCGACGGGGATAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGCAGTGAT CACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTCCTGGGGAACACCGATAGACACAGCATCAAGAAAAATCTCATCGGAG CCCTGCTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAATCGC ATCTGCTATCTGCAAGAGATCTTTTCGAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGT GGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTTGGAAACATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCT ACCATCTGCGGAAGAAGTTGGTTGACTCAACTGACACAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGATTCAAATTC CGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGACGTGGATAAGCTTTTCATTCAACTGGTGCAGACCTACAA CCAACTGTTCGAAGAAAACCCAATCAATGCTAGCGGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCC TCGAAAACCTGATCGCCAGCTGCCGGGAGAGAAAAGAACGGACTTTTCGGCAACTTGATCGCTCTCTCACTGGGACTCACTCCC	341

suitable for inclusion in fusion protein coding sequence)	AATTTCAAGTCCAATTTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGACGACGACTTGGACAATTTGCT GGCACAAATTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTGCGCG TGAACACCGAAATAACCAAAGCGCCCGCTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAA GCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAATGGGTACGCAGGGTACATCGATGG AGGCGCTAGCCAGGAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTCAAGCTGA ACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGATCCATTCCCCACCAGATCCATCTGGGTGAGCTGCACGCCATC TTGCGGCGCCCAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGAAAAGATCGAGAAAATTCTGACGTTCCGCATCCCGTATTA CGTGGGCCCACTGGCGCGCCGGCAATTCGCGCTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGGAATTTCGAGG AAGTTGTGGATAAGGGAGCTTCGGCACAAAGCTTCATCGAACGAATGACCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTT CCTAAGCACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGTGAAATACGTTACTGAAGGAATGAGGAAGCC GGCCTTTCTGTCCGGAGAACAGAAGAAAGCAATTGTCGATCTGCTTCAAGACCAACCGCAAGGTGACCGTCAAGCAGCTTAAAG AGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTAT CATGATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAACGAGGACATCCTGGAAGATATCGTCCTGACCTT GACCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAGGTCATGAAACAACTCA AGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTG GATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAATTGATCCACGACGACTCCTTGACCATTTAAGGAGGACATCCA AAAAGCACAAGTGTCCGGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCCGGTTCGCCGGCGATTAAGAAGGGAATTC TGCAAACTGTGAAGGTGGTCGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCCGAGAA AACCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGAT CCTGAAAGAGCACCCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTGCAAAATGGACGGGACATGTACG TGGACCAAGAGCTGGACATCAATCGGTTGTCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGACTCGATC GATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAA TTACTGGCGGCAGCTCCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGACTCTCAG AGCTGGATAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGTGGCGCAGATTACCAAGCACGTGGCGCAGATCTTGGACTCCCGC ATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCG GAAGGACTTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGACGCATACCTCAACGCTGTGGTCGGTACCG CCCTGATCAAAAAGTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAGATGATAGCCAAG TCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTTTACTCAAACATCATGAACTGAAATTACGCTGGC CAATGGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGGGACTTCGCAA CTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTCAAAGGAATCGATC CTCCCAAAGAGAAATAGCGACACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGT CGCATACTCCGTCCTCGTGGTGGCCAAGGTGGAAAGGGAAAGAGCAAAAAGCTCAAATCCGTCAAAGAGCTGCTGGGGATTACCA TCATGGAACGATCCTCGTTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATC AAACTCCCCAAGTACTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGA GCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGAAAAACTCAAGGGTCACCGGAAGATAACGAAC AGAAGCAGCTTTTCGTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTCAAAGCGCGTGATCCTC GCCGACGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACACAGGCCGAGAACATTATCCA CTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTACTTCGATACTACTATCGATCGCAAAGATACACGTCCACCA AGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATCGATCTGTCGCAGCTGGGTGGCGAT

		342
Cas9 ORF using long half life codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACTCTATCGGTTTGGACATCGGTACCAACTCTGTCGGTTGGGCCGTCATCACCGACGAATACAAGGTCCCATCTAA GAAGTTCAAGGTCTTGGGTAACACCGACAGACACTCTATCAAGAAGAACTTGATCGGTGCCTTGTTGTTCGACTCTGGTGAAACCG CCGAAGCCACCCAGATTGAAGAGAACCGCCAGAAGAAGATACACCAGAAGAACAGAATCTGCTACTTGCAAGAAATCTTCTCT AACGAAATGGCCAAGGTCGACGACTCTTTCTTCCACAGATTGGAAGAATCTTTCTTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCAATCTTCGGTAACATCGTCGACGAAGTCGCCTACCACGAAAAGTACCCAACCATCTACCACTTGAGAAAGAAGTTGGTCGACT CTACCGACAAGGCCGACTTGAGATTGATCTACTTGGCCTTGGCCCACATGATCAAGTTCAGAGGTCACTTCTTGATCGAAGGTGAC TTGAACCCAGACAACTCTGACGTCGACAAGTTGTTCATCCAATTGGTCCAAACCTACAACCAATTGTTCGAAGAAAACCCAATCAA CGCCTCTGGTGTCGACGCCAAGGCCATCTTGTCTGCCAGATTGTCTAAGAGCAGAAGATTGGAAAACTTGATCGCCCAATTGCCAG GTGAAAAGAAGAACGGTTTGTTCGGTAACTTGATCGCCTTGTCTTTGGGTTTGACCCCAAACTTCAAGTCTAACTTCGACTTGGCC GAAGACGCCAAGTTGCAATTGTCTAAGGACACCTACGACGACGACTTGGACAACTTGTTGGCCCAAATCGGTGACCAATACGCGGA CTTGTTCTTGGCCGCCAAGAACTTGTCTGACGCCATCTTGTTGTCTGACATCTTGAGAGTCAACACCGAAATCACCAAGGCCCCAT TGTCTGCCTCTATGATCAAGAGATACGACGAACACCACCAAGACTTGACCTTGTTGAAGGCCTTGGTCAGACAACAATTGCCAGAA AAGTACAAGGAAATCTTCTTCGACCAATCTAAGAACGGTTACGCCGGTTACATCGACGGTGGTGCCTCTCAAGAAGAATTCTACAA GTTCATCAAGCCAATCTTGGAAAAGATGGACGGTACCGAAGAATTGTTGGTCAAGTTGAACAGAGAAGACTTGTTGAGAAAGCAAA GAACCTTCGACAACGGTTCTATCCCACACCAAATCCACTTGGGTGAATTGCACGCCATCTTGAGAAGACAAGAAGACTTCTACCCA TTCTTGAAGGACAACAGAGAAAAGATCTTGACCTTCAGAATCCCATACTACGTCGGTCCATTGGCCAGAGGTAACAG CAGATTCGCCTGGATGACCAGAAAGTCTGAAGAAACCATCACCCCATGGAACTTCGAAGAAGTCGTCGACAAGGGTGCCTCTGCCC AATCTTTCATCGAAGAATGACCAACTTCGACAAGAACTTGCCAAACGAAAAGGTCTTGCCAAGCACTCTTTGTTGTACGAATAC TTCACCGTCTACAACGAATTGACCAAGGTCAAGTACGTCACCGAAGGTATGAGAAAGCCAGCCTTCTTGTCTGGTGAACAAAAGAA GGCCATCGTCGACTTGTTGTTCAAGACCAACAGAAAGGTCACCGTCAAGCAATTGAAGGAAGACTACTTCAAGAAGATCGAATGCT TCGACTCTGTCGAAATCTCTGGTGTCGAAGACAGATTCAACGCCTCTTTGGGTACCTACCACGACTTGTTGAAGATCATCAAGGAC AAGGACTTCTTGGACAACGAAGAAAACGAAGACATCTTGGAAGACATCGTCTTGACCTTGACGTTGTTCGAAGACAGAGAAATGAT CGAAGAAGATTGAAGACCTACGCCCACTTGTTCGACGACAAGGTCATGAAGCAATTGAAGAGAAGAAGATACACCGGTTGGGGTA GATTGAGCAGAAAGTTGATCAACGGTATCAGAGACAAGCAATCTGGTAAGACCATCTTGGACTTCTTGAAGTCTGACGGTTTCGCC AACAGAAACTTCATGCAATTGATCCACGACGACTCTTTGACCTTCAAGGAAGACATCCAAAAGGCCCAAGTCTCTGGTCAAGGTGA CTCTTTGCACGAACACATCGCCAACTTGGCCGGTTCTCCAGCCATCAAGAAGGGTATCTTGCAAACCGTCAAGGTCGTCGACGAAT TGGTCAAGGTCATGGGTAGACACAAGCCAGAAAACATCGTCATCGAAATGGCCAGAGAAAACCAAACCACACCCAAAGGGTCAAAAG AACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGTATCAAGGAATTGGGTTCTCAAATCTTGAAGGAACACCCAGTCGAAAACAC CCAATTGCAAAACGAAAAGTTGTACTACTTGCAAAACGGTAGAGACATGTACGTCGACCAAGAATTGGACATCAACAGAT TGTCTGACTACGACGTCGACCACATCGTCCCACAATCTTTCTTGAAGGACGACTCTATCGACAACAAGGTCTTGACCAGATCTGAC AAGAACAGAGGTAAGTCTGACAACGTCCCATCTGAAGAAGATGAAGAACTACTGGAGACAATTGTTGAACGCCAA GTTGATCACCCAAAGAAAGTTCGACAACTTGACCAAGGCCGAAAGAGGTGGTTTGTCTGAATTGGACAAGGCCGGTTTCATCAAGA GACAATTGGTCGAACCAGACAAATCACCAAGCACGTCGCCCAAATCTTGGACAGCAGAATGAACACCACGAGTACGACGAAAACGAC AAGTTGATCAGAGAAGTCAAGGTCATCACCTTGAAGTCTAAGTTGGTCTCTGACTTCAGAAAGGACTTCCAATTCTACAAGGTCAG AGAAATCAACAACTACCACCACGCCCACGACGCCTACTTGAACGCCGTCGTCGGTACCGCCTTGATCAAGAAGTACCCAAAGTTGG AATCTGAATTCGTCTACGGTGACTACAAGGTCTACGACGTCAGAAAGATGATCGCCAAGTCTGAACAAGAAATCGGTAAGGCCACC GCCAAGTACTTCTTCTACTCTAACATCATGAACTTCTTCAAGACCGAAATCACCTTGGCCAACGGTGAAATCAGAAAGAGACCATT GATCGAAACCAACGGTGAAACCGGTGAAATCGTCTGGGACAAGGGTAGAGACTTCGCCACCGTCAGAAAGGTCTTGTCTATGCCAC AAGTCAACATCGTCAAGAAGACCGAAGTCCAAACCGGTGGTTTCTCTAAGGAATCTATCTTGCCAAAGAGAAACTCTGACAAGTTG	

	ATCGCCAGAAAGAAGGACTGGGACCCAAAGAAGTACGGTGGTTTCGACTCTCCAACCGTCGCCTACTCTGTCTTGGTCGTCGCCAA GGTCGAAAAGGGTAAGTCTAAGAAGTTGAAGTCTGTCAAGGAATTGTTGGGTATCACCATCATGGAAAGATCTTCTTTCGAAAAGA ACCCAATCGACTTCTTGGAAGCCAAGGGTTACAAGGAAGTCAAGAAGGACTTGATCATCAAGTTGCCAAAGTACTCTTTGTTCGAA TTGGAAAACGGTAGAAAGAGAATGTTGGCCTCTCGGCGGTGAATTGCAAAAGGGTAACGAATTGGCCTTGCCATCTAAGTACGTCAA CTTCTTGTACTTGGCCTCTCACTACGAAAAGTTGAAGGGTTCTCCAGAAGACAACGAACAAAGCAATTGTTCGTCGAACAACACA AGCACTACTTGGACGAAATCATCGAACAAATCTCTGAATTCTCTAAGAGAGTCATCTTGGCCGACGCCAACTTGGACAAGGTCTTG TCTGCCTACAACAAGCACAGAGACAAGCCGAAAACATCATCCACTTGTTCACCTTGACCAACTTGGGTGC CCCAGCCGCCTTCAAGTACTTCGACACCACCATCGACAGAAGAGATACACCTCTACCAAGGAAGTCTTGGACGCCACCTTGATCC ACCAATCTATCACCGGTTTGTACGAAACCAGAATCGACTTGTCTCAATTGGGTGGTGACGGTGGTGGTTCTCCAAAGAAGAAGAGA AAGGTC
Cas9 ORF using U rich codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GATAAAAAATATTCTATTGGTTTAGATATTGGTACTAATTCTGTTGGTTGGGCTGTTATTACTGATGAATATAAAGTTCCTTCTAA AAAATTTAAAGTTTTAGGTAATACTGATCGTCATTCTATTAAAAAAAATTTAATTGGTGCTTTATTATTTGATTCTGGTGAAACTG CTGAAGCTACTCGTTTAAAACGTACTGCTCGTCGTTATACTCGTCGTAAAAATCGTATTTGTTATTTACAAGAAATTTTTCT AATGAAATGGCTAAAGTTGATGATTCTTTTTTCATCGTTTAGAAGAATCTTTTTTAGTTGAAGAAGATAAAAAACATGAACGTCA TCCTATTTTTGGTAATATTGTTGATGAAGTTGCTTATCATGAAAAATATCCTACTATTTATCATTTACGTAAAAAATTAGTTGATT CTACTGATAAAGCTGATTTACGTTTTAATTTATTTAGCTTTAGCTCTATATGATTTAAATTTCGTGGTCATTTTTTTAATTGAAGGTGAT TTAAATCCTGATAATTCTGATGTTGATAAATTATTTATTCAATTAGTTCAAACTTATAATTCAATTATTTGAAGAAAATCCTATTAA TGCTTCTGGTGTTGATGCTAAAGCTATTTTATCTGCTCGTTTATCTAAATCTCGTCGTTTAGAAAATTTAATTGCTCAATTACCTG GTGAAAAAAAAATGGTTTATTTGGTAATTTAATTGCTTTATCTTTAGGTTTAACTCCTAATTTTAAATCTAATTTTGATTTAGCT GAAGATGCTAAATTACAATTATCTAAAGATACTTATGATGATTTAGATAATTTATTAGCTCAAATTGGTGATCAATATGCTGA TTTATTTTTAGCTGCTAAAAATTTATCTGATGCTATTTTATCTGATATTTTACGTGTTAATACTGAAATTACTAAAGCTCCTTT TATCTGCTTCTATGATTAAACGTTATGATGAACATCATCAAGATTTAACTTTATTAAAAGCTTTAGTTCGTCAACAATTACCTGAA AAATATAAAGAAATTTTTTTTTGATCAATCTAAAAATGGTTATGCTGGTTATATTGATGGTGGTGCTTCTCAAGAAGAATTTTATAA ATTTATTAAACCTATTTTAGAAAAAATGGATGGTACTGAAGAATTATTAGTTAAATTAAATCGTGAAGATTTATTACGTAAACAAC GTACTTTTGATAATGGTTCTATTCCTCATCAAATTCATTTAGGTGAATTACATGCTATTTTACGTCGTCAAGAAGATTTTTATCCT TTTTTAAAGATAATCGTGAAAAATTGAAAAATTTTAACTTTTCGTATTCCTTATTATGTTGGTCCTTTAGCTCGTGGTAATTC TCGTTTTGCTTGGATGACTCGTAAATCTGAAGAAACTATTACTCCTTGGAATTTTGAAGAAGTTGTTGATAAAGGTGCTTCTGCTC AATCTTTTATTGAACGTATGACTAATTTTGATAAAAATTTACCTAAATGAAAAAGTTTTACCTAAACATTCTTTATTATATGAATAT TTTACTGTTTATAATGAATTAACTAAAGTTAAATATGTTACTGAAGGTATGCGTAAACCTGCTTTTTTATCTGGTGAACAAAAAA AGCTATTGTTGATTTATTATTTAAAACTAATCGTAAAGTTACTGTTAAACAATTAAAAGAAGATTATTTTAAAAAATTGAATGTT TTGATTCTGTTGAAATTTCTGGTGTTGAAGATCGTTTTAATGCTTCTTTAGGTACTTATCATGATTTATTAAAAATTATTAAGAT AAAGATTTTTTAGATAATGAAGAAAATGAAGATATTTTAGAAGATATTGTTTTAACTTTATTGAAGATCGTGAAAATGAT TGAAGAACGTTTAAAAACTTATGCTCATTTATTTGATGATAAAGTTATGAAACAATTAAAACGTCGTCGTTATACTGGTTGGGGTC GTTTATCTCGTAAATTAATTGGTATTCGTGATAAACAATCTGGTAAACTATTTTAGATTTTTAAATCTGATGGTTTTTGCT AATCGTAATTTTATGCAATTAATTCATGATGATTCTTTAACTTTAAAGAAGATATTCAAAAAGCTCAAGTTTCTGGTCAAGGTGA TTCTTTACATGAACATATTGCTAATTTAGCTGGTTCTCCTGCTATTAAAAAAGGTATTTTACAAACTGTTAAAGTTGTTGATGAAT TAGTTAAAGTTATGGGTCGTCATAAACCTGAAAATATTGTTATTGAAATGGCTCGTGAAAATCAAACTACTCAAAAAGGTCAAAAA AATTCTCGTGAACGTATGAAACGTATTGAAGAAGGTATTAAAGAATTAGGTTCTCAAATTTTAAAAGAACATCCTGTTGAAAATAC TCAATTACAAAATGAAAAATTATATTATTTACAAAATGGTCGTGATATGTATGTTGATCAAGAATTAGATATTAATCGTT
	343

	344
Cas9 ORF using low G codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAAAAATACTCCATCGGCCTCGACATCGGCACCAACTCCGTCGGCTGGGCCGTCATCACCGACGAATACAAGGTCCCCTCCAA AAAATTCAAAGTCCTCGGCAACACCGACAGACACTCCATCAAAAAAAACCTCATCGGCGCCCTCCTTCGACTCCGGCGAAACCG CCGAAGCCACCAGACTCAAAAGAACCGCCAGAAGAAGATACACCAGAAGAAAAAACAGAATCTGCTACCTCCAAGAAATCTTCTCC AACGAAATGGCCAAAGTCGACGACTCCTTCTTCCACAGACTCGAAGAATCCTTCCTCGTCGAAGAAGACAAAAAACACGAAAGACA CCCCATCTTCGGCAACATCGTCGATGAAGTCGCCTACCACGAAAAATACCCCACCATCTACCACCTCAGAAAAAAACTCGTCGACT CCACCGACAAAGCCGACCTCATCTACCTCGCCCTCGCCCACATGATCAATTCAGAGGCCACTTCCTCATCGAAGGCGAC CTCAACCCCGACACAACTCCGACGTCGACAAACTCTTCATCCAACTCGTCCAAATCCAGACTCTTCGAAGAAAACCCCATCAA CGCCTCCGGCGTCGACGCCAAGCCATCCTCTCGCCAGACTCTCCAAATCCAGAAGACTCGAAAACCTCATCGCCCAACTCCCCG GCGAAAAAAAAAACGGCCTCTTCGGCAACCTCATCGCCCTCTCCCTCGGCCTCACCCCCAACTTCAAATCCAACTTCGACCTCGCC GAAGACGCCAAACTCCAACTCTCCAAAGACACCTACGACGACGACCTCGACAACCTCCTCGCCCAAATCGGCGACCAATACGCCGA CCTCTTCCTCGCCGCCAAAAACCTCTCCGACGCCATCCTCCTCTCCGACATCCTCCAGAGTCAACACGAAATCACCAAGCCCCCC TCTCCGCCTCCATGATCAAAGATACGACGAACACCACCAAGACCTCACCCTCCTCCTCAAAGCCCTCGTCAGACACAACACTCCCCGAA AAATACAAAGAAATCTTCTTCGACCAATCCAAAAACGGCTACGCCGGTACATCGACGGCGGCGCCTCCCAAGAAGAATTCTACAA ATTCATCAAACCCATCCTCGAAAAAATGGACGGCACCGAAGAACTCCTCGTCAAACTCAACAGAGAAGACCTCCTCAGAAAACAAA GAACCTTCGACAACGGCTCCATCCCCCACCAAATCCACCTCGGCGAACTCCACGCCATCCTCCAGAAGACAAGAAGACTTCTACCCC TTCCTCAAAGACAACAGAGAAAAAATCGAAAAAATCCTCACCTTCAGAATCCCCTACTACGTCGGCCCCCTCGCCAGAGGCAACTC CAGATTCGCCTGGATGACCAGAAAATCCGAAGAAACCATCACCCCCTGGAACTTCGAAGAAGTCGTCGACAAAGGCGCCTCCGCCC AATCCTTCATCGAAAGAATGACCAACTTCGACAAAAACCTCCCCAACGAAAAAGTCCTCCCCCAAACACTCCCTCCTCTACGAATAC TTCACCGTCTACAACGAACTCACCAAAGTCAAATACGTCACCGAAGGCATGAGAAAACCCGCCTTCCTGTCCGGCGAACAAAAAAA

	345
Cas9 ORF using low C codons of Table 4 (no start or stop codons; suitable for inclusion in fusion	GATAAGAAGTATATGATTATTGGATTGGATATTGGAACAAATAGTGTGGGATGGGCTGTGATTACAGATGAGTATAAGGTGCCTAGTAA GAAGTTTAAGGTGTTGGGAAATACAGATAGACATAGTATTAAGAAGAATTTGATTGGAGCTTTGTTGTTTGATAGTGGAGAGACAG CTGAGGCTACACAAGATTGAAGAGAAACAGCTAGAAGAAGATATACAAGAAGAAATAGAATTTGTTATTTGCAGGAGATTTTTTAGT AATGAGATGGCTAAGGTGGATGATAGTTTTTTCATAGATTGGAGGAGAGTTTTTTGGTGGAGGAGATAAGAAGCATGAGAGACA TCCTATTTTTGGAAATATTGTGGATGAGGTGGCTTATCATGAGAAGTATCCTACAATTTATCATTTGAGAAAGAAGTTGGTGGATA GTACAGATAAGGCTGATTTGAGATTGATTTATTTGGCTTTGGCTCATATGATTAAGTTTAGAGGACATTTTTTGATTGAGGGAGAT TTGAATCCTGATAATAGTGATGTGGATAAGTTGTTTATTCAGTTGGTGCAGACATATAATCAGTTGTTTGAGGAGAATCCTATTAA TGCTAGTGGAGTGGATGCTAAGGCTATTTTGAGTCTAGATTGAGTAAGAGTAGAAGATTGGAGAATTTGATTGCTCAGTTGCCTG GAGAGAAGAAGAATGGATTGTTTGAAATTTGATTGCTTTGAGTTTGGGATTGACACCTAATTTTAAGAGTAATTTTGATTTGGCT

protein coding sequence)	GAGGATGCTAAGTTGCAGTTGAGTAAGGATACATATGATGATTTGGATAATATTGTTGGCTCAGATTGGAGATCAGTATGCTGA TTTGTTTTTGGCTGCTAAGAATTTGAGTGATGCTATTTTGTTGAGTGATATTTTGAGAGTAAATACAGAGATTACAAAGGCTCCTT TGAGTGCTAGTATGATTAAGAGATATGATGAGCATCATCAGGATTTGACATTGTTGAAGGCTTTGGTGATGAGACAGCAGTTGCCTGAG AAGTATAAGGAGATTTTTTTTGATCAGAGTAAGAATGGATATGCTGGATATATTGATGGAGGAGCTAGTCAGGAGGAGTTTTATAA GTTTATTAAGCCTATTTTTGGAGAAGATGGATGGAACAGAGGAGTTGTTGGTGAAGTTGAATAGAGAGGATTTGTTGAGAAAGCAGA GAACATTTGATAATGGAAGTATTCCTCATCAGATTCATTTGGGAGAGTTGCATGCTATTTTGAGAAGACAGGAGGATTTTTATCCT TTTTTGAAGGATAATAGAGAGAAGATTTGAGAAGATTTTGACATTTAGAATTCCTTATTATGTGGGACCTTTGGCTAGAGGAAATAG TAGATTTGCTTGGATGACAAGAAAGAGTGAGGAGACAATTACACCTTGGAATTTTGAGGAGGTGGTGGATAAGGAGCTAGTGCTC AGAGTTTTATTGAGAGAATGACAAATTTTGATAAGAATTTGCCTAAGCATAGTTTGTTGTATGAGTAT TTTACAGTGTATAATGAGTTGACAAAGGTGAAGTATGTGACAGAGGGAATGAGAAGCCTGCTTTTTTGAGTGGAGAGCAGAAGAA GGCTATTGTGGATTTGTTGTTTAAGACAAATAGAAAGGTGACAGTGAAGCAGTTGAAGGAGGATTATATTTTAAGAAGATTGAGTGTT TTGATAGTGTGGAGATTAGTGGAGTGGAGGATAGATTTAATGCTAGTTTGGGAACATATCATGATTTGTTGAAGATATTATTAAGGAT AAGGATTTTTTTGGATAAATGAGGAGAATATTTGTGTTGACATTGACATTGTTTGAGGATAGAGAGATGAT TGAGGAGAGATTGAAGACATATGCTCATTTGTTTGATGATAAGGTGATGAAGCAGTTGAAGAAGAAGATATACAGGATGGGGAA GATTGAGTAGAAAGTTGATTAATGGAATATAAGCAGAGTGGAAAGACAATTTTGGATTTTTTGAAGAGTGATGGATTTGCT AATAGAAATTTTATGCAGTTGATTCATGATAGTTTGACATTTAAGGAGGATATTCAGAAGGCTCAGGTGAGTGGACAGGGAGA TAGTTTGCATGAGCATATTGCTAATTTGGCTGGAAGTCCTGCTATTTAAGAAGGGAATTTTGCAGACAGTGAAGGTGGTGGATGAGT TGGTGAAGGTGATGGGAAGACATAAGCCTGAGAATATTGTGATTGAGATGGCTAGAGAGAATCAGACAACACACAGAAGGGACAGAAG AATAGTAGAGAGAGAATTGAGGAGGGAATTAAGGAGTTGGGAAGTCAGATTTTGAAGGAGCATCCTGTGGAGAATAC ACAGTTGCAGAATGAGAAGTTGTATTTGTATTATTTGCAGAATGGAAGAGATATGTATGTGGATCAGGAGTTGGATATTAATAGAT TGAGTGATTATGATGTGGATCATATTGTGCCTCAGATTTTTTGAAGGATGATAGTATTGATAATAAGGTGTTGACAAGAAGTGAT AAGAATAGAGGAAAGAGTGATAATGTGCCTAGTGAGGAGGTGGTGAAGAAGATGAAGAATTATTGGAGACAGTTGTTGAATGCTAA GTTGATTACACAGAGAAAGTTTGATAATTTGACAAAGGCTGAGAGAGGAGATTGAGTGAGTTGGATAAGGCTGGATTTATTAAGA GACAGTTGGTGGAGACAAGACAGATTACAAAGCATGTGGCTCAGATTTTGGATAGTAGAATGAATACAAGTATGATGAGAATGAT AAGTTGATTAGAGAGGTGAAGGTGATTACATTGAAGAGTAAGTTGGTGAGTGATTTTAGAAAGGATTTTCAGTTTTATAAGGTGAG AGAGATTAATAATTATCATCATGCTCATTATTTGAATGCTTGTGGTGGGAACAGCTTTGATTAAGAAGTATCCTAAGTTGG AGAGTGAGTTTGTGTATGGAGATTATAAGGTGTATGAGAAAGATGATTGCTAAGAGTGAGCAGAGGATTGGAAAGGCTACA GCTAAGTATTTTTTTTATAGTAATATTATGAATTTTTTTAAGACAGAGATTTACATTGGCTAATGGAGATTAGAAAGAGACCTTT GATTGAGACAAATGGAGAGACAGGAGATTGTGTGGGATAAGGGAAGAGATTTGCTACAGTGAGAAAGTATAGTTGTTGAGTATGCCTC AGGTGAATATTGTGAAGAAGACAGGAGGTGCAGACAGGAGGATTTAGTAAGGAGAGTATTTTGCCTAAGAGAAATAGTGATAAGTTG ATTGCTAGAAAGAAGGATTGGGATCCTAAGAAGTATGGAGGATTTGATAGTCCTACAGTGGCTTATAGTGTGTTGGTGGTGGCTAA GGTGGAGAAGGGAAAGAGTAAGAAGTTGAAGAGTGTGTTGGGAATTACAATTATGGAGAGAAGTAGTTTTGAGAAGA ATCCTATTGATTTTTTGGAGGCTAAGGATATAAGGAGGTGAAGAAGGATTTGATTATTAAGTTGCCTAAGTATAGTTGTTGAG TTGGAGAATGGAAGAAAGAGAATGTTGGCTAGTGCTGGAGAGTTGCAGAAGGGAAATGAGTTGGCTTTGCCTAGTAAGTATGTGAA TTTTTTGTATTTGGCTAGTCATTATGAGAAGTTGAAGGGAAGTCCTGAGGATAATGAGCAGAAGCAGTTGTTTGTGGAGCAGCATA AGCATTATTTGGATGAGATTATTGAGCAGATTAGTGAGTTTAGTAAGAGAGTGATTTTGGCTGATGCTAATTTGGATAAGGTGTTG AGTGCTTATAATAAGCATAGAGATAAGCCTATTAGAGAGCAGGCTGAGAAATATTATTCATTTGTTTACATTGACAAATTTGGGAGC TCCTGCTGCTTTTAAGTATTTTGATACAACAATTGATAGAAAGAGATATACAAGTACAAAGGAGGTGTTGGATGCTACATTGATTC ATCAGAGTATTACAGGATTGTATGAGACAAGAATTGATTTGAGTCAGTTGGGAGGAGATGGAGGAGGAAGTCCTAAGAAGAAGAGA

	AAGGTG	
Cas9 ORF using low A codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACT CCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTC CCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCC AGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGTACACCGGCTGGGGCC GGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGA CTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGAC AAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGC	346

GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG AGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTG ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAA CTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG TCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGG AAGGTG	347

	CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCC	348
	GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGCC	
	AACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA	
	CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC	
	TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG	
	AACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC	
	CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC	
	TGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGAC	
	AAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA	
	GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGC	
	GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGAC	
	AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG	
	GGAGATCAACAACTACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGG	
	AGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACC	
	GCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT	
	GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC	
	AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTG	
	ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAA	
	GGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGA	
	ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAG	
	CTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAA	
	CTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA	
	AGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG	
	AGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC	
	CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCC	
	ACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAGCGG	
	AAGGTG	
Cas9 ORF using low A codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion	GACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAA	
	GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCG	
	CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC	
	AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA	
	CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACT	
	CCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC	
	CTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA	
	CGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCG	
	GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCC	
	GAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA	
	CCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC	
	TGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCTGCCCGAG	

protein coding sequence)	
	AAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAA
	GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC
	GGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC
	TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTC
	CCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCC
	AGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTAC
	TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAA
	GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT
	TCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC
	AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT
	CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCCGGTACACCGGCTGGGGCC
	GGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCC
	AACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGA
	CTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC
	TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG
	AACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC
	CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGGCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC
	TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGAC
	AAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA
	GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGC
	GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGAC
	AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG
	GGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG
	AGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACC
	GCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT
	GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGAAGGGTGCTGTCCATGCCCC
	AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGATCCTGCCCAAGCGGAACTCCGACAAGCTG
	ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAA
	GGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTCCTTCGAGAAGA
	ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAG
	CTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAA
	CTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA
	AGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG
	TCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC
	CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCC
	ACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGG
	AAGGTGGACGGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGC

Cas9 nickase ORF using low A codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACT CCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGAGCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGTCCGCCTCCATGATCAAGCGCTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCGTCGTGCGCCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGATTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTC CCGGTTCGCCTGGATGACCGCGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCC AGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCCGGTACACCGGCTGGGGCC GGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGA CTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGAC AAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTCCGAAGGAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG AGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTG	349

	ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAA CTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG TCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCC CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGG AAGGTG	350
Cas9 nickase ORF using low A codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGATCTGCTACCTGCAGGAGATCTTCTCC AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACT CCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGAGGGCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTC CCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCC AGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGATGATT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGGGCC GGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGA CTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC	

TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGAC AAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACGACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG AGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGATCCTGCCCAAGCGGAACTCCGACAAGCTG ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAAGCGAGCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAA CTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG TCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGAC	351
Cas9 nickase ORF using low A codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACT CCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAA CGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC AGTCCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC GGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTC CCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCC AGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT	

TCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC		
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CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCC		
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AACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGA		
CTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC		
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AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG		
GGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG		
AGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACC		
GCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT		
GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCC		
AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGCGGAACTCCGACAAGCTG		
ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAA		
GGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTTCGAGAAGA		
ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAG		
CTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAA		
CTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA		
AGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG		
TCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC		
CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCC		
ACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGG		
AAGGTGGACGGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGC		
dCas9 ORF using low A codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACT CCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGCCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA	352

coding sequence)	CCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGTCCGCCTCCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACTC CCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCC AGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGGGCC GGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGATTCCTGAAGTCCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGA CTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGAC AAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGG AGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGATCCTGCCCAAGCGGAACTCCGACAAGCTG ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAA CTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG TCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCGGCGGCTCCCCCAAGAAGAAGCGG AAGGTG

dCas9 ORF using low A codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGAAGAAGCTGGTGGACT CCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGGGGCGCCTCCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGGCCCGGGGCAACTC CCGGTTCGCCTGGATGACCGCGAGGAGTCCGAAGTCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCC AGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCCGGTACACCGGCTGGGGCC GGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGA CTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACCACATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGAC AAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTCCGAAGGAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG AGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCATCCTGCCCAAGAGCGGAACTCCGACAAGCTG	353

	ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAA CTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG TCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCC CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGAC	354
dCas9 ORF using low A codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACTCCATCGGCCTGGCCATCGGCACCAACTCCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACTCCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCTCC AACGAGATGGCCAAGGTGGACGACTCCTTCTTCCACCGGCTGGAGGAGTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACT CCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACTCCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCTCCGGCGTGGACGCCAAGGCCATCCTGTCCGCCCGGCTGTCCAAGTCCCGGCGCCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGTCCCTGGGCCTGACCCCCAACTTCAAGTCCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGTCCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGTCCGCCTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCTCCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGGCCCCGGGGCAACTC CCGGTTCGCCTGGATGACCCGGAAGTCCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCC AGTCCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACTCCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGTCCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAACGCCTCCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCCGGTACACCGGCTGGGGCC GGCTGTCCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACTCCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGTCCGGCCAGGGCGA CTCCCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACTCCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCTCCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGTCCGACTACGACGTGGACGCCATCGTGCCCCAGTCCTTCCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCCGGTCCGAC	

AAGAACCGGGGCAAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGGCCGGCTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGTCCAAGCTGGTGTCCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG AGTCCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGTCCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGATCCTGCCCAAGCGGAACTCCGACAAGCTG ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACTCCCCCACCGTGGCCTACTCCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGTCCAAGAAGCTGAAGTCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGTCCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCTCCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCTCCAAGTACGTGAA CTTCCTGTACCTGGCCTCCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG TCCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGTCCATCACCGGCCTGTACGAGACCCGGATCGACCTGTCCCAGCTGGGCGGCGACGGCTCCGGCTCCCCCAAGAAGAAGCGG AAGGTGGACGGCTCCCCCAAGAAGAAGCGGAAGGTGGACTCCGGC	GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGC AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACA GCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAA CGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAG GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT	355
Cas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)		

		356
Cas9 ORF using low A/U codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion	GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGC AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACA GCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA	

protein coding sequence)	CCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC
	TGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG
	AAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAA
	GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC
	GGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC
	TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAG
	CCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCC
	AGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTAC
	TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAA
	GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT
	TCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC
	AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT
	CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGGGCC
	GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGACCGGCAAGACCATCCTGGATTCCTGAAGAGCGACGGCTTCGCC
	AACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA
	CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC
	TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG
	AACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC
	CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC
	TGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGAC
	AAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA
	GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGC
	GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGAC
	AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG
	GGAGATCAACAACTACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGG
	AGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACC
	GCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT
	GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC
	AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTG
	ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAA
	GGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGA
	ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAG
	CTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAA
	CTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA
	AGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG
	AGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC
	CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCC
	ACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGAC

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Cas9 nickase ORF using low A/U codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGC AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACA GCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGCCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCGTCGTGCGGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAG CCGGTTCGCCTGGATGACCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCC AGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCCGGTACACCGGCTGGGGCC GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGATTCCCTGAAGAGCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGAC AAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG AGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTG	

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Cas9 nickase ORF using low A/U codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGATCTGCTACCTGCAGGAGATCTTCAGC AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACA GCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGAGGGCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCC TGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAG CCGGTTCGCCTGGATGACCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCC AGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGGGCC GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC

TGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGAC AAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACGACGCCCACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG AGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTG ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAA CTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG AGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCAAGAAGAAGCCCGCCACCAAG AAGGTGGACGGCGCAGCCCCAAGAAGAAGCGGAAGGTGGACAGCGGC	359

GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT		
TCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC		
AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT		
CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCC		
GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGCC		
AACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA		
CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC		
TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG		
AACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC		
CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC		
TGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGAC		
AAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA		
GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGC		
GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGAC		
AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG		
GGAGATCAACAACTACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGG		
AGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACC		
GCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT		
GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC		
AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTG		
ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAA		
GGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGA		
ACCCCATCGACTTCCTGGAGGCTAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAG		
CTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAA		
CTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA		
AGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG		
AGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC		
CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCC		
ACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGAC		360
dCas9 ORF using low A/U codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein	GACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAA	
	GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCG	
	CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGC	
	AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA	
	CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACA	
	GCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC	
	CTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA	
	CGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGCCTGGAGAACCTGATCGCCCAGCTGCCCG	
	GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCC	
	GAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA	

coding sequence)	CCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAG CCGGTTCGCCTGGATGACCCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCC AGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGCTACACCGGCTGGGGCC GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGAGCGACTACGACGTGGACgcCATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGAC AAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGATAAGGCCGGTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGG AGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACC GCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTG ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAA CTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG AGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGC CCCCGCCGCCTTCAAGTACTTCGACACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAGCGG AAGGTG

dCas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGC AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACA GCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGCCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCC TGAGCGCCAGCATGATCAAGCGCTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAG CCGGTTCGCCTGGATGACCGCGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCC AGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGAT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTGGGGCC GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGATTCCCTGAAGAGCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC TGAGCGACTACGACGTGGACCACATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGGAGCGAC AAGAACCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAA GCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGC GGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACGAC AAGCTGATCCGGGAGGTGAAGGTGATCACCCTGAAGAGCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCG GGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGG AGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCAC GCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAACGGCGAGATCCGGAAGCGGCCCCT GATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGCAAGGTGCTGAGCATGCCCC AGGTGAACATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTG	361

	ATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAA GGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGAGCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGA ACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAG CTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCCAGCAAGTACGTGAA CTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA AGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG AGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCC CCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCC ACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACGGCAAGCCCAAGAAGAAGCGG AAGGTGGACGGCGCAGCCCCCAAGAAGAAGCGGAAGGTGGACAGCGGC	362
dCas9 ORF using low A/U codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGCCTGGcCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAA GAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCG CCGAGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGC AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCA CCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAAGTACCCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACA GCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGAGGGCACTTCCTGATCGAGGGCGAC CTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAA CGCCAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCG GCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCC GAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGCCCCCC TGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAG AAGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTACAA GTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGC GGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCC TTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAG CCGGTTCGCCTGGATGACCGGAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCC AGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTAC TTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAA GGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCT TCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGTTCGAGGACCGGGAGATGATT CGAGGAGCGGCTGAAGACCTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCGGCTACACCGGCTGGGGCC GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAGAGCGACGGCTTCGCC AACCGGAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGC TGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAG AACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACAC CCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGC	

	363
Nme Cas9 ORF using low A codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GCCGCTTCAAGCCCAACTCCATCACTACATCCTGGGCCTGGACATCGGCATCGCCTCCGTGGGCTGGGCCATGGTGGAGATCGA CGAGGAGGAGAACCCCATCCGGCTGATCGACCTGGGCGTGCGGGTGTTCGAGCGGGCCGAGGTGCCCAAGACCGGCGACTCCCTGG CCATGGCCCGGCGGCTGGCCCGGTCCGTGCGCGGCTGACCCGGCGGGCCCACCGGCTGCTGCGCTGCTGCTGCTGAAG CGGGAGGGCGTGCTGCAGGCCGCCAACTTCGACGAGAACGGCCTGATCAAGTCCCTGCCCAACACCCCCTGGCAGCTGCGGCGGCCGC CGCCCTGGACCGGAAGCTGACCCCCCTGGAGTGGTCCGCCGTGCTGCTGCACCTGATCAAGCACCGGGGCTACCTGTCCCAGCGGA AGAACGAGGGCGAGACCCGCCGACACAAGGAGCTGGGCGCCCTGCTGAAGGGCGTGGCCGGCAACGCCCACGCCCTGCAGACCGGCGAC TTCCGGACCCCCGCCGAGCTGGCCCTGAACAAGTTCGAGAAGGAGTCCGGCCACATCCGGAACCAGCGTCCGAGCACTACTCCCACAC CTTCTCCCGGAAGGACCTGCAGGCCGAGCTGATCCTGTTCGAGAAGCAGAAGGAGTTCGGCAAGGATGCTGGCCACTGCACC TGAAGGAGGGCATCGAGACCCTGCTGATGACCCAGCGGCCCGCCCTGTCCGGCGACGCCGTGCAGAAGATGCTGGGCCACTGCACC TTCGAGCCCGCCGAGGCCCAAGAACACCTACACCGCCGAGCGGTTCATCTGGCTGACCAAGCTGAACAACCTGCGGAT CCTGGAGCAGGGCTCCGAGCGGCCCCTGACCGACACCGAGCGGGCCACCCTGATGGACGAGCCCTACCGAAGTCCAAGCTGACCT ACGCCCAGGCCCGGAAGCTGCTGGGCCTGGAGGACACCGCCTTCTTCAAGGGCCTGCGGTACGGCAAGGACAACGCCGAGGCCTCC ACCCTGATGGAGATGAAGGCCTACCACGCCATCTCCCGGGCCCTGAAGGAGGAGGCCTGAAGGACAAGAAGTCCCCCCTGAACCT GAAGATCTACCTGCCCCCCATCCCCGCCGACGAGATCCGGAACCCCGTGGTGCTGCGGGCCCTGTCCCAGGCCCGGAAGGTGATCA ACGGCGTGGTGCGGCGGTACGGCTCCCCCGCCCGGATCCACATCGAGACCGCCCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCGTGGG GAGATCGAGAAGCGGCAGGAGGAGAACCGGAAGGACCGGGAGAAGGCCGCCGCCAAGTTCCGGGAGTACTTCCCCAACTTCGTGGG CGAGCCCAAGTCCAAGGACATCCTGAAGCTGCGGCTGTACGAGCAGCACGGCAAGTGCCTGTACTCCGGCAAGGAGATCAACC

	TGGGCCGGCTGAACGAGAAGGGCTACGTGGAGATCGACCACGCCCTGCCTTCTCCCGGACCTGGGACGACTCCTTCAACAACAAG GTGCTGGTGCTGGGCTCCGAGAACCAGAACAAGGGCAACCAGACCCCCTACGAGTACTTCAACGGCAAGGACAACTCCCGGGAGTG GCAGGAGTTCAAGGCCCGGGTGGAGACCTCCCGGTTCCCCAAGAAGCAGCGGATCCTGCTGCAGAAGTTCGACGAGGACG GCTTCAAGGAGCGGAACCTGAACGACACCCGGTACGTGAACCGGTTCCTGTGCCAGTTCGTGGCCGACCGGATGCGGCTGACCGGC AAGGGCAAGAAGCGGGTGTTCGCCTCCAACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGGCCTGCGGAAGGTGCGGGCCGA GAACGACCGGCACCACGCCCTGGACGCCGTGGTGGTGGCCATGCAGCAGAAGATCACCCGGTTCGTGCGGT ACAAGGAGATGAACGCCTTCGACGGCAAGACCATCGACAAGGAGACCGGCGAGGTGCTGCACCAGAAGACCCACTTCCCCCAGCCC TGGGAGTTCTTCGCCCAGGAGGTGATGATCCGGGTGTTCGGCAAGCCCGAGTTCGAGGAGGCCGACACCCTGGA GAAGCTGCGGAACCCTGCTGGCCGAGAAGCTGTCCTCCCGGCCCGAGGCCGTGCACGAGTACGTGACCCCCCTGTTCGTGTCCCGGG CCCCCAACCGGAAGATGTCCGGCCACGGCCACATGGAGACCGTGAAGTCCGCCAAGCGGCTGGACGAGGGCGTGTCCGTGCTGCGG GTGCCCCTGACCCAGCTGAAGCTGAAGGACTTGGAGAAGATGGTGAACCGGGAGCGGGAGCCCAAGCTGTACGAGGCCCTGAAGGC CCGGCTGGAGGCCCACAAGGACGACCCCGCCAAGGCCTTCGCCGAGCCTTCTACAAGTACGACAAGGCCGGCAACCGGACCCAGC AGGTGAAGGCCGTGCGGGTGGAGCAGGTGCAGAAGACCGGCGTGTGGTGCGGAACCACACAACGGCATCGCCGACAACGCCACCATG GTGCGGGTGGACGTGTTCGAGAAGGGCGACAAGTACTACCTGGTGCCCATCTACTCCTGGCAGGTGGCCAAGGGCATCCTGCCCGA CCGGGCCGTGGTGCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACTCCTTCAACTTCAAGTTCTCCCTGCACCCCAACG ACCTGGTGGAGGTGATCACCAAGAAGGCCCGGATGTTCGGCTACTTCGCCTCCTGCCACCGGGGCACCGGCAACATCAACATCCGG ATCCACGACCTGGACCACAAGATCGGCAAGAACGGCATCCTGGAGGGCATCGGCGCGTGAAGACCGCCCTGTCCTTCCAGAAGTACCA GATCGACGAGCTGGGCAAGGAGATCCGGCCCTGCCGGCTGAAGAAGCGGCCCCCCGGTGCGGTCCGGCAAGCGGACCGCCGACGGCCT CCGAGTTCGAGTCCCCCAAGAAGAAGCGGAAGGTGGAG	364

	TGGGCCGGCTGAACGAGAAAGGGCTACGTGGAGATCGACCACGCCCTGCCTTCAGCCGGACCTGGGACGACAGCTTCAACAACAAG GTGCTGGTGCTGGGCAGCCGAGAACCAGAACAAGGGCAACCAGACCCCCTACGAGTACTTCAACGGCAAGGACAACACAGCCGGGAGTG GCAGGAGTTCAAGGCCCGGGTGGAGACCAGCCGGTTCCCCCGGAGCAAGAAGCAGCGGATCCTGCTGCAGAAGTTCGACGAGGACG GCTTCAAGGAGCGGAACCTGAACGACACCCGGTACGTGAACCGGTTCCTGTGCCAGTTCGTGGCCGACCGGATGCGGCTGACCGGC AAGGGCAAGAAGCGGGTGTTCGCCAGCAACGGCCAGATCACCAACCTGCTGCGGGGCTTCTGGGGCCTGCGGAAGGTGCGGGCCGA GAACGACCGGCACCACGCCCTGGACGCCGTGGTGGTGGCCATGCAGCAGAAGATCACCCGGTTCGTGCGGT ACAAGGAGATGAACGCCTTCGACGGCAAGACCGACGAGGTGCTGCACCAGAAGACCCACTTCCCCCAGCCC TGGGAGTTCTTCGCCCAGGAGGTGATGATCCGGGTGTTCGGCAAGCCCGAGTTCGAGGAGGCCGACACCCTGGA GAAGCTGCGGAACCCTGCTGGCCGAGAAGCTGAGCAGCCGTGCACGAGTACGTGACCCCCCTGTTCGTGAGCCGGG CCCCCAACCGGAAGATGAGCGGCCACATGGAGACCGTGAAGAGCGCCAAGCGGCTGGACGAGGGCGTGAGCGTGCTGCGG GTGCCCCTGACCCAGCTGAAGCTGGAGGAAGATGGTGAACCGGGAGCGGGAGCCCAAGCTGTACGAGGCCCTGAAGGC CCGGCTGGAGGCCCACAAGGACGACCCCGCCAAGGCCTTCGCCGAGCCTTCTACAAGTACGACAAGGCCGGCAACCGGACCCAGC AGGTGAAGGCCGTGCGGGTGGAGCAGGTGCAGAAGACCGGCGTGTGGTGCGGAACCACAACGGCATCGCCGACAACGCCACCATG GTGCGGGTGGACGTGTTCGAGAAGGGCGACAAGTACTACCTGGTGCCCATCTACAGCTGGCAAGGGCATCCTGCCCGA CCGGGCCGTGGTGCAGGGCAAGGACGAGGAGGACTGGCAGCTGATCGACGACAGCTTCAACTTCAAGTTCAGCCTGCACCCCCAACG ACCTGGTGGAGGTGATCACCAAGAAGGCCCGGATGTTCGGCTACTTCGCCAGCTGCCACCGGGGCACCGGCAACATCAACATCCGG ATCCACGACCTGGACCACAAGATCGGCAAGAACGGCATCCTGGAGGGCATCGGCGTGAAGACCGCCCTGAGCTTCCAGAAGTACCA GATCGACGAGCTGGGCAAGGAGATCCGGCCCTGCCGGCTGAAGAAGCGGCCCCCCGTGCGGAGCGGCAAGCGGACCGCCGACGGCA GCGAGTTCGAGAGCCCCAAGAAGAAGCGGAAGGTGGAG	365
Open reading frame for Cas9 with NLS1 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGACTGGACATCGGCACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGG GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATTGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGC TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA GAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT	

	366
Open reading frame for Cas9 with NLS2 (no start or stop codons; suitable for inclusion in fusion protein	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAAGAACAGCAAGAAGAAAGAAGAACACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGG GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATTGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA	

coding sequence)	CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAGGCACCGC
	TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA
	AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA
	GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA
	GAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG
	TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG
	CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC
	AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC
	TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA
	GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT
	TCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCCAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC
	AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGAT
	CGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAA
	GACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGATTCCTGAAGAGCGACGGATTCGCA
	AACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA
	CAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAAC
	TGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG
	AACAGCAGAGAGAAGAATGAAGAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACAC
	ACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGAC
	TGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGAC
	AAGAACAGAGGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAA
	GCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGGAGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA
	GACAGCTGGTCGAACACAAGACACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGAC
	AAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG
	AGAAATCAACAACTACCACGCACACGACGCATACCTGAACGCAGTCGTTGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG
	AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACA
	GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT
	GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC
	AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTG
	ATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCTACACAGCGTCCTGGTCGTCGCAAA
	GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGA
	ACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAA
	CTGGAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA
	CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACA
	AGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTG
	AGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGC
	ACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCC
	ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCAGGCAGCAAAGAGA
	AGCAGAACAACA

		367
Open reading frame for Cas9 with NLS3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAAGAAGATACACAAGAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCCACTCATGAGCTGAAGAAGGGCAACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGG GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGC TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA GAACATTCGACAACGGAAGCATCCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT TCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGAT CGAAGAAAGACTGAAGACATACGCCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAA GACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGCAAAGACAATCCTGGATCCTTCCTGAAGAGCGACGGATTCGCA AACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA CAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAAC TGGTCAAGGTCATGGGAAGACACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG AACAGCAGAGAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACAC ACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGAC TGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGAC AAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAA GCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA GACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGAC AAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG AGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACA GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTG	

	ATCGCAAGAAAGAAGGACTGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGA ACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAA CTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAAGCTGAAGGGAAGCCCGGAAGACAACGAAGCAGCTGTTCGTCGAACAGCACA AGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTG AGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGC ACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCCACAAAGGCAGATCCTGGACGCAACACTGATCC ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGGCACCGGCAAAG AGAGAAAGAACAACA	368
Open reading frame for Cas9 with NLS4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACGCACATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGACTGAGAAGCAGAAGACTGGAAAACCTGATCGCCCTGCCGGG GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATTGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGC TGAGCGCCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGATTCTACAA GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA GAACATTCGACAACGGAAGCATCCCCCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGATCCCGTACTACGTCGGACCGCTGGCAAGGGAGCAAGCGCAC CAGATTCGCATGGATGACAGAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT TCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAACGCCTG AAAACATACGCTCACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGCTGAGCAGAAAG CTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCCAACAGAAACTTC ATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGATCTCTGCACGAA CATATCGCCAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAAGTG ATGGGACGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAACAGACCACTACCAAGGGACAGAAGAACAGCCGCGAG AGAATGAAGCGGATCGAAGAGGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACTCAGCTG CAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTG AGCGACTACGACGTCGATCACATCGTCCCACAGAGCTTTCCTGAAGGACGACTCCATCGACAACAAGGTCCTGACCAGAAGC GACAAGAACAGAGGCAAGAGCGACAACGTCCCCTCCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGCGGCAGCTGCTG AACGCCAAGCTGATCACCCAGAGAAAGTTCGACAACCTGACCAAGGCCGAGAGAGGCGGACTGAGCGAACTGGACAAGGCC GGCTTCATCAAGAGACAGCTGGTCGAGACCAGACAGATCACAAAGCACGTCGCCCAGATCCTGGACTCCCGGATGAACACC AAGTACGACGAGAACGACAAGCTGATCCGGGAAGTCAAGGTCATCACCCTGAAGTCCAAGCTGGTCTCCGACTTCCGGAAG GACTTCCAGTTCTACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTCGGAACC GCCCTGATCAAAAAGTACCCCAAGCTGGAAAGCGAGTTCGTCTACGGCGACTACAAGGTCTACGACGTCCGCAAGATGATC GCCAAGAGCGAGCAGGAAATCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAG ATCACCCTGGCCAACGGCGAGATCCGCAAGCGCCCCCTGATCGAGACCAACGGCGAAACCGGGGAGATCGTGTGGGACAAG GGCCGCGACTTCGCCACCGTGCGCAAGGTCCTGTCCATGCCCCAAGTCAACATCGTGAAAAAGACCGAGGTGCAGACCGGC GGCTTCAGCAAAGAGTCCATCCTGCCCAAGAGGAACAGCGACAAGCTGATCGCCAGAAAGAAGGACTGGGACCCCAAGAAG TACGGCGGCTTCGACAGCCCCACCGTGGCCTACTCCGTGCTGGTCGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTG AAGAGCGTGAAAGAGCTGCTGGGCATCACCATCATGGAACGCAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAAGCC AAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCCAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAG AGAATGCTGGCCAGCGCCGGCGAACTGCAGAAGGGCAACGAACTGGCCCTGCCCAGCAAATACGTGAACTTCCTGTACCTG GCCAGCCACTACGAGAAGCTGAAGGGCTCCCCCGAGGACAACGAACAGAAACAGCTGTTCGTGGAACAGCACAAGCACTAC CTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCCAATCTGGACAAGGTGCTGTCC GCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGA GCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGATACACCAGCACCAAAGAGGTGCTGGACGCCACC CTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAGGCGACAAAAGACCGGCCGCC ACCAAGAAAGCCGGACAGGCCAAAAAGAAGAAA	

TGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGAC AAGAACAGAGGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAA GCTGATCACACAGAAGTTCGACAACCTGACAAAGGCAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA GACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGAC AAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG AGAAATCAACAACTACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAAGGATGATCGCAAAGAGAGCGAACAGGAAATCGGAAAGGCAACA GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAAGAAAGGTCCTGAGCATGCCGC AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTG ATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA GGTCGAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGA ACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAA CTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACA AGCACTACCTGGACGAAATCATCGAGCAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTG AGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGC ACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCC ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGACGGAGGAGGAAGCCAGGCAGCAAAGAGA CCGAGAACAACA	369
Open reading frame for Cas9 with NLS5 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAA GAAGTTCAAGGTCCTGGGAAACACAGACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGATACACAAGAAGAAACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCCAGACCTGATCTACCTGGCCCTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAGGGCGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGG GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGC TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACACAGCAGCTGCCGGAA AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA GAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA

Open reading frame for Cas9 with NLS6 (no start or stop codons; suitable for inclusion in fusion	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCACAAGACTGAAGAGAAGCAGAAGATACACAAGAAGAAAGAACACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGG GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATTGCTCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA	370

protein coding sequence)	GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA
	CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACACAGAAATCACACAAGGCACCGC
	TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA
	AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA
	GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA
	GAACATTCGACAACGGAAGCATCCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG
	TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG
	CAGATTCGCATGGATGACAAGAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC
	AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC
	TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA
	GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT
	TCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCCAAGCCTGGGACACATCCACGACCTGCTGAAGATCATCAAGGAC
	AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGAT
	CGAAGAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAA
	GACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGATTCCTGAAGAGCGACGGATTCGCA
	AACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA
	CAGCCTGCACGAACACATCGCAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAAC
	TGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAATGGCAAGAAAACCAGACACAACACAGAAGGGACAGAAG
	AACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACAC
	ACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGAC
	TGAGCGACTACGACGTCGACCACATCGTCCCCCAGAGCTTCCTGAAGGACGACATCGACAACAAGGTCCTGACAAGAAGCGAC
	AAGAACAGAGGAAAGAGCGACAACGTCCCCAGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAA
	GCTGATCACACAGAAGTTCGACAACCTGACAAAGGCAGAGAGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA
	GACAGCTGGTCGAAACAAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGAC
	AAGCTGATCAGAGAAGTCAAGGTCACCCTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG
	AGAAATCAACAACTACCACGCCCACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG
	AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACA
	GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT
	GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC
	AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAATCCTGCCGAAGAGAAACAGCGACAAGCTG
	ATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA
	GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGA
	ACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAA
	CTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAACGAACTGGCACTGCCGAGCAAGTACGTCAA
	CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACA
	AGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTG
	AGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGC
	ACCGGCAGCATTTCAAGTACTTCGACACAACAATCGACAAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCC
	ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCGCAGCAGCAAAGAGA

Open reading frame for Cas9 with NLS7 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	AGCTGGAGCATGGCAGCA	371
	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA	
	GAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG	
	CAGAAGCAACAAGACTGAAGAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGC	
	AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA	
	CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACA	
	GCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC	
	CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA	
	CGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCAGCTGCCGG	
	GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA	
	GAAGACGCCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCCAGATCGGAGACCAGTACGCAGA	
	CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGC	
	TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA	
	AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA	
	GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA	
	GAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG	
	TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG	
	CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC	
	AGAGCTTCATCGAAAGAATGACCAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC	
	TTCACAGTCTACAACGAACTGACCAAAGTCAAATACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGCGAACAGAAGAA	
	GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGATCTACTTCAAGAAGATCGAATGCT	
	TCGACAGCGTCGAAATCAGCGGAGTCGAAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC	
	AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGAT	
	CGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAA	
	GACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCA	
	AACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA	
	CAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAAC	
	TGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAACCAGACAACACAGAAGGGACAGAAG	
	AACAGCGAGAGAACGAAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA	
	ACAGCTGCAGAAACGAAAAGCTGTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACGAC	
	GACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGAC	
	AAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG	
	AGAAATCAACAACTACCACGCCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG	
	AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCCAAAGAGCGAACAGGAAATCGGAAAGGCAACA	
	GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT	
	GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC	

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Open reading frame for Cas9 with NLS8 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACGACATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAAGCAGAAGAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCCAGACCTGATCTACCTGGCCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGACTGGAAAACCTGATCGCACAGCTGCCGG GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACACAGAAATCACACAAGGCACCGC TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGGTCAGACACAGCAGCTGCCGGAA AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA GAACATTCGACAACGGAAGCATCCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGATGACTTCTACCCG TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT TCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGAT CGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAA GACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCA AACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA CAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAAC TGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG AACAGCAGAGAAGAAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACAC

ACAGCTGCAGAACGAAAAGCTGTACCTGTACCTGCAGAACGGAAGACATGTACGTCGACCAGGAACTGGACATCAACAGAC TGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGAC AAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAA GCTGATCACACAGAAGTTCGACAACCTGACAAAGGCAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA GACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGAC AAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG AGAAATCAACAACTACCACCACGCACACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACA GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTG ATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGA ACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAA CTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCGAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCCGGAAGACAACGAAGCAGCTGTTCGTCGAACAGCACA AGCACTACCTGGACGAAATCATCGAGCAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTG AGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGC ACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACGAGCACAAAGGAAGTCCTGGACGCAACACTGATCC ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGACGGAGGAGGAAGCGCAGCAGCAAAAGAGA AGCTGGAGCATGGCATTC	GACAAGAAGTACAGCATCGGACTGGACATCGGAAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCAGCTGCCGG GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGC TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA GAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC	373

TTCACAGTCTACAACGAACTGACAAAGGTCACAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT TCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGAT CGAAGAAAGACTGAAGACATACGCCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAA GACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGACGGCAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCA AACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA CAGCCTGCACGAACACATCGCAAACCTGGCAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAAC TGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG AACAGCAGAGAAAGAATGAAGAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACAC ACAGCTGCAGAACGAAAAGCTGTACCTGCAGAACGGAAGACATGTACGTCGACCAGGAACTGGACATCAACAGAC TGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAACAAGAGC AAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAA GCTGATCACACAGAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA GACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCGAATGAACACAAAGTACGACGAAAACGAC AAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG AGAAATCAACAACTACCACGCCCACGACGCATACCTGAACGCCGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCCAAAGAGCGAACAGGAAATCGGAAAGGCAACA GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTG ATCGCAAGAAAGAAGGATGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCTAAA GGTCGAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGA ACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAA CTGGAAAACGGACGTAAGAGAAAGATGCTGGCAAGCGCAGGAAGAACTGCAGAAGGGAAACGATGTCCTGGACGCAACACTGATCC CTTCCTGTACCTGGCAAGCCACTACGAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACA AGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTG AGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGC ACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCC ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGGACGGAAGGCGCAGCAGCAAAGAGA AAGTACTTCGCAGCA	374
Open reading frame for Cas9 with NLS10 (no start or stop codons; suitable for inclusion in	GACAAGAAGTACAGCATCGGACTGGACATCGGAACACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACAGCACATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAAGAACAGCAAGAAGAAAGAACACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACATGAATTCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGG	

fusion protein coding sequence)	GAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGA CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGC TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAA GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA GAACATTCGACAACGGAAGCATCCCCCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG CAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAAGACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT TCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAGGAACGC CGAAGAAAGACTGAAGACATACGCACACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACACGAGGATGGGGAA GACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGATTCCTGAAGAGCGACGGATTCGCA AACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA CAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAAC TGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAATGGCAAGAAAACCAGACACACACAGAGGGACAGAAG AACAGCAGAGAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACAC ACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGAC TGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGAC AAGAACAGAGGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAA GCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA GACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGATGTACGACGAAAACGAC AAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG AGAAATCAACAACTACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACA GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT GATCGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC AGGTCAACATCGTCAAGAAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTG ATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAGA ACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAA CTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAA CTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACA AGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTG AGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGC ACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCC

Open reading frame for Cas9 with NLS11 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	ACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCAGAGCAGCAAAGAGA AAGGCATTCGCAGCA GACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAA GAAGTTCAAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAG CAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAAGATACACAAGAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGC AACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACA CCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACACAATCTACCACCTGAGAAAGAAGCTGGTCGACA GCACAGACAAGGCAGACCTGAGACTATCTACCTGGCCACTACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGAC CTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAA CGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCAGCTGCCGG GAGAAAGAAGAACGGACTGTTCGGAAACCTGATTGCCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGAGACCAGTACGCAGA CCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGC TGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAA AAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGATTCTACAA GTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGA GAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCG TTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAG CAGATTCGCATGGATGACAAGAAAGAGCGAAGAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC AGAGCTTCATCGAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATAC TTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAA GGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCT TCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGAC AAGGACTTCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGAT CGAAGAAAGACTGAAGACATACGCCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAA GACTGAGCAGAAAGCTGATCAACGGCATTAGAGACAAGCAGTCCGGCAAAACAATCCTGGATTCCTTCAAGAGCGACGGATTCGCA AACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGA CAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAAC TGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAG AACAGCAGAGAAGAAATGAAGAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACAC ACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGAC TGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGAC AAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAA GCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGGAGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGA GACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGAC AAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAG AGAAATCAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGG AAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACA GCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCT	375

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mRNA transcript with XBG UTRs and Cas9 ORF with low U 1 codons of Table 4	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACAAGAAGUACAGCAUCGGCCUGGACAUCGGCACCAAC AGCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCCAGCAAGAAGUUCAAGGUGCUGGGCAACACCGACAGACACAGCAU CAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACAGCGGCGAGACCGCCGAGGCCACCAGACUGAAGAGAACCGCCAGAAGAUAU ACACCAGAAGAAAGAACACAGAAUCUGCUACCUGCAGGAGAUCUUCAGCAACGAGAUGGCCAAGGUGGACGACAGCUUCUUCCACAGA CUGGAGGAGAGCUUCCUGGUGGAGGAGGACAAGAAGCACGAGAGACACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCA CGAGAAGUACCCCACCAUCUACCACCUGAGAAAGAAGCUGGUGGACAGCACCGACAAGGCCGACCUGAGACUGAUCUACCUGGCCCC UGGCCCACAUGAUCAAGUUCAGAGGCCACUUCCUGAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUCAUC CAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGCGUGGACGCCAAGGCCAUCCUGAGCGCCAG ACUGAGCAAGAGCAGAAGACUGGAGAACCUGAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCC UGAGCCUGGGCCUGACCCCCAACUUCAAGAGCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGAGCAAGGACACCUACGAC GACGACCUGGACAACCUGCUGGCCCAGAUCGGCGACCAGUACGCCGACCUGUUUCCUGGCCGCCAAGAACCUGAGCGACGCCAUCCU GCUGAGCGACAUCCUGAGAGUGAACACCGAGAUCACCAAGGCCCCCCUGAGCGCCAGCAUGAUCAAGAGAUACGACGAGCACCACC AGGACCUGACCCUGCUGAAGGCCGUGGUGAGACAGCAGCUGCCCGAGAAGUACAAGGAGAUCUUCUUCGACCAGAGCAAGAACGGC UACGCCGGCUACAUCGACGGCGGCGCCAGCCAGGAGGAGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGA GGAGCUGCUGGUGAAGCUGAACAGAGAGGACCUGCUGAGAAAGCAGAGAACCUUCGACAACGGCAGCAUCCCCCACCAGAUCCACC UGGGCGAGCUGCACGCCAUCCUGAGAAGACAGGAGGACUUCUACCCCUUCCUGAAGGACAACAGAGAGAAGAUCGAGAAGAUCCUG ACCUUCAGAAUCCCCUACUACGUGGGCCCCCUGGCCAGAGGCAACAGCAGAUUCGCCUGGAUGACCAGAAAGAGCGAGGAGACCAU CACCCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCAGCGCCUCAGAGCAGUUCAUCGAGAGAAUGACCAACUUCGACAAGAACC UGCCCAACGAGAAGGUGCUGCCCAAGCACAGCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUG ACCGAGGGCAUGAGAAAGCCCGCCUUCCUGAGCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAAGACCAACAGAAAGGU GACCGUGAAGCAGCUGAAGGAGGACUACUUCAAGAAGAUCGAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGACAGAUUCA ACGCCAGCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAGGAGAACGAGGACAUCCUG GAGGACAUCGUGCUGACCCUGACCCUGUUCGAGGACAGAGAUGAUCGAGGAGAGACUGAAGACCUACGCCCACCUGUUCGACGA CAAGGUGAUGAAGCAGCUGAAGAGAAGAUACACCGGCUGGGGCAGACUGAGCAGAAAGCUGAUCAACGGCAUCAGAGACAAGC AGAGCGGCAAGACCAUCCUGGACUUCCUGAAGAGCGACGGCUUCGCCAACAGAAACUUCAUGCAGCUGAUCCACGACGACAGCCUG ACCUUCAAGGAGGACAUCCAGAAGGCCCAGGUGAGCGGCCAGGGCGACAGCCUGCACGAGCACAUCGCCAACCUGGCCGGCAGCCC CGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCAGACACAAGCCCGAGAACAUCG

	377
mRNA transcript with XBG UTRs and Cas9 ORF with low A codons of Table 4	GGGAAGCUCAGAAUAAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACAAGAAGUACUCCAUCGGCCUGGACAUCGGCACCAAC UCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCCUCCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACUCCAU CAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACUCCGGCGAGACCGCCGAAGCGGACCGCCGCCGGCGGCGGU ACACCCGGCGGAAGAACCGGAUCUACCUGCAGGAGAUCUUCUCCAACGAGAUGGCCAAGGUGGACGACUCCUUCUUCCACCGG CUGGAGGAGUCCUUCCUGGUGGAGGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCA CGAGAAGUACCCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUCUACCUGGCCCU UGGCCCACAUGAUCAAGUUCCGGGGCCACUUCCUGAUCGAGGGCGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUUCAUC CAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCUCCGGCGUGGACGCCAAGGCCAUCCUGUCCGCCCG GCUGUCCAAGUCCCGGCGGCUGGAGAACCUGAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCC UGUCCCUGGGCCUGACCCCCAACUUCAAGUCCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGUCCAAGGACACCUACGAC GACGACCUGGACAACCUGCUGGCCCAGAUCGGCGACCAGUACGCCGACCUGUUUCCUGGCCGCCAAGAACCUGUCCGACGCCAUCCU GCUGUCCGACAUCCUGCGGGUGAACACCGAGAUCACCAAGGCCCCCCUGUCCGCCUCCAUGAUCAAGCGGUACGACGAGCACCACC AGGACCUGACCCUGCTGAAGGCCCUGGTGCGGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGC UACGCCGGCTACATCGACGGCGGCGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGA

GGAGCUGCUGGUGAACCGGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGGCUCCAUCCCCCACCAGAUCCACC UGGGCGAGCUGCACGCCAUCCUGCGGCAGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCCUG ACCUUCCGGAUCCCCUACUACGUGGGCCCCCUGGCCCGGGGCAACUCCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAU CACCCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCUCCGCCCAGUCCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACC UGCCCAACGAGAAGGUGCUGCCCAAGCACUCCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUG ACCGAGGGCAUGCGGAAGCCCGCCUUCCUGUCCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAAGACCAACCGGAAGGU GACCGUGAAGCAGCUGAAGGAGGACUACUUCAAGAAGAUCGAGUGCUUCGACUCCGUGGAGAUCUCCGGCGUGGAGGACCGGUUCA ACGCCUCCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAGGAGAACGAGGACAUCCUG GAGGACAUCGUGCUGACCCUGACCCUGUUCGAGGACCGGGAGAUGAUCGAGGAGCGGCUGAAGACCUACGCCCACCUGUUCGACGA CAAGGUGAUGAAGCAGCUGAAGCGGCGCCGGUACACCGGCUGGGGCCGGCUGUCCCGGAAGCUGAUCAACGGCAUCCGGGACAAGC AGUCCGGCAAGACCAUCCUGGACUUCCUGAAGUCCGACGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUCCACGACGACUCCCUG ACCUUCAAGGAGGACAUCCAGAAGGCCCAGGUGUCCGGCCAGGGCGACUCCCUGCACGAGCACAUCGCCAACCUGGCCGGCUCCCCC CGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCG UGAUCGAGAUGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACUCCCGGGAGCGGAUGAAGCGGAUCGAGGAGGGCAUC AAGGAGCUGGGCUCCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACGAGAAGCUGUACCUGUACUACCUGCA GAACGGCCGGGACAUGUACGUGGACCAGGAGCUGGACAUCAACCGGCUGUCCGACUACGACGUGGACCACAUCGUGCCCCAGUCCU UCCUGAAGGACGACUCCAUCGACAACAAGGUGCUGACCCGGUCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCCUCCGAGGAG GUGGUGAAGAAGAUGAAGAACUACUGGCGGCAGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGC CGAGCGGGGCGGCCUGUCCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGAUCACCAAGCACGUGG CCCAGAUCCUGGACUCCCGGAUGAACACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGAAGGUGATCACCCUGAAGUCC AAGCUGGUGUCCGACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACGCCUACCU GAACGCCGUGGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUACGACG UGCGGAAGAUGAUCGCCAAGUCCGAGCAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUC AAGACCGAGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAACGGCGAGACCGGCGAGAUCGUGUGGGA CAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCGGCG GCUUCUCCAAGGAGUCCAUCCUGCCCAAGCGGAACUCCGACAAGCUGAUCGCCCGGAAGAAGGACUGGGACCCCAAGAAGUACGGC GGCUUCGACUCCCCCACCGUGGCCUACUCCGUGCUGGUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGUGAA GGAGCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGGCUACAAGGAGG UGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACUCCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCUCCGCCGGC GAGCUGCAGAAGGGCAACGAGCUGGCCCUGCCCUCCAAGUACGUGAACUUCCUGUACCUGGCCUCCCACUACGAGAAGCUGAAGGGG CUCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUCUCCGAGUU UCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGAG CAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUACUUCGACACCACCAUCGACCG GAAGCGGUACACCUCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACCGGCCUGUACGAGACCCGGAUCGACC UGUCCCAGCUGGGCGGCGACGGCGGCUCCCCCCAAGAAGAAGCGGAAGGUGUGACUAGCACCAGCCUCAAGAACACCCGAAUGG AGUCUCUAAGCUACAUAAUACCAACUUUACACAAAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUAUCUGCUCCUAAUAAAAA GAAAGUUUCUUCACAUUCUCCGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAA	mRNA	378
	GGGAAGCUCAGAAUAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGGACAAGAAGUACAGCAUCGGCCUGGACAUCGGCACCAAC	

transcript with XBG UTRs and Cas9 ORF with low U/A codons of Table 4	AGCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCCAGCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACAGCAU CAAGAAGAACCUGAUCGGCGCCCUGCUGUUCGACAGCGGCGAGACCGCCGAGGCCACCCGGCUGAAGCGGACCGCCCGGCGGCGGU ACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAGGAGAUCUUCAGCAACGAGAUGGCCAAGGUGGACGACAGCUUCUUCCACCGG CUGGAGGAGAGCUUCCUGGUGGAGGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUACCA CGAGAAGUACCCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACAGCACCGACAAGGCCGACCUGCGGCUGAUCUACCUGGCCCC UGGCCCACAUGAUCAAGUUCCGGGGCCACUUCCUGAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUCAUC CAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGCGUGGACGCCAAGGCCAUCCUGAGCGCCCG GCUGAGCAAGAGCCGGCGGCUGGAGAACCUGAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCC UGAGCCUGGGCCUGACCCCCAACUUCAAGAGCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGAGCAAGGACACCUACGAC GACGACCUGGACAACCUGCUGGCCCAGAUCGGCGACCAGUACGCCGACCUGUUUCCUGGCCGCCAAGAACCUGAGCGACGCCAUCCU GCUGAGCGACAUCCUGCGGGUGAACACCGAGAUCACCAAGGCCCCCCUGAGCGCCAGCAUGAUCAAGCGGUACGACGAGCACCACC AGGACCUGACCCUGCUGAAGGCCCUGGUGCGGCAGCUGCCCGAGAAGUACAAGGAGAUCUUCUUCGACCAGAGCAAGAACGGC UACGCCGGCUACAUCGACGGCGGCGCCAGCCAGGAGGAGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGA GGAGCUGCUGGUGAAGCUGAACCGGGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGGCAGCAUCCCCCACCAGAUCCACC UGGGCGAGCUGCACGCCAUCCUGCGGCGGCAGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCCUG ACCUUCCGGAUCCCCUACGUGGGCCCCCUGGCCCGGGGCAACAGCCGGUUCGCCUGGAUGACCCGGAAGAGCGAGGAGACCAU CACCCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCAGCGCCCAGAGCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACC UGCCCAACGAGAAGGUGCUGCCCAAGCACAGCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAAGUACGUG ACCGAGGGCAUGCGGAAGCCCGCCUUCCUGAGCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAAGACCAACCGGAAGGU GACCGUGAAGCAGCUGAAGGAGGACUACUUCAAGAAGAUCGAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGACCGGUUCA ACGCCAGCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAGGAGAACGAGGACAUCCUG GAGGACAUCGUGCUGACCCUGUUCGAGGACCGGGAGAUGAUCGAGGAGCGGCUGAAGACCUACGCCCACCUGUUCGACGA CAAGGUGAUGAAGCAGCUGAAGCGGCGCGGUACACCGGCUGGGGCCGGCUGAGCCGGAAGCUGAUCAACGGCAUCAGGGACAAGC AGAGCGGCAAGACCAUCCUGGACUUCCUGAAGAGCGACGGCUUCGCCAACCGGAACUUCCAUGCAGCUGAUCCACGACGACAGCCUG ACCUUCAAGGAGGACAUCCAGAAGGCCCAGGUGAGCGGCCAGGGCGACAGCCUGCACGAGCACAUCGCCAACCUGGCCGGCAGCCC CGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCG UGAUCGAGAUGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCCGCGAGCGGAUGAAGCGGAUCGAGGAGGGCAUC AAGGAGCUGGGCAGCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACGAGAAGCUGUACCUGUACCUGCA GAACGGCCGGGACAUGUACGUGGACCAGGAGCUGGACAUCAACCGGCUGAGCGACUACGUGGACCACATCGUGCCCCAGAGCUU UCCUGAAGGACGACAGCAUCGACAACAAGGUGCUGACCCGGAGCGACAAGAACCGGGGCAAGAGCGACAACGUGCCCAGCGAGGAG GUGGUGAAGAAGAUGAAGAACUACUGGCGCCAGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGC CGAGCGGGGCGGCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGAUCACCAAGCACGUGG CCCAGAUCCUGGACAGCCGGAUGAACACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGAAGGUGACCACCCUGAAGAGC AAGCUGGUGAGCGACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACGCCUACCU GAACGCCGUGGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGAGCGAGUUCGUGUACGGCGACUACAAGGUGUACGACG UGCGGAAGAUGAUCGCCAAGAGCGAGCAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC AAGACCGAGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAACGGCGAGACCGGCGAGAUCGUGUGGGGA CAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUGCUGAGCAUGCCCCAGGUGAACAUCGUGAAGAAGACCGAGGUGCAGACCGGCG GCUUCAGCAAGGAGAGCAUCCUGCCCAAGCGGAACAGCGACAAGCUGAUCGCCCGGAAGAAGGACUGGGACCCCAAGAAGUACGGC

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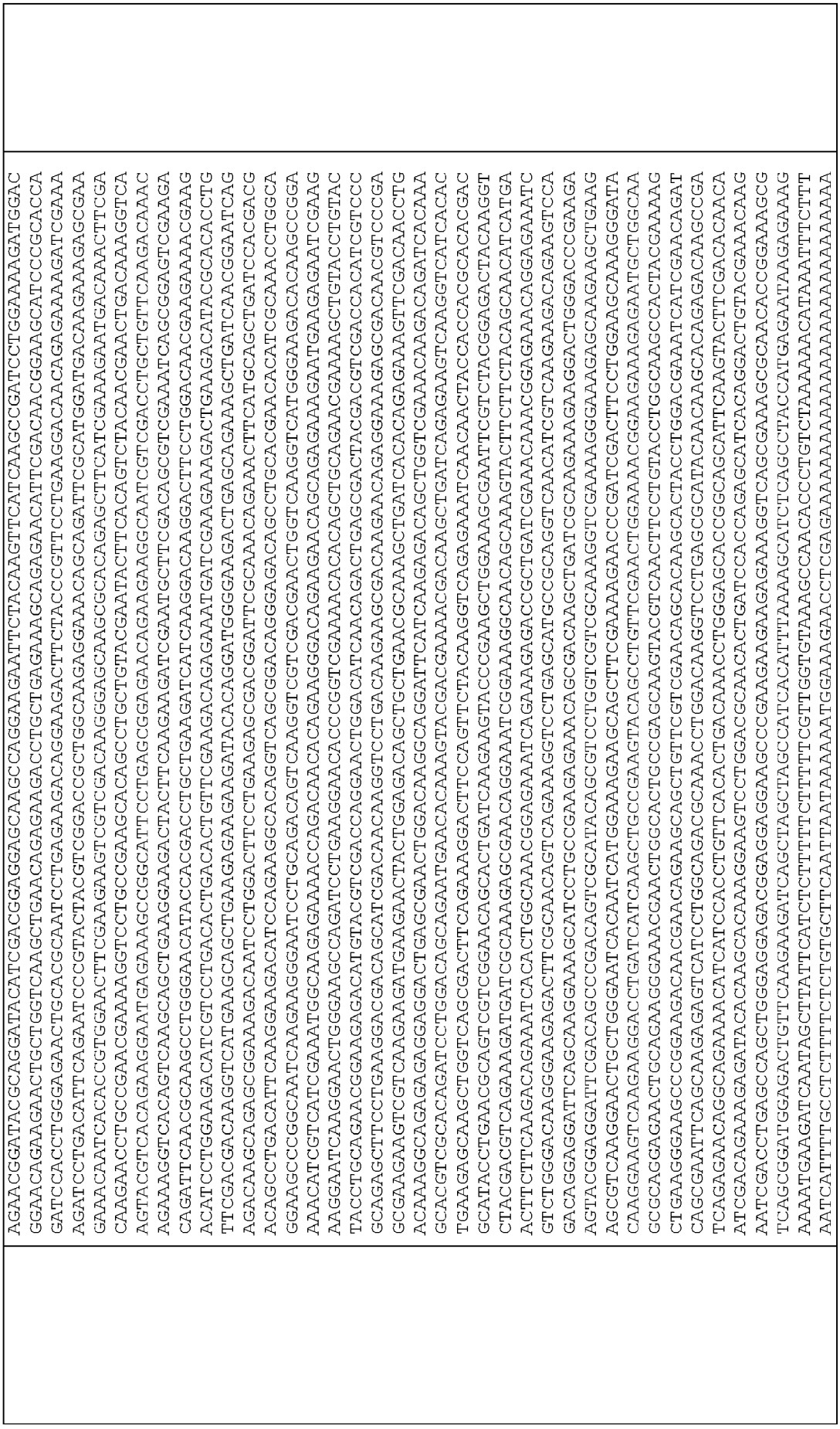
mRNA transcript with ORF encoding Cas9 with HiBiT tag, HSD 5′ UTR and human ALB 3′ UTR	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTCGTTGCAGGCCTTATTCGGATCCGCCACCATGGACAAGA AGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTC AAGGTCCTGGGAAACACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGC AACAAGACTGAAGAGAACAGCAAGAAGATACACAAGAAAGAACACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAA TGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATC TTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCCACTCACATGAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCC CGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAA GAAGAACGGACTGTTCGGAAACCTGATCGCCTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACG CAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTC CTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGC AAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACACGAGCTGCCGGAAAAGTACA AGGAAATCTTCTTCGACCAGAGCAAGAACGGATACGCCGGTACATCGACGGAGGAGCCAAGCCAGGAAATTCTACAAGTTCATC AAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATT CGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGATCTTCTACCCGTTCCTGA AGGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTC GCATGGATGACAAGAAAAGAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTT CATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAG TCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATC GTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAG CGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACT TCCTGGACAACGAAGAAAACGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAA AGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAG CAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAA ACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTG CACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAA

mRNA transcript with ORF encoding Cas9 with HiBiT tag, CMV-1 5′ UTR and human ALB 3′ UTR	GGGCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCG GAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGA CACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACACAAGATGGCAAGAACAGCAAG AAGAAGATACACAAGAAGAAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAGGTCGACGACAGCTTCTT TCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTC GCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTA CCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGC TGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTG AGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCT GATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACA CATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGAC GCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGA ACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCA	380



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mRNA transcript with ORF encoding Cas9 with HiBiT tag, CMV-3 5′ UTR and human ALB 3′ UTR	GGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACTCACCGCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCG GAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGA CACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGAGACTGAAGAGAACAGCAAG AAGAAGATACACAAGAAGAAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCT TCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTC GCATACCACGAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTA CCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGC TGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTG AGCGCCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCT GATCGCCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCCAAAGCTGCAGCTGAGCAAGGACA CATACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCCTGGCAGCCAAAAACCTGTCGGACGCC ATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACCAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGA ACACCACCAGGACCTGACACTGCTGAAGGCCATCGTGCGACAGCAGCTGCCGGAAAGTACAAGGAAATCTTCTTCGACCAGAGCA AGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGAC GGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCA GATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACGAGAAAGATCGAAA AGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGGGAAACAGCAGATTCGCATGGATGACAAGAAGAGCGAA GAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGA CAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCA AGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAAC AGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGA CAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAG ACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACCTG TTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAG	382

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mRNA transcript with ORF encoding Cas9 with HiBiT tag, HBA 5′ UTR and human ALB 3′ UTR	GGGCATAAACCCTGGCGCGCTCGCGGCCCGGCACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACCCGCCACCATGGACAAGA AGTACAGCATCGGACTGGACATCGGAACACAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTC AAGGTCCTGGGAAACACAGACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGC AACAAGACTGAAGAGAACAGCAAGAAGATACACACAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAA TGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATC TTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGAGACCTTCCTGATCGAAGGAGACCTGAACC CGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCCCAGCTGCCGGGAGAAAA GAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACG

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mRNA transcript with ORF encoding Cas9 with HiBiT tag, XBG 5′ UTR and human ALB 3′ UTR	GGGAAGCTCAGAATAAACGCTCAACTTTGGCCGGATCTCGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAA CAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCA TCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACACAAGATGAACAGCAAGAAGAGA TACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAG ACTGGAAGAAAAGCTTCCTGTCGAAGAAGACAAGAAGCACACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACC ACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCA CTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTGGACAAGCTGTTCAT CCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCCAGCGGAGTCGACGCCAAGGCCATCCTGAGCGCCAA GACTGAGCAAGAGCAGAAGAACTGGAAAACCTGATCGCCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCA CTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGA CGACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCC TGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCAC CAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGG ATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGATTTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAG AAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCAC CTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCT GACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAA TCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAAC CTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACGT CACAGAAGGAATGAGAAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGG TCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTC

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AAAAAAAAAAAAAAAAAAAA

DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSLAAK RSRTT	387
Amino acid sequence for Cas9 with NLS2	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSQAAK RSRTT	388
Amino acid sequence for Cas9 with NLS3	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA	

	TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPAPA KRERTT	389
Amino acid sequence for Cas9 with NLS4	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSQAAK RPRTT	390
Amino acid sequence for Cas9 with NLS5	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSRAAK RPRTT	

Amino acid sequence for Cas9 with NLS6	391
	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF
	SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG
	DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL
	AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP
	EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY
	PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE
	YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK
	DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF
	ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
	KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS
	DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN
	DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA
	TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK
	LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF
	ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
	LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAK
	RSWSMAA
Amino acid sequence for Cas9 with NLS7	392
	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF
	SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG
	DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL
	AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP
	EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY
	PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE
	YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK
	DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF
	ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
	KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS
	DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN
	DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA
	TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK
	LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF
	ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
	LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAK
	RVWSMAF"
Amino acid sequence for Cas9 with NLS8	393
	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF
	SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG
	DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL
	AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP
	EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY

PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAK RSWSMAF	394
Amino acid sequence for Cas9 with NLS9	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSAAAK RKYFAA	395
Amino acid sequence for Cas9 with NLS10	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS	

	DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSRAAK RKAFAA	396
Amino acid sequence for Cas9 with NLS11	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL AEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIK DKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKA TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSRAAK RKYFAV	

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

What is Claimed is:

1. A method of treating amyloidosis associated with TTR (ATTR), comprising administering a corticosteroid and a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. 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,

thereby treating ATTR.

2. A method of reducing TTR serum concentration, comprising administering a corticosteroid and a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. 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,

thereby reducing TTR serum concentration.

3. A method for reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, comprising administering a corticosteroid and a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. 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,

thereby reducing accumulation of amyloids or amyloid fibrils.

4. A composition comprising a guide RNA comprising:
   a. a guide sequence selected from SEQ ID NOs: 5-82;
   b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
   c. 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,

for use in combination with a corticosteroid in a method of 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.

5. A composition comprising a vector encoding a guide RNA, wherein the guide RNA comprises:
   a. a guide sequence selected from SEQ ID NOs: 5-82;
   b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
   c. 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,

for use in combination with a corticosteroid in a method of 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.

6. A composition comprising:

(i) a guide RNA comprising:
   a. a guide sequence selected from SEQ ID NOs: 5-82;
   b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
   c. 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, and

(ii) an mRNA that encodes 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;

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;
c. the open reading frame consists of a set of codons of which at least 75% of the codons are codons listed in Table 1;
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;

for use in combination with a corticosteroid in a method of 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.

7. A composition comprising:

(i) a vector encoding a guide RNA, wherein the guide RNA comprises:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. 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, and

(ii) an mRNA that encodes 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;
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;
c. the open reading frame consists of a set of codons of which at least 75% of the codons are codons listed in Table 1;
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;

for use in combination with a corticosteroid in a method of 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.

8. The composition for use or method of any one of claims 1-3 or 5-7, wherein the method comprises administering the composition by infusion for more than 30 minutes, e.g. more than 60 minutes or more than 120 minutes.

9. The composition or method of any one of claims 1-8, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82.

10. The composition or method of any one of the preceding claims, 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 .

11. The composition of any one of claims 4-10, 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, or reducing TTR serum concentration in a subject, or reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

12. The method or composition for use of any one of claims 1-11, wherein the corticosteroid is dexamethasone, betamethasone, prednisone, prednisolone, methylprednisolone, cortisone, hydrocortisone, triamcinolone, or ethamethasoneb.

13. The method or composition for use of any one of claims 1-12, wherein the corticosteroid is dexamethasone.

14. The method or composition for use of any one of claims 1-13, wherein the corticosteroid is administered before the composition.

15. The method or composition for use of any one of claims 1-14, wherein the corticosteroid is administered after the composition.

16. The method or composition for use of any one of claims 1-15, wherein the corticosteroid is administered simultaneously with the composition.

17. The method or composition for use of any one of claims 1-16, wherein the corticosteroid is administered about 5 minutes to within about 168 hours before the composition is administered.

18. The method or composition for use of any one of claims 1-17, wherein the corticosteroid is administered about 5 minutes to within about 168 hours after the composition is administered.

19. The method or composition for use of any one of claims 1-18, wherein the corticosteroid is administered 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 18 hours, 1 day, 1.5 days, 2 days, 3 days, 4 days, 5 days, 6 days, or one week before the composition is administered.

20. The method or composition for use of any one of claims 1-19, wherein at least two doses of the corticosteroid are administered before or after the administration of the composition.

21. The method or composition for use of any one of claims 1-20, wherein at least two doses of the corticosteroid and at least two doses of the composition are administered.

22. The method or composition for use of any one of claims 1-21, wherein the corticosteroid is administered to the subject at a dose of 0.75 mg to 20 mg, or at a dose of about 0.01 – 0.4 mg/kg, such as 0.1 – 0.35 mg/kg or 0.25 – 0.35 mg/kg.

23. The method or composition for use of any one of claims 1-22, wherein the corticosteroid is administered to the subject via an intravenous injection.

24. The method or composition for use of any one of claims 1-23, wherein the corticosteroid is administered to the subject orally, optionally wherein the corticosteroid is administered to the subject orally before the composition is administered to the subject by intravenous injection.

25. The method or composition for use of claim 24, wherein the corticosteroid is dexamethasone, and the dexamethasone is administered to the subject orally in the amount of 20 mg 6 to 12 hour before the composition is administered to the subject, or the dexamethasone is administered to the subject intravenously in the amount of 20 mg for 30 minutes 6 to 12 hour before the composition is administered to the subject.

26. The method or composition for use of any one of claims 1-25, 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.

27. The method or composition for use of any one of claims 1-26, wherein the corticosteroid is dexamethasone.

28. The method or composition for use of any one of claims 1-27, wherein the method further comprises administering an infusion prophylaxis, wherein the infusion prophylaxis comprises one or more of acetaminophen, an H1 blocker, or an H2 blocker, optionally

wherein the one or more of the acetaminophen, H1 blocker, or H2 blocker are concurrently administered with the corticosteroid and/or before the composition.

29. The method or composition for use of claim 28, wherein each of the acetaminophen, H1 blocker, and H2 blocker are administered.

30. The method or composition for use of claim 28 or 29, wherein the H1 blocker and/or the H2 blocker are administered orally.

31. The method or composition for use of any one of claims 28-30, wherein the infusion prophylaxis comprises an intravenous corticosteroid (such as dexamethasone 8-12 mg, or 10 mg or equivalent) and acetaminophen (such as oral acetaminophen 500 mg).

32. The method or composition for use of any one of claims 28-31, wherein the infusion prophylaxis is administered as a required premedication prior to administering a guide RNA-containing composition, e.g. an LNP composition.

33. The method or composition for use of any one of claims 28-32, wherein H1 blocker is diphenhydramine.

34. The method or composition for use of any one of claims 28-33, wherein the H2 blocker is ranitidine.

35. The method or composition for use of any one of claims 1-35, wherein a first dose of the corticosteroid is administered at about 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered at about 1-2 hours before the composition is administered.

36. The method or composition for use of claim 35, wherein the method further comprises administering one or more of acetaminophen, an H1 blocker, or an H2 blocker, optionally wherein the one or more of the acetaminophen, H1 blocker, or H2 blocker are concurrently administered with the second dose of the corticosteroid.

37. The method or composition for use of any one of claims 1-36, wherein a first dose of the corticosteroid is administered orally at about 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered intravenously at about 1-2 hours before the composition is administered.

38. The method or composition for use of any one of claims 1-37, wherein a first dose of the corticosteroid is administered orally at about 8-24 hours before the composition is administered and a second dose of the corticosteroid is administered intravenously concurrently with administration of acetaminophen, H1 blocker and H2 blocker at about 1-2 hours before the composition is administered.

39. The method or composition for use of any one of claims 1-38, wherein the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of about 6-10 mg is administered to the subject orally at about 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the amount of about 8-12 mg is intravenously administered to the subject concurrently with oral administration of acetaminophen and intravenous administration of an H1 blocker and an H2 blocker, at about 1-2 hours before the composition is administered to the subject, optionally wherein the H1 blocker is diphenhydramine and the H2 blocker is ranitidine, and/or optionally wherein the subject is human.

40. The method or composition for use of any one of claims 1-39, wherein the corticosteroid is dexamethasone, and a first dose of dexamethasone in the amount of 8 mg is administered to the subject orally at about 8-24 hours before the composition is administered to the subject, and a second dose of dexamethasone in the amount of 10 mg is intravenously administered to the subject concurrently with oral administration of acetaminophen and intravenous administration of an H1 blocker and an H2 blocker, at about 1-2 hours before the composition is administered to the subject, optionally wherein the H1 blocker is diphenhydramine and the H2 blocker is ranitidine.

41. The method or composition for use of any one of claims 1-40, wherein the composition is administered in the amount of 3 mg/kg by infusion for about 1.5-6 hours; a first dose of the corticosteroid is administered orally at about 8-24 hours before infusion of the composition; and a second dose of the corticosteroid is administered intravenously at about 1-2 hours before infusion of the composition.

42. The method or composition for use of any one of claims 1-41, wherein administering the corticosteroid improves tolerability of the composition comprising the guide RNA.

43. The method or composition for use of any one of claims 1-42, wherein administering the corticosteroid reduces the incidence or severity of one or more of inflammation, nausea, vomiting, elevated ALT concentration in blood, hyperthermia, and/or hyperalgesia in response to the composition comprising the guide RNA.

44. The method or composition for use of any one of claims 1-43, wherein administering the corticosteroid reduces or inhibits production or activity of one or more interferons and/or inflammatory cytokines in response to the composition comprising the guide RNA.

45. The method or composition for use of any one of claims 1-44, wherein the composition reduces serum TTR levels.

46. The method or composition for use of claim 45, wherein the serum TTR levels are reduced by at least 50% as compared to serum TTR levels before administration of the composition.

47. The method or composition for use of any one of claims 1-46, wherein the composition results in editing of the TTR gene.

48. The method or composition for use of claim 47, wherein the editing is calculated as a percentage of the population that is edited (percent editing), optionally wherein the percent editing is between 30 and 99% of the population..

49. The method or composition for use of claims 1-48, 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.

50. The method or composition for use of claims 1-49, wherein the composition is administered or delivered at least two times.

51. The method or composition for use of claim 50, 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, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 months.

52. The method or composition of any one of claims 1-51, wherein the guide sequence is selected from SEQ ID NOs: 5-82.

53. The method or composition of any one of claims 1-52, wherein the guide RNA is at least partially complementary to a target sequence present in the human *TTR* gene.

54. The method or composition of claim 53, wherein the target sequence is in exon 1, 2, 3, or 4 of the human *TTR* gene.

55. The method or composition of any one of claims 1-54, wherein the guide sequence is complementary to a first target sequence in the positive strand of the *TTR* gene, and wherein the composition further comprises a second guide sequence that is complementary to a second target sequence in the negative strand of the *TTR* gene.

56. The method or composition of any one of claims 1-55, wherein the guide RNA is a single guide (sgRNA).

57. The method or composition of claim 56, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-82 and nucleotides 21-100 of SEQ ID NO: 3.

58. The method or composition of claim 56, wherein the sgRNA 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: 87-124.

59. The method or composition of claim 58, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-124.

60. The method or composition of any one of claims 1-59, wherein the guide RNA comprises at least one modification.

61. The method or composition of claim 60, 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.

62. The method or composition of any one of claims 60-61, wherein the at least one modification includes PS bonds between the first four nucleotides, PS bonds between the last four nucleotides, 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.

63. The method or composition of any one of claims 60-62, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

64. The method or composition of any one of claims 1-63, wherein the guide RNA is associated with a lipid nanoparticle (LNP).

65. The method or composition of claim 64, wherein the LNP comprises an ionizable lipid.

66. The method or composition of any one of claims 64-65, wherein the LNP comprises a biodegradable ionizable lipid.

67. The method or composition of any one of claims 64-65, wherein the LNP comprises an amine lipid, e.g., a CCD lipid.

68. The method or composition of any one of claims 64-66, wherein the LNP comprises a helper lipid.

69. The method or composition of any one of claims 64-67, wherein the LNP comprises a stealth lipid, optionally 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.

70. The method or composition of any one of claims 64-69, wherein the LNP comprises a neutral lipid.

71. The method or composition of any one of claims 64-70, 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 lipid 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.

72. The method or composition of any one of claims 64-71, 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.

73. The method or composition of any one of claims 1-72, wherein the composition further comprises an RNA-guided DNA binding agent.

74. The method or composition of any one of claims 1-72, wherein the composition further comprises a polynucleotide that encodes an RNA-guided DNA binding agent.

75. The method or composition of claim 74, wherein the polynucleotide is an mRNA.

76. The method or composition of any one of claims 73-75, wherein the RNA-guided DNA binding agent is a Cas cleavase.

77. The method or composition of any one of claims 74-76, wherein the polynucleotide comprises 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;
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;
c. the open reading frame consists of a set of codons of which at least 75% of the codons are codons listed in Table 4;
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.

78. The composition or method of any one of claims 74-77, wherein the polynucleotide comprises a 5' UTR with at least 90% identity to any one of SEQ ID NOs: 232, 234, 236,

238, 241, or 275-277; and/or a 3' UTR with at least 90% identity to any one of SEQ ID NOs: 233, 235, 237, 239, or 240.

79. The composition or method of any of claims 74-78, wherein the polynucleotide is an mRNA and at least 10% of the uridine in the mRNA is substituted with a modified uridine.

80. The method or composition of any one of claims 73-79, wherein the RNA-guided DNA binding agent is modified.

81. The method or composition of claim 80, wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

82. The method or composition of any one of claims 1-81, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

83. The method or composition for use of any one of claims 1-82, wherein the composition reduces or prevents amyloids or amyloid fibrils comprising TTR.

84. The method or composition for use of any one of claims 1-83, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the *TTR* gene.

85. The method or composition of any one of claims 1-84, 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.

86. The method or composition for use of any one of claims 1-85, wherein administering the composition reduces levels of TTR in the subject, optionally wherein the levels of TTR are reduced by at least 50%.

87. The method or composition for use of claim 86, wherein the levels of TTR are measured in serum, plasma, blood, cerebral spinal fluid, or sputum, or liver, choroid plexus, and/or retina, optionally wherein the levels of TTR are measured via enzyme-linked immunosorbent assay (ELISA).

88. The method or composition for use of claims 1-87, wherein the subject has ATTR, familial amyloid polyneuropathy or familial amyloid cardiomyopathy.

89. The method or composition for use of claims 1-88, wherein the subject is human.

90. The method or composition for use of claims 1-89, wherein the subject is tested for specific mutations in the *TTR* gene before administering the composition or formulation.

91. Use of a composition or formulation of any of claims 1-90 for the preparation of a medicament for treating a human subject having ATTR.

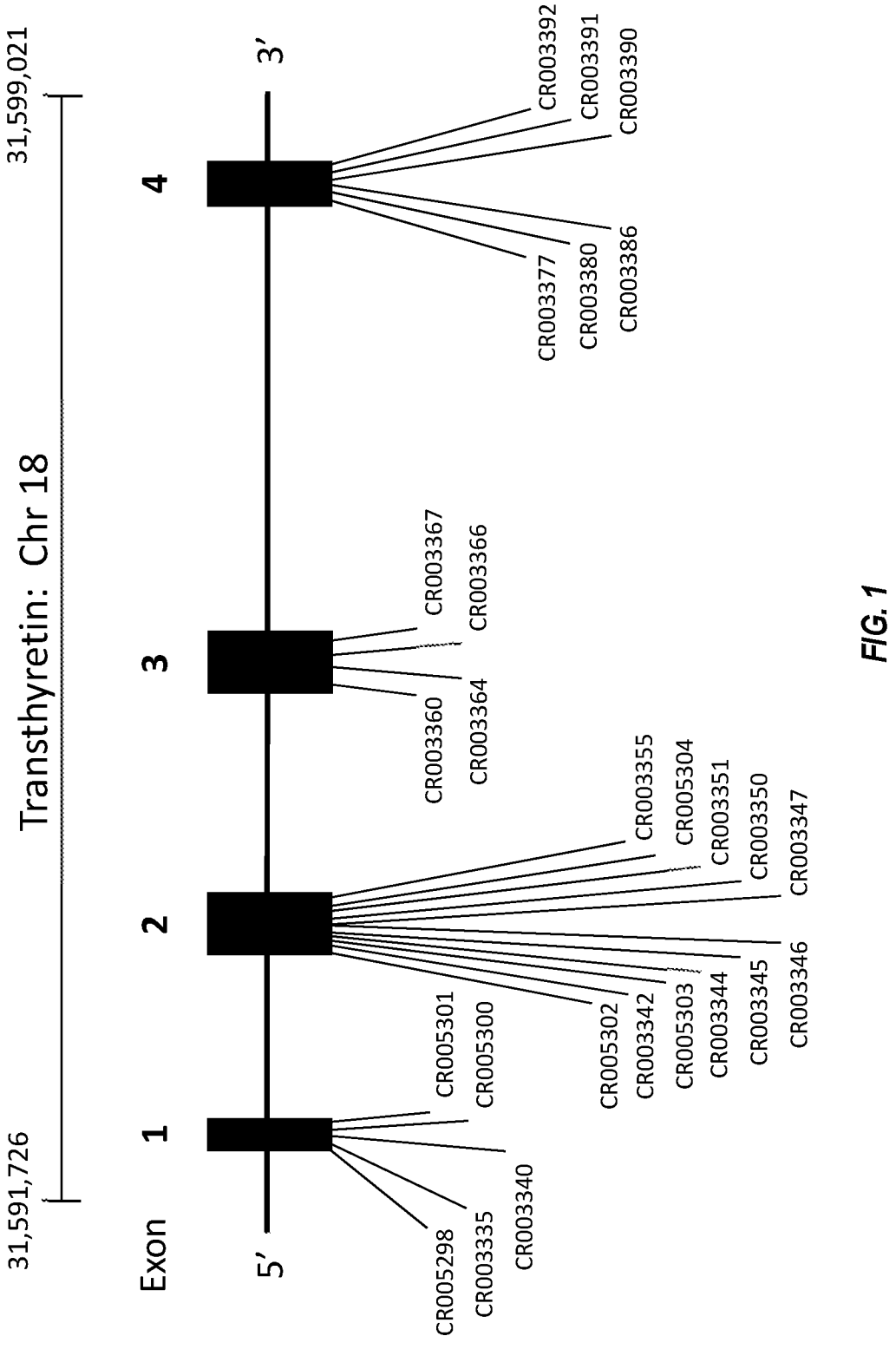
31,599,021
Transthyretin: Chr 18
31,591,726
Exon 1 2 3 4
5'
3'
CR005298
CR003335
CR003340
CR005301
CR005300
CR005302
CR003342
CR005303
CR003344
CR003345
CR003346
CR003347
CR003350
CR003351
CR005304
CR003355
CR003360
CR003364
CR003367
CR003366
CR003377
CR003380
CR003386
CR003392
CR003391
CR003390

FIG. 1

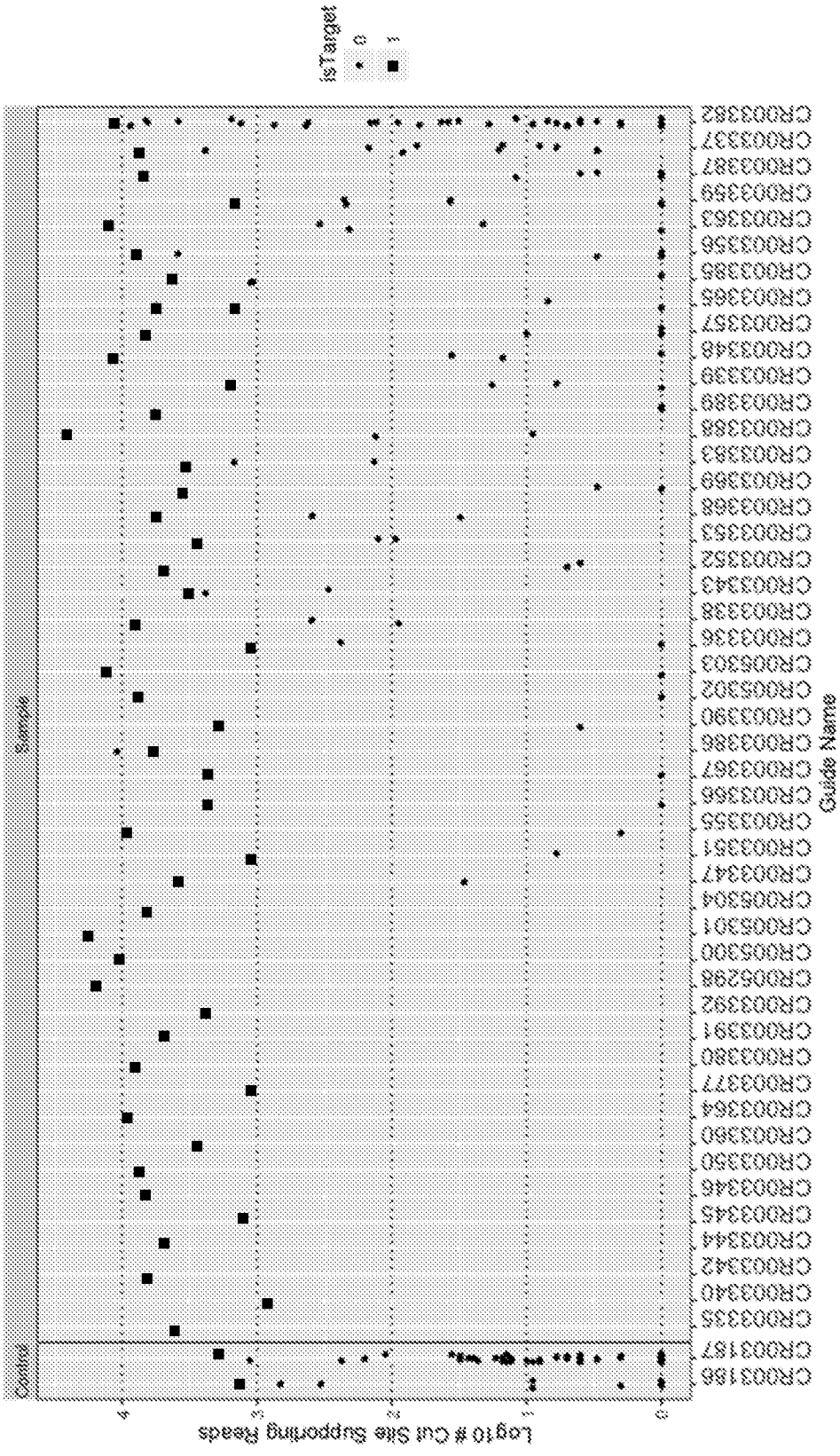
isTarget
0
1
Sample
Control
log10 # Cut Site Supporting Reads
0
1
2
3
4
Guide Name
CR003152
CR003187
CR003335
CR003340
CR003342
CR003344
CR003345
CR003346
CR003350
CR003360
CR003364
CR003377
CR003380
CR003391
CR003392
CR005298
CR005300
CR005301
CR005304
CR003347
CR003351
CR003355
CR003366
CR003367
CR003386
CR003390
CR005302
CR005303
CR003336
CR003338
CR003343
CR003352
CR003353
CR003368
CR003369
CR003383
CR003388
CR003389
CR003339
CR003348
CR003357
CR003365
CR003385
CR003356
CR003363
CR003359
CR003387
CR003337
CR003382

FIG. 2

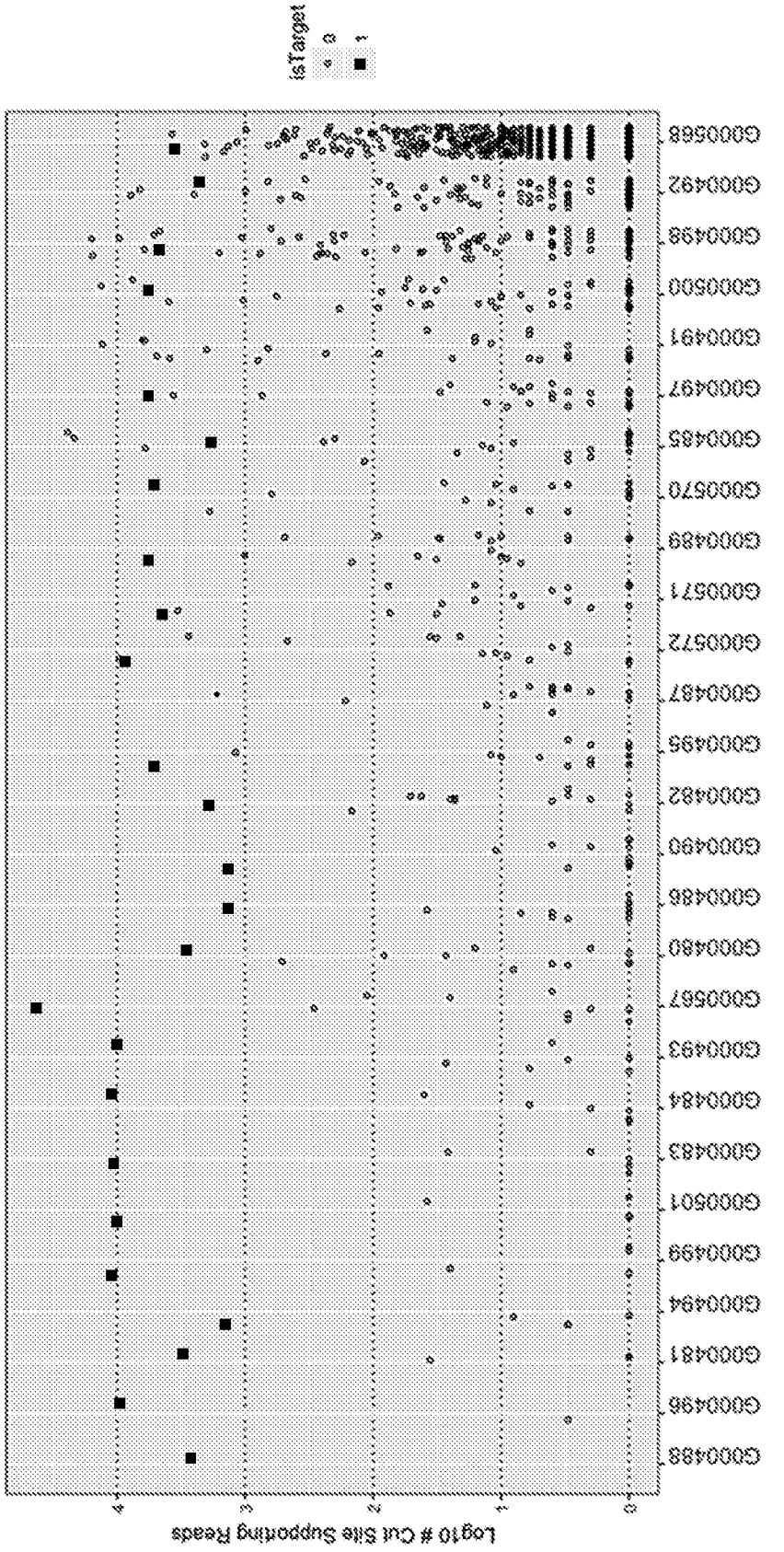
isTarget
0
1
log10 # Cut Site Supporting Reads
0
1
2
3
4
G000488
G000496
G000481
G000494
G000499
G000501
G000483
G000484
G000493
G000567
G000480
G000486
G000490
G000482
G000495
G000487
G000572
G000571
G000489
G000570
G000485
G000497
G000491
G000500
G000498
G000492
G000568

FIG. 3

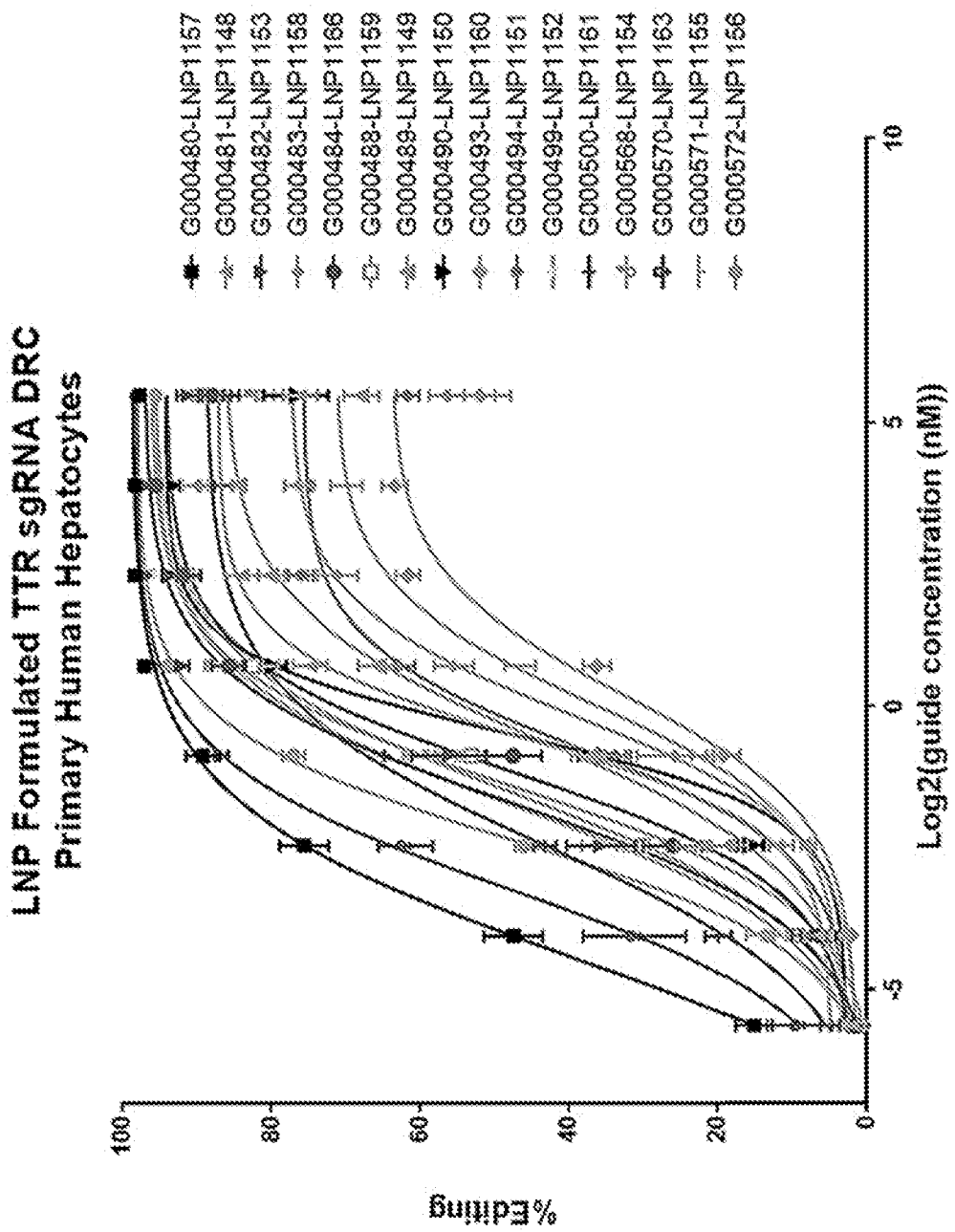
LNP Formulated TTR sgRNA DRC
Primary Human Hepatocytes
G000480-LNP1157
G000481-LNP1148
G000482-LNP1153
G000483-LNP1158
G000484-LNP1166
G000488-LNP1159
G000489-LNP1149
G000490-LNP1150
G000493-LNP1160
G000494-LNP1151
G000499-LNP1152
G000500-LNP1161
G000568-LNP1154
G000570-LNP1163
G000571-LNP1155
G000572-LNP1156
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 4

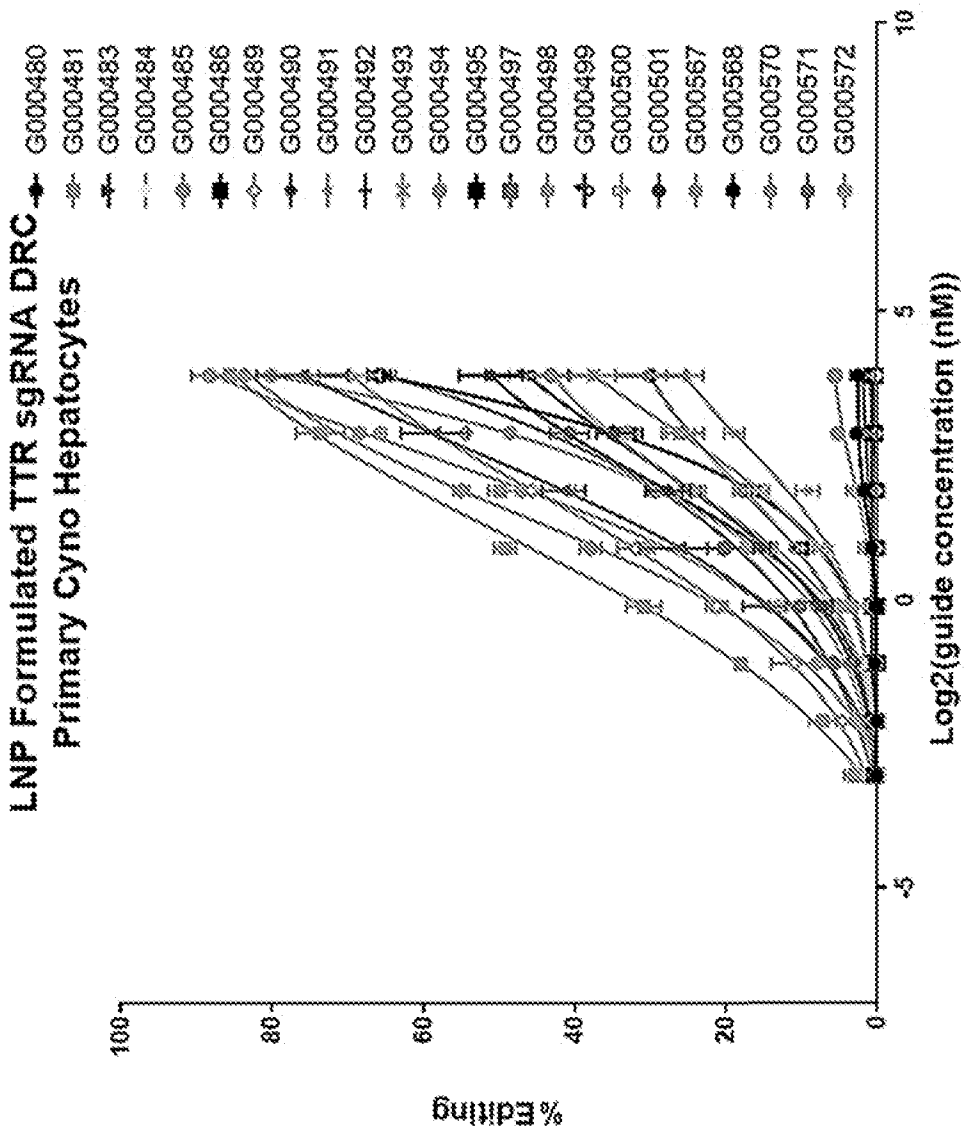
LNP Formulated TTR sgRNA DRC
Primary Cyno Hepatocytes
G000480
G000481
G000483
G000484
G000485
G000486
G000489
G000490
G000491
G000492
G000493
G000494
G000495
G000497
G000498
G000499
G000500
G000501
G000567
G000568
G000570
G000571
G000572
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 5

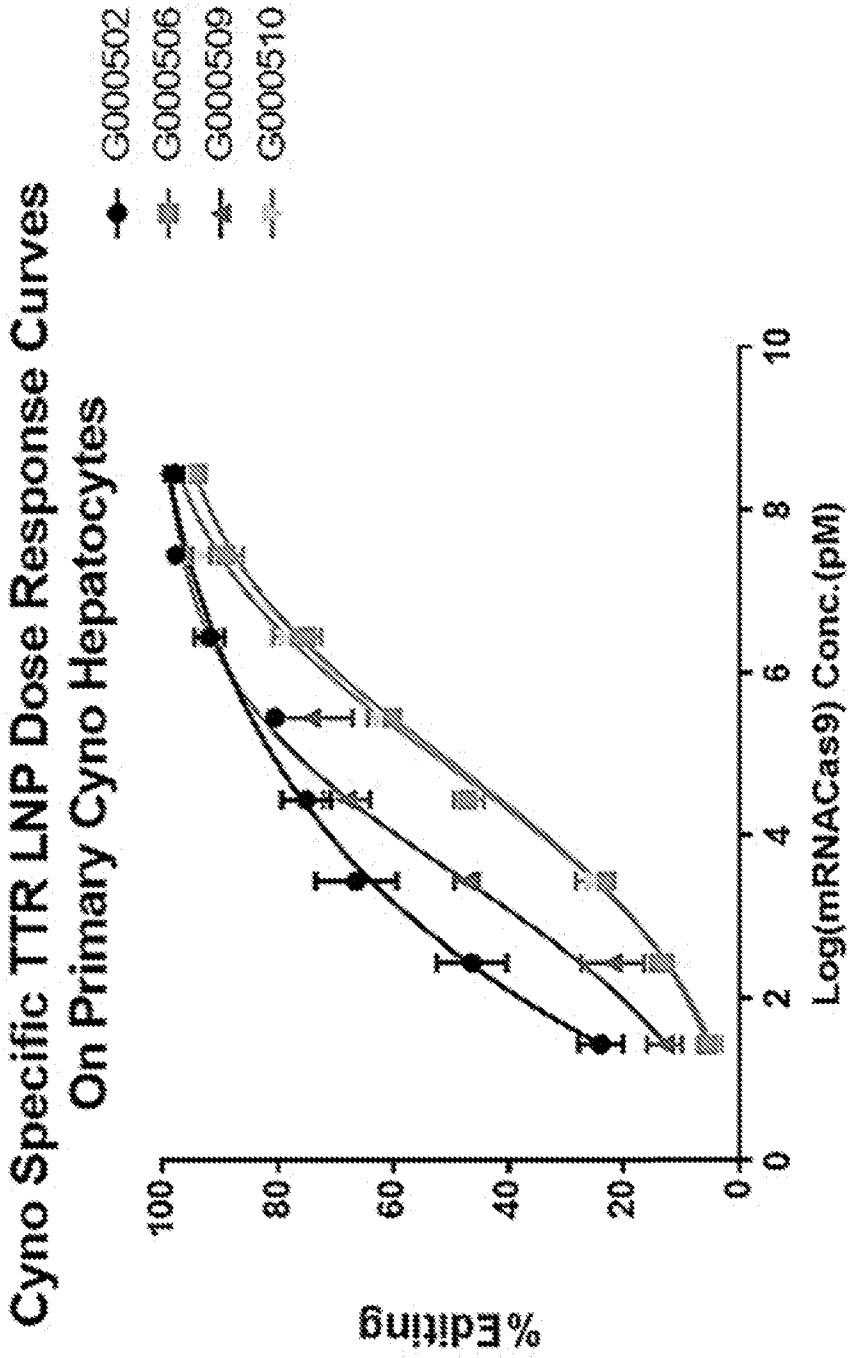
Cyno Specific TTR LNP Dose Response Curves
On Primary Cyno Hepatocytes
G000502
G000506
G000509
G000510
% Editing
100
80
60
40
20
0
0
2
4
6
8
10
Log(mRNACas9) Conc.(pM)

FIG. 6

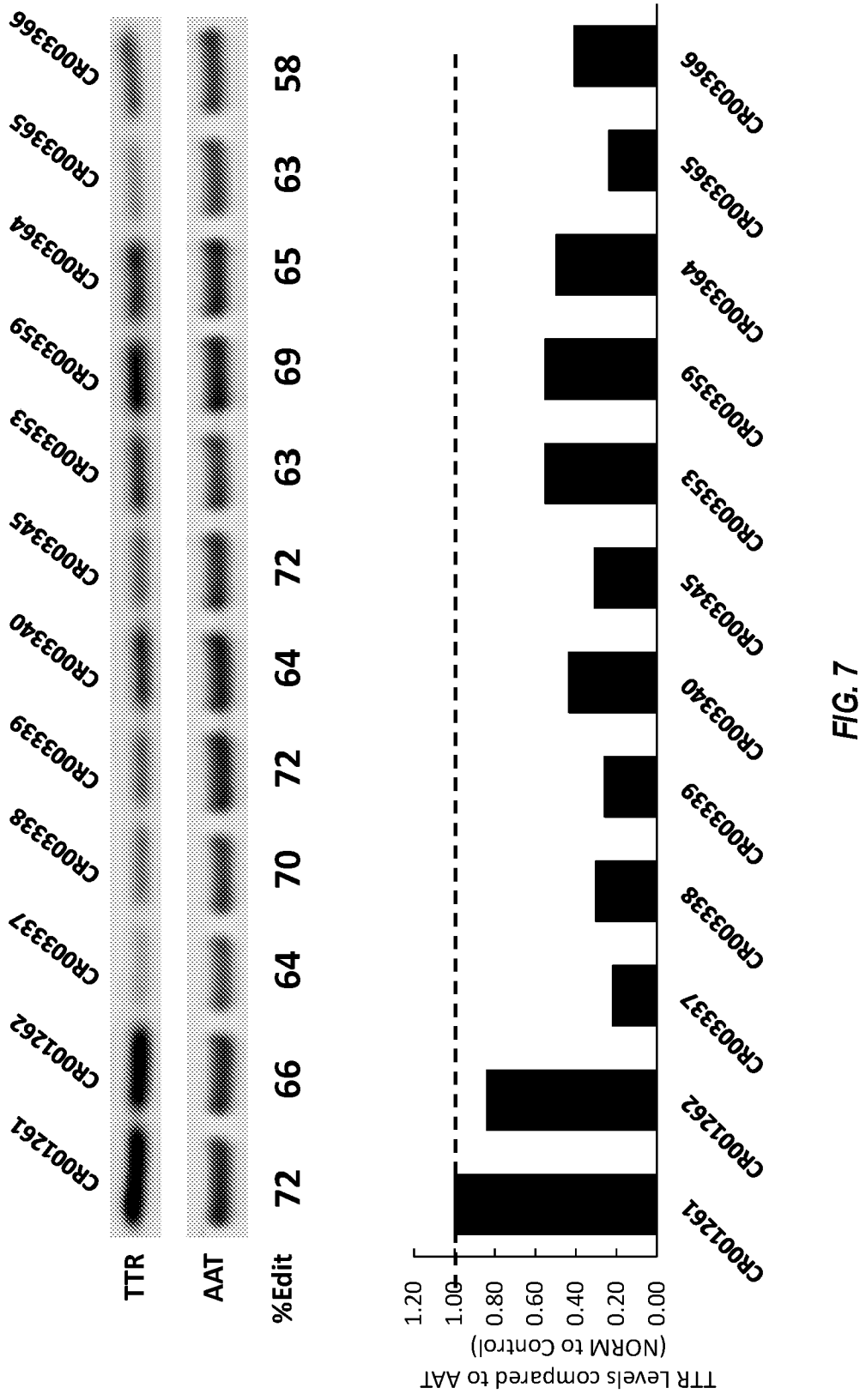
CR001261
CR001262
CR003337
CR003338
CR003339
CR003340
CR003345
CR003353
CR003359
CR003364
CR003365
CR003366
TTR
AAT
%Edit
72
66
64
70
72
64
72
63
69
65
63
58
TTR Levels compared to AAT
(NORM to Control)
1.20
1.00
0.80
0.60
0.40
0.20
0.00

FIG. 7

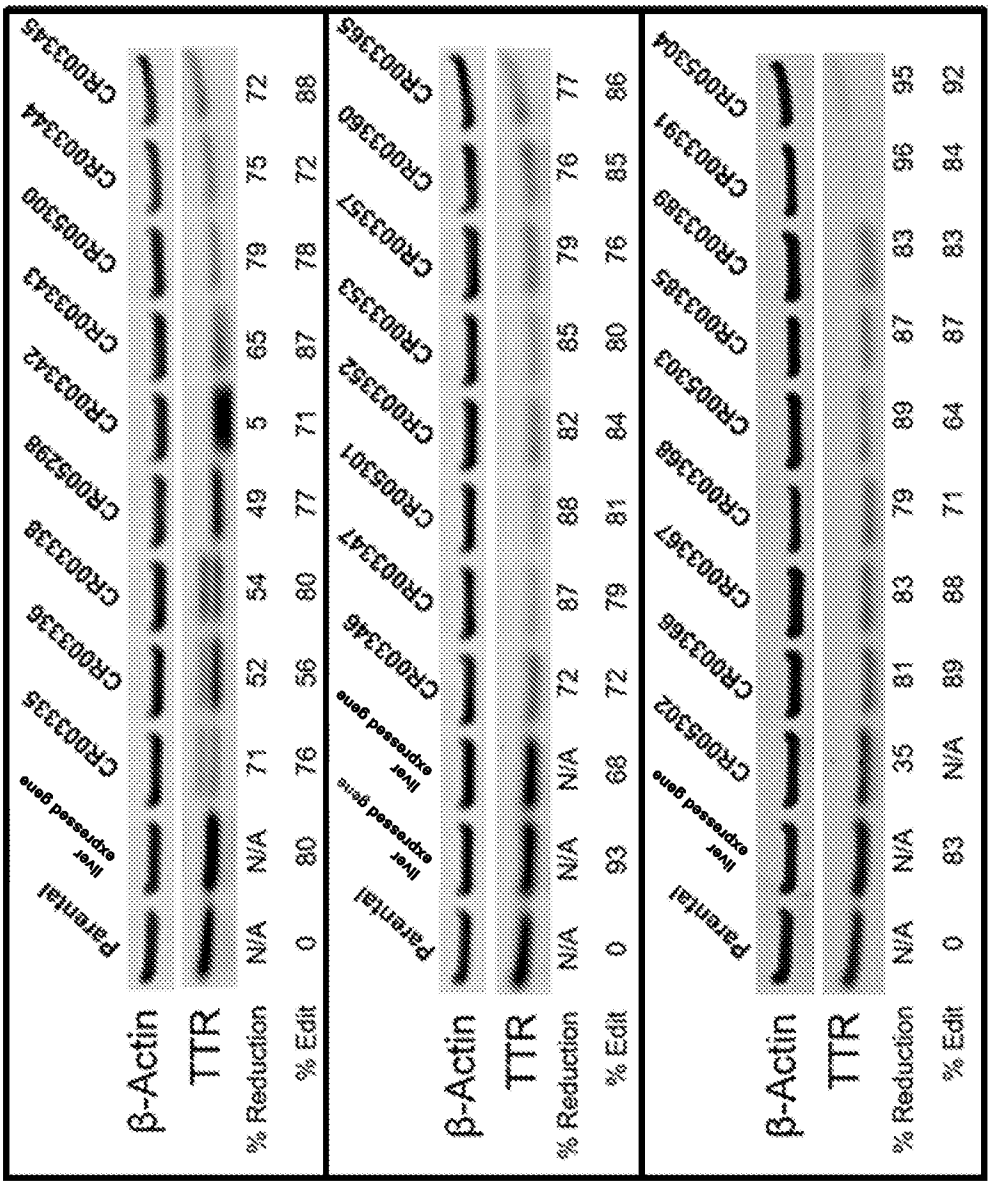
β-Actin
TTR
% Reduction
% Edit
Parental
liver expressed gene
CR003335
CR003336
CR003338
CR005298
CR003342
CR003343
CR005300
CR003344
CR003345
liver expressed gene
CR003346
CR003347
CR005301
CR003352
CR003357
CR003360
CR003363
liver expressed gene
CR003302
CR003366
CR003367
CR003368
CR005303
CR003385
CR003389
CR003391
CR005304

FIG. 8

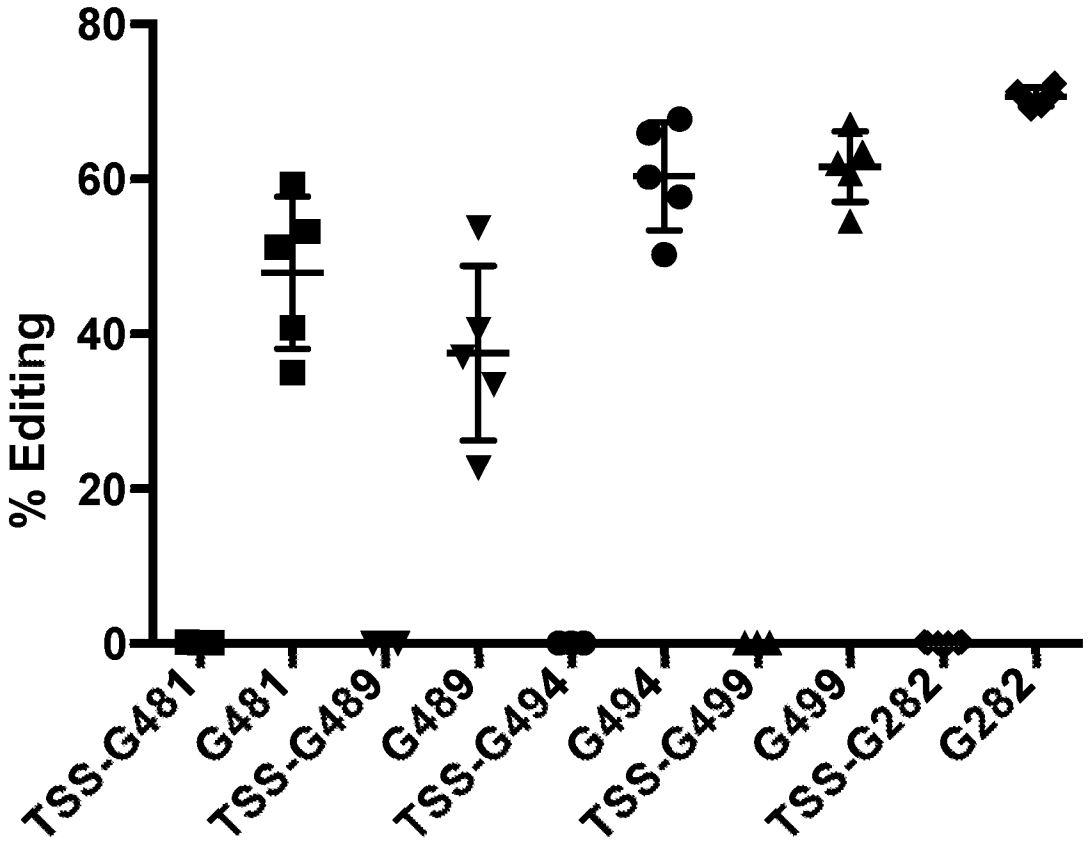
80
60
40
20
0
% Editing
TSS-G481
G481
TSS-G489
G489
TSS-G494
G494
TSS-G499
G499
TSS-G282
G282

FIG. 9

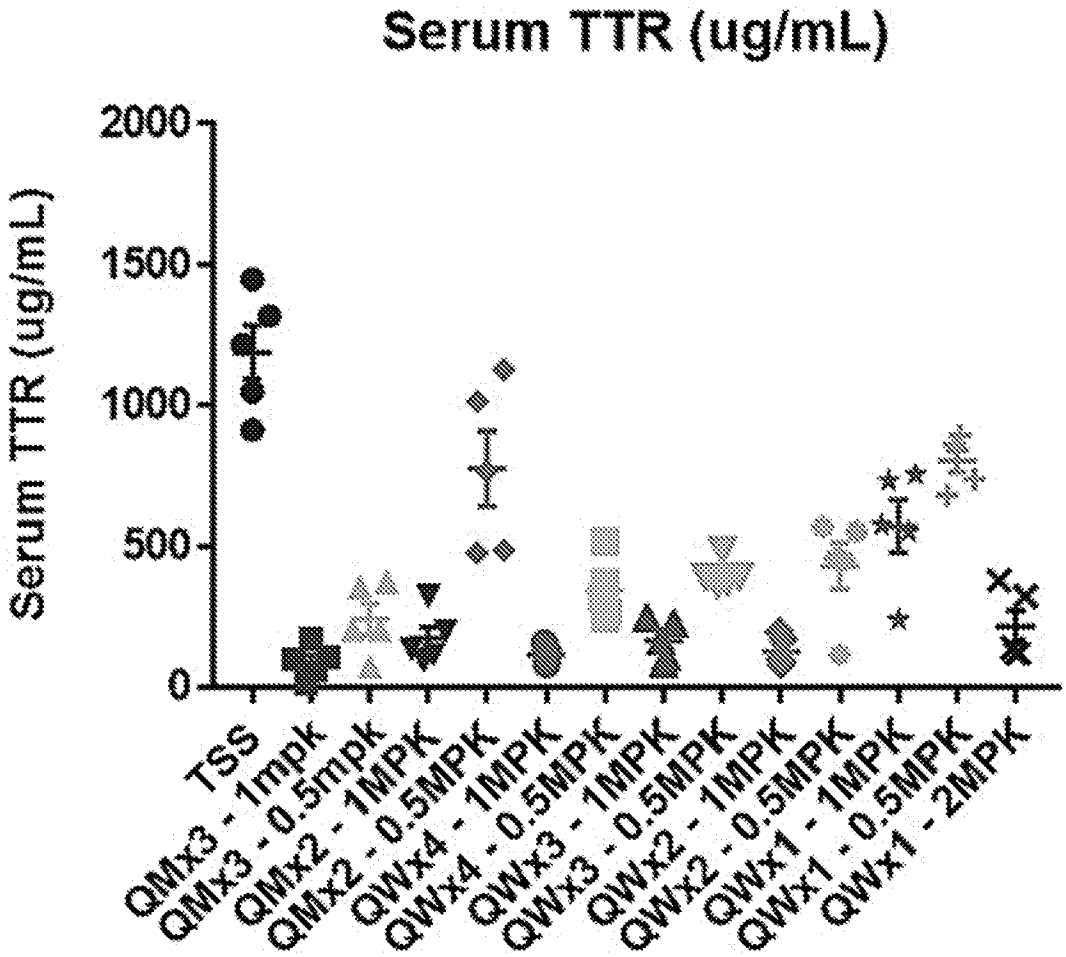
Serum TTR (ug/mL)
Serum TTR (ug/mL)
2000
1500
1000
500
0
TSS
QMx3 - 1mpk
QMx3 - 0.5mpk
QMx2 - 1MPK
QMx2 - 0.5MPK
QWx4 - 1MPK
QWx4 - 0.5MPK
QWx3 - 1MPK
QWx3 - 0.5MPK
QWx2 - 1MPK
QWx2 - 0.5MPK
QWx1 - 1MPK
QWx1 - 0.5MPK
QWx1 - 2MPK

FIG. 10A

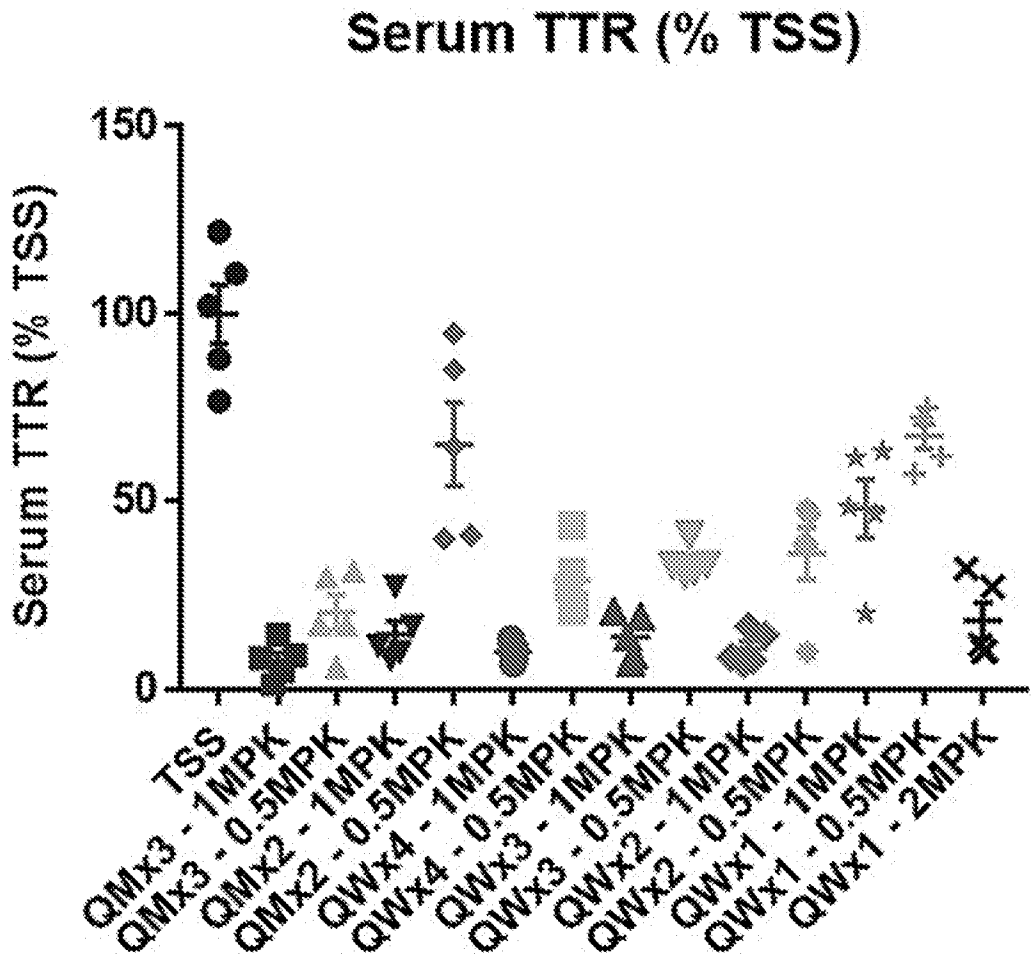
Serum TTR (% TSS)
Serum TTR (% TSS)
150
100
50
0
TSS
QMx3 - 1MPK
QMx3 - 0.5MPK
QMx2 - 1MPK
QMx2 - 0.5MPK
QWx4 - 1MPK
QWx4 - 0.5MPK
QWx3 - 1MPK
QWx3 - 0.5MPK
QWx2 - 1MPK
QWx2 - 0.5MPK
QWx1 - 1MPK
QWx1 - 0.5MPK
QWx1 - 2MPK

FIG. 10B

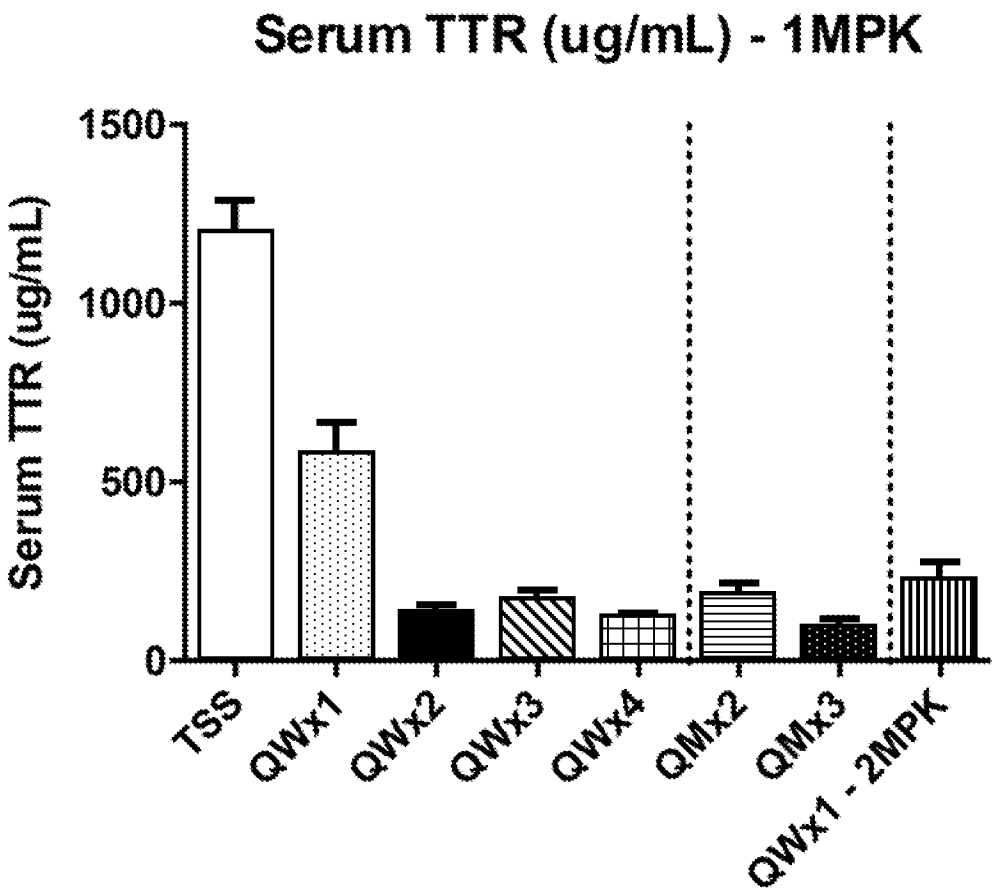
Serum TTR (ug/mL) - 1MPK
Serum TTR (ug/mL)
1500
1000
500
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 11A

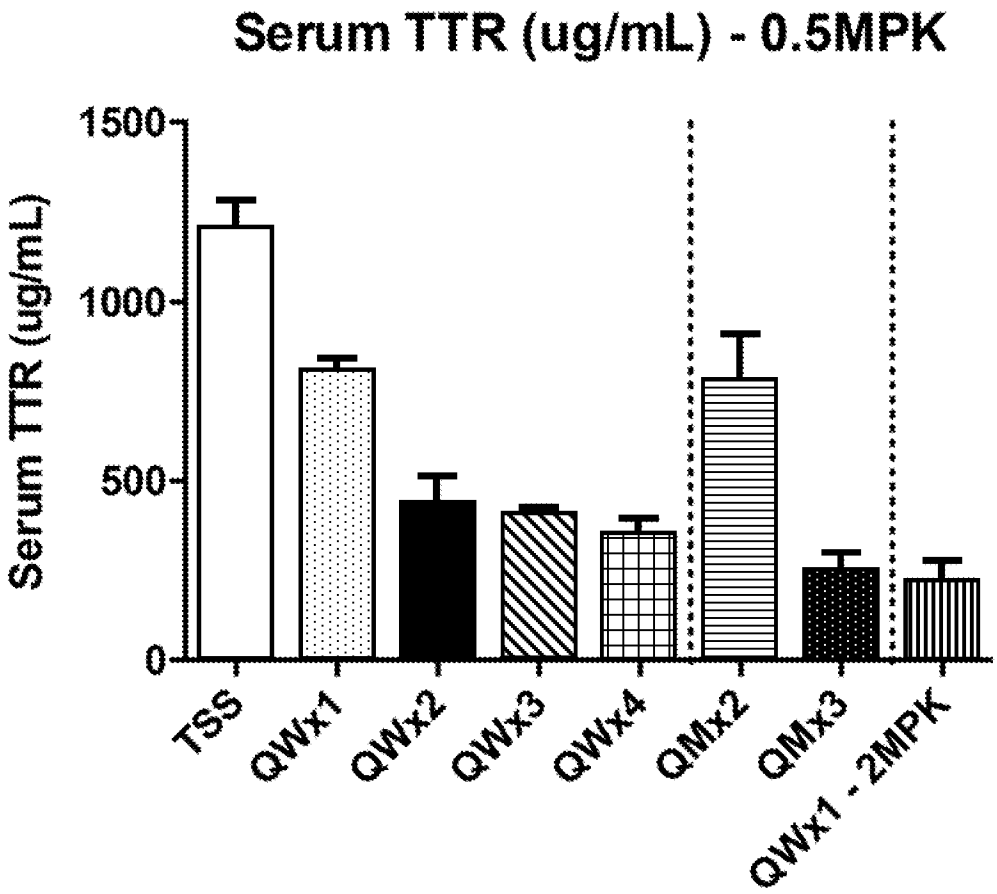
Serum TTR (ug/mL) - 0.5MPK
Serum TTR (ug/mL)
1500
1000
500
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 11B

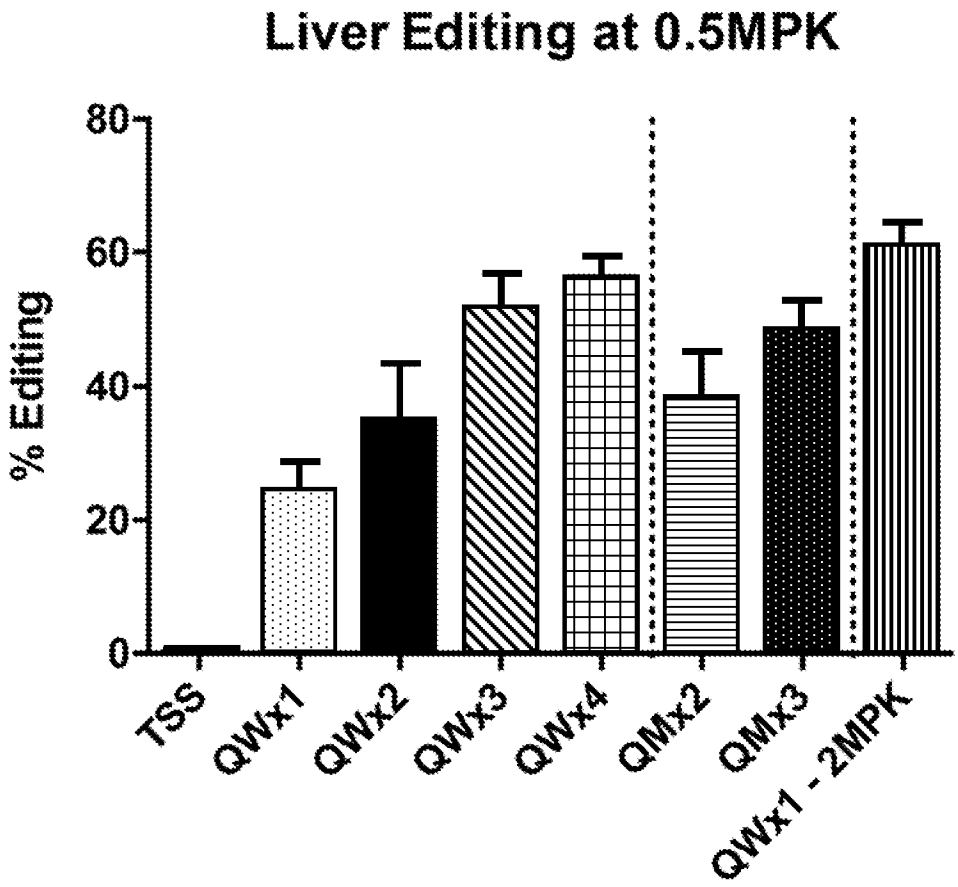
Liver Editing at 0.5MPK
% Editing
80
60
40
20
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 12A

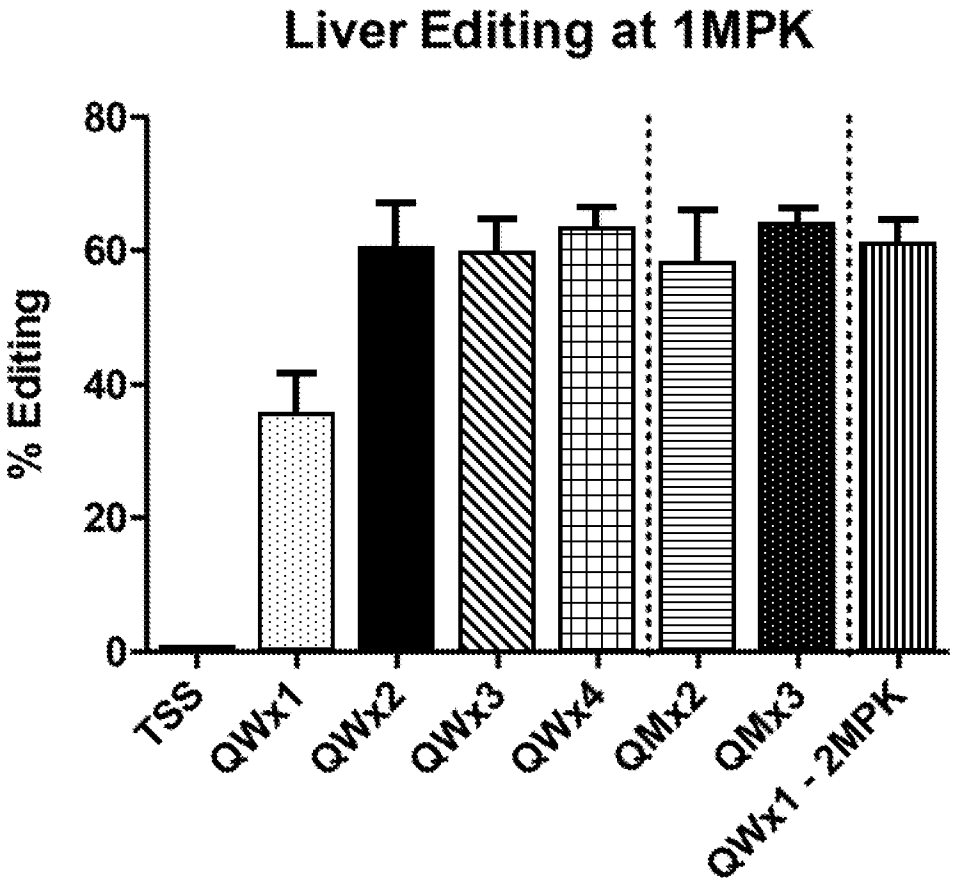
Liver Editing at 1MPK
% Editing
80
60
40
20
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 12B

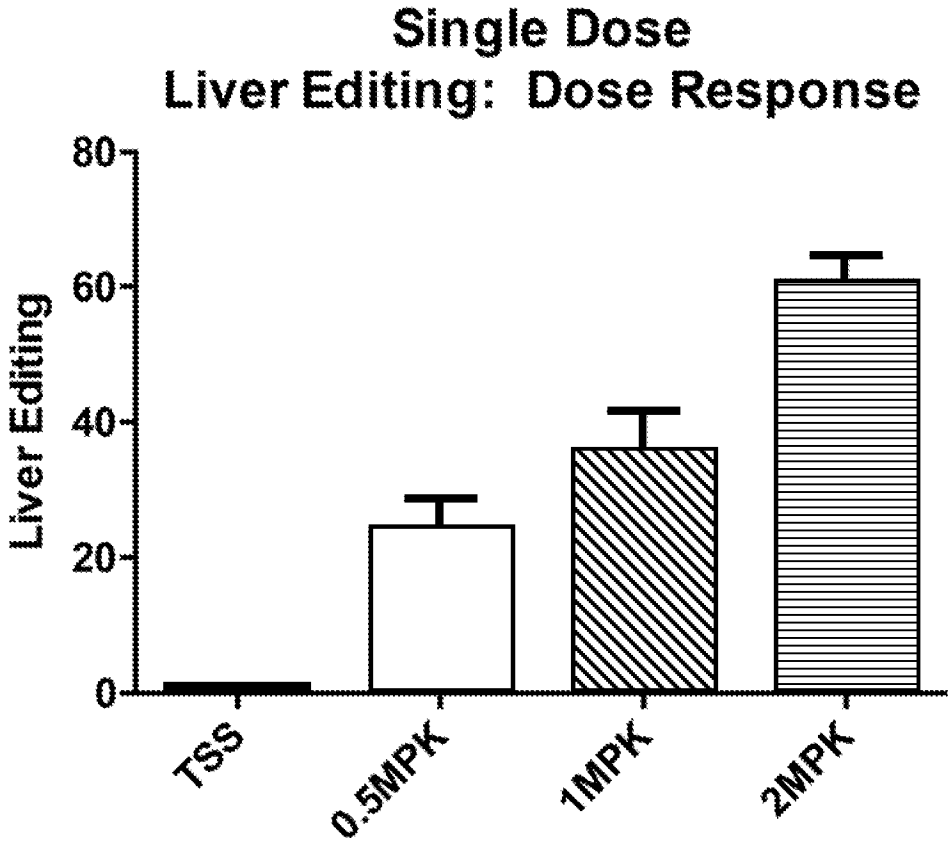
Single Dose
Liver Editing: Dose Response
Liver Editing
80
60
40
20
0
TSS
0.5MPK
1MPK
2MPK

FIG. 12C

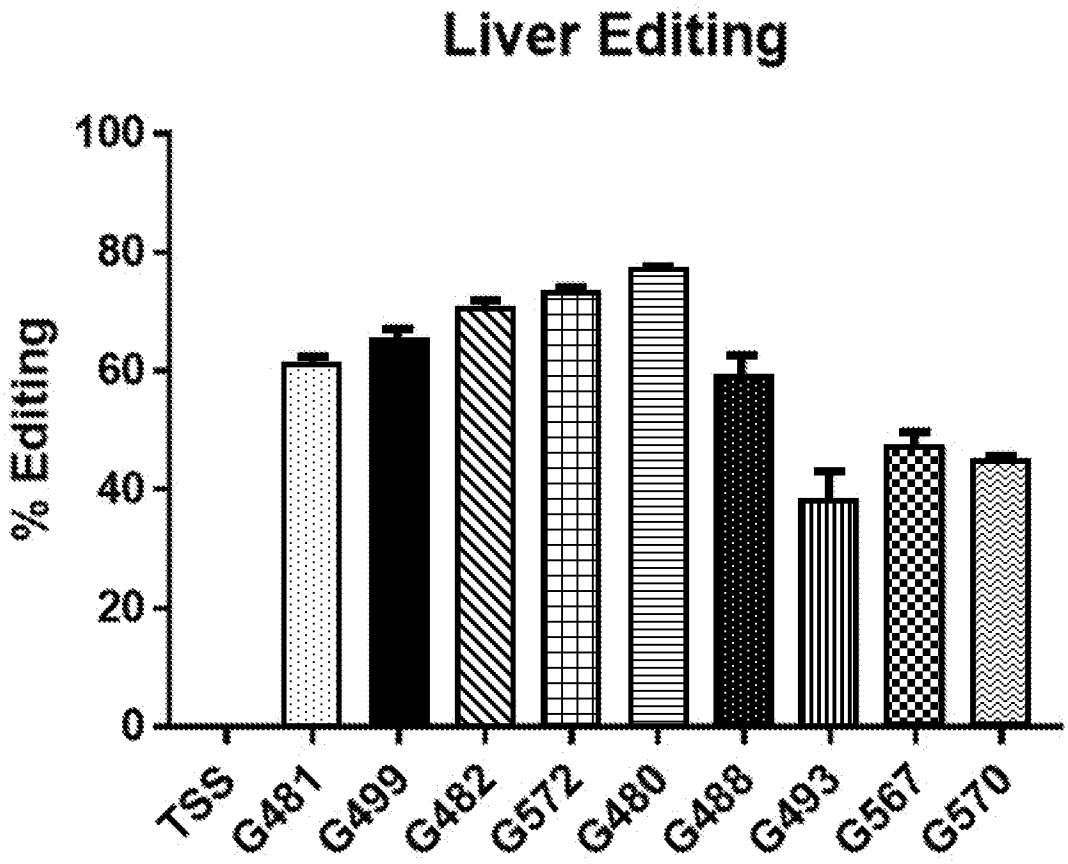
Liver Editing
% Editing
100
80
60
40
20
0
TSS
G481
G499
G482
G572
G480
G488
G493
G567
G570

FIG. 13

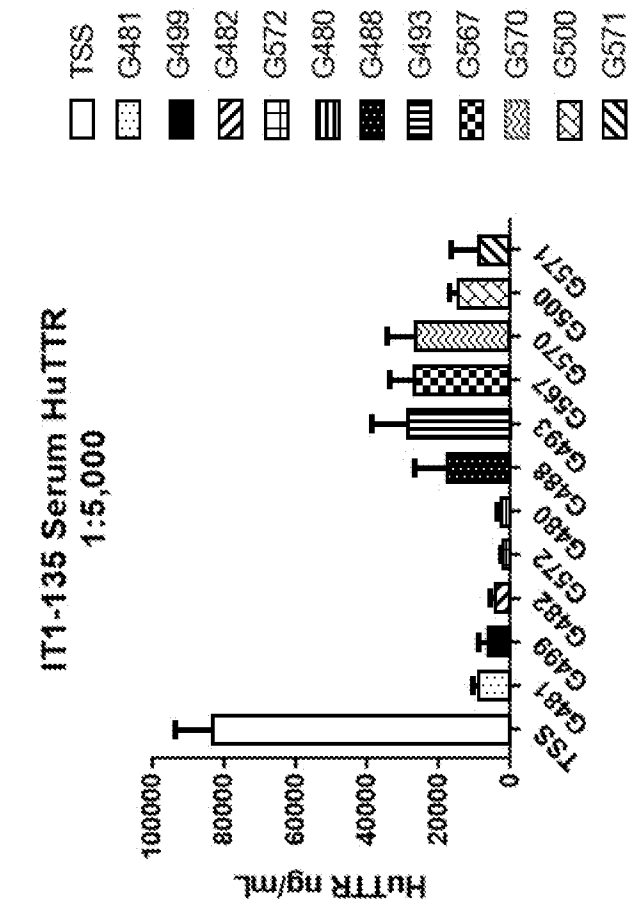

FIG. 14B

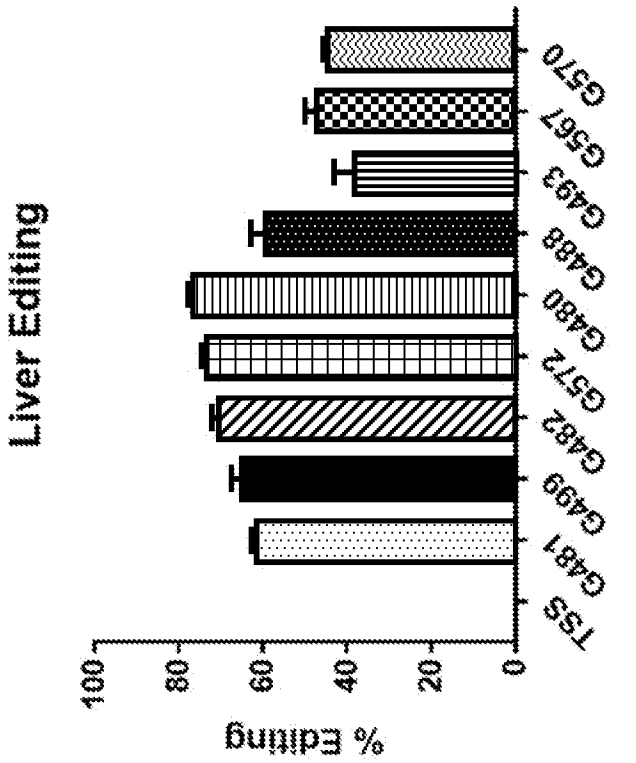

FIG. 14A

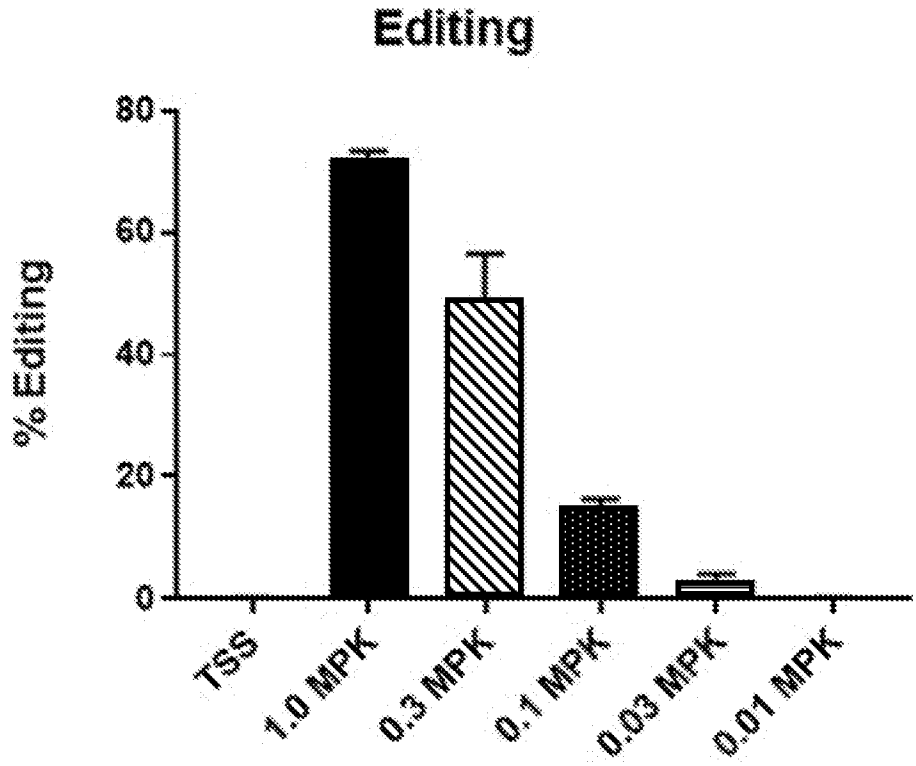
Editing
% Editing
80
60
40
20
0
TSS
1.0 MPK
0.3 MPK
0.1 MPK
0.03 MPK
0.01 MPK

FIG. 15A

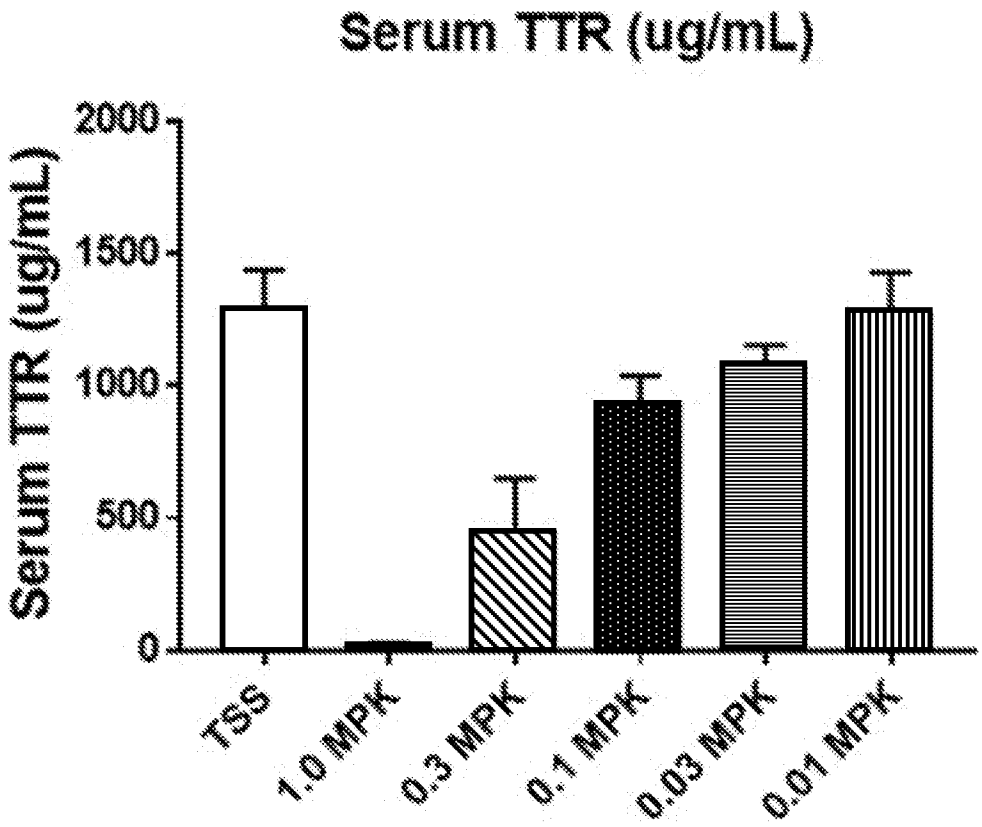
Serum TTR (ug/mL)
Serum TTR (ug/mL)
2000
1500
1000
500
0
TSS
1.0 MPK
0.3 MPK
0.1 MPK
0.03 MPK
0.01 MPK

FIG. 15B

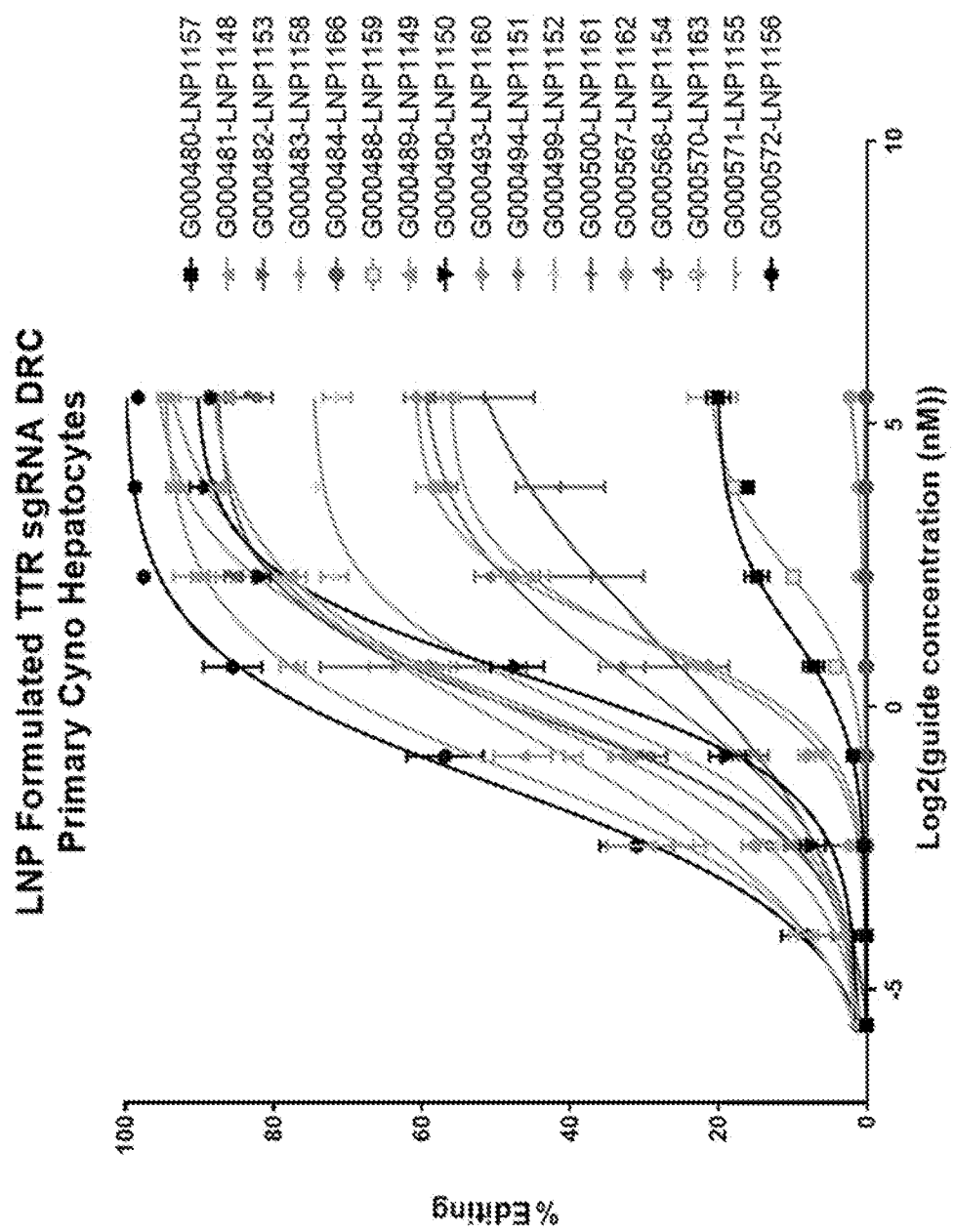
LNP Formulated TTR sgRNA DRC
Primary Cyno Hepatocytes
G000480-LNP1157
G000481-LNP1148
G000482-LNP1153
G000483-LNP1158
G000484-LNP1166
G000488-LNP1159
G000489-LNP1149
G000490-LNP1150
G000493-LNP1160
G000494-LNP1151
G000499-LNP1152
G000500-LNP1161
G000567-LNP1162
G000568-LNP1154
G000570-LNP1163
G000571-LNP1155
G000572-LNP1156
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 16

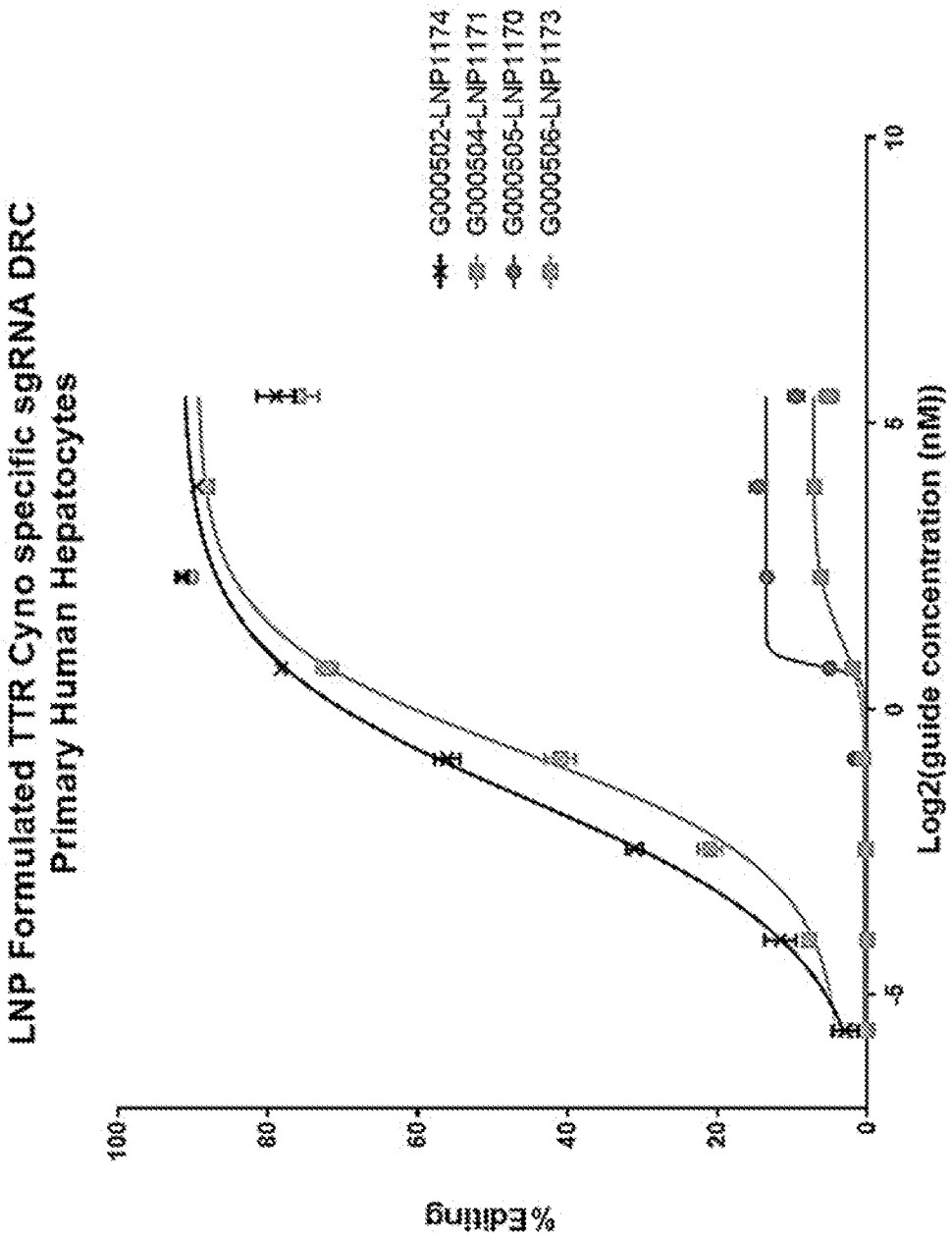
LNP Formulated TTR Cyno specific sgRNA DRC
Primary Human Hepatocytes
G000502-LNP1174
G000504-LNP1171
G000505-LNP1170
G000506-LNP1173
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 17

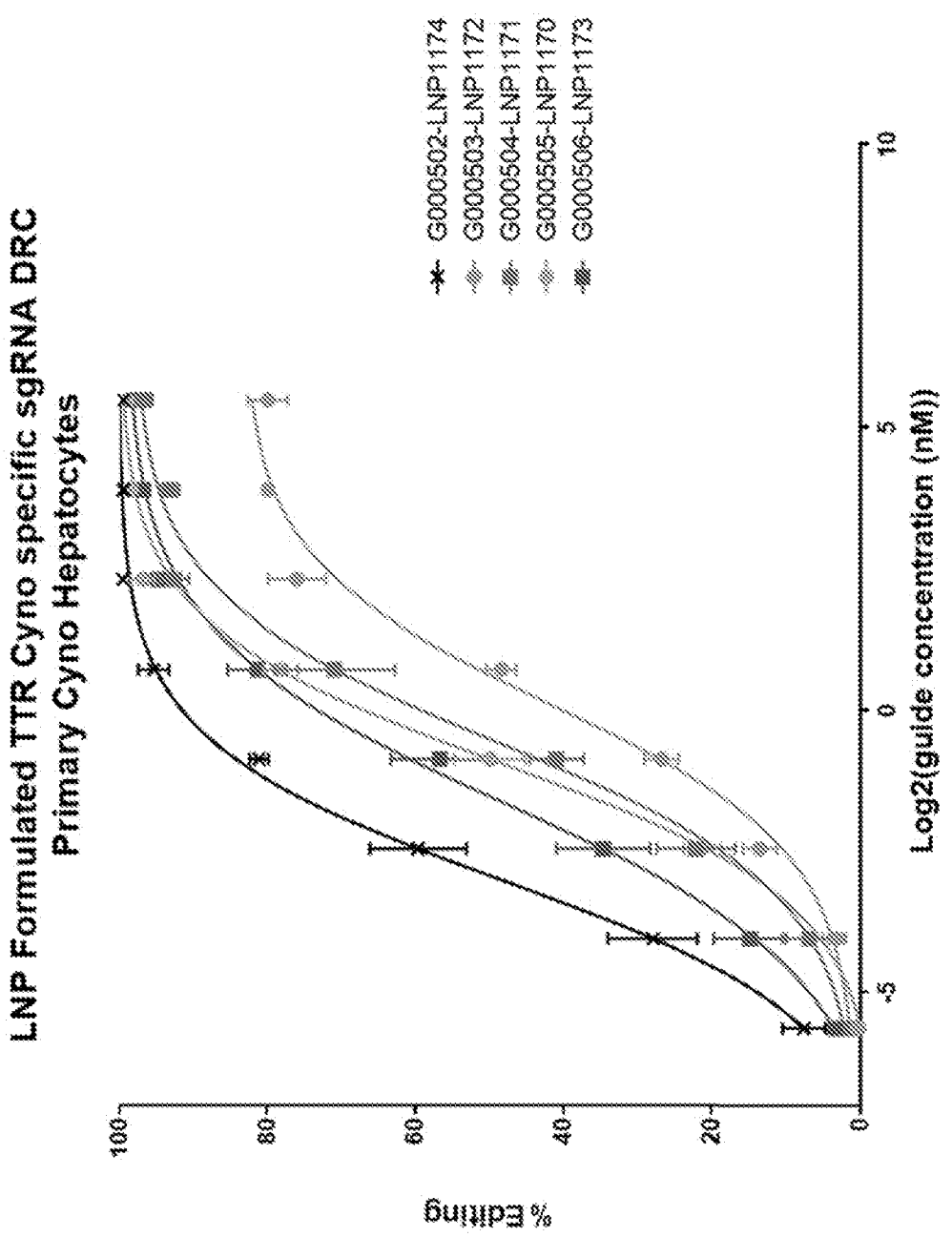
LNP Formulated TTR Cyno specific sgRNA DRC
Primary Cyno Hepatocytes
G000502-LNP1174
G000503-LNP1172
G000504-LNP1171
G000505-LNP1170
G000506-LNP1173
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 18

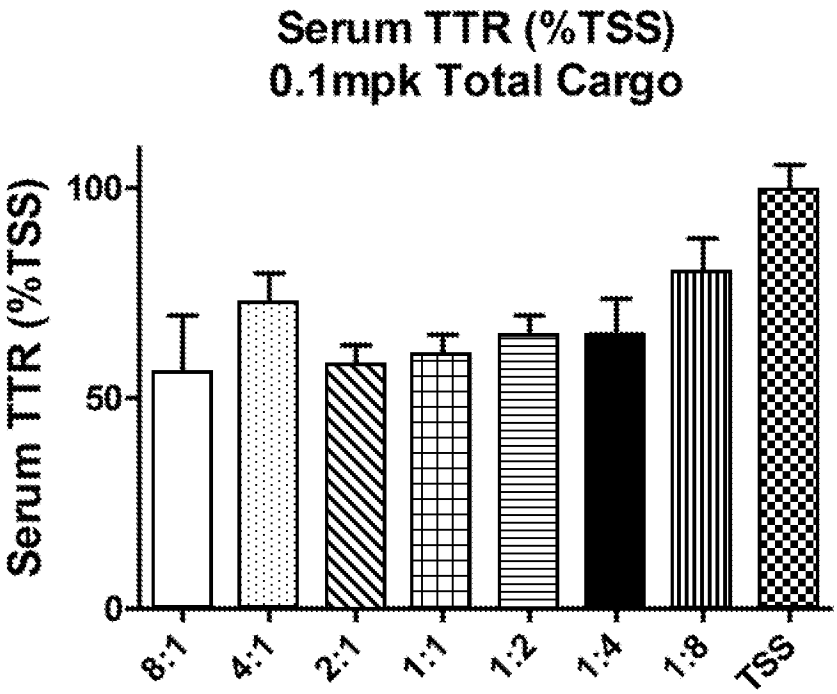

FIG. 19A

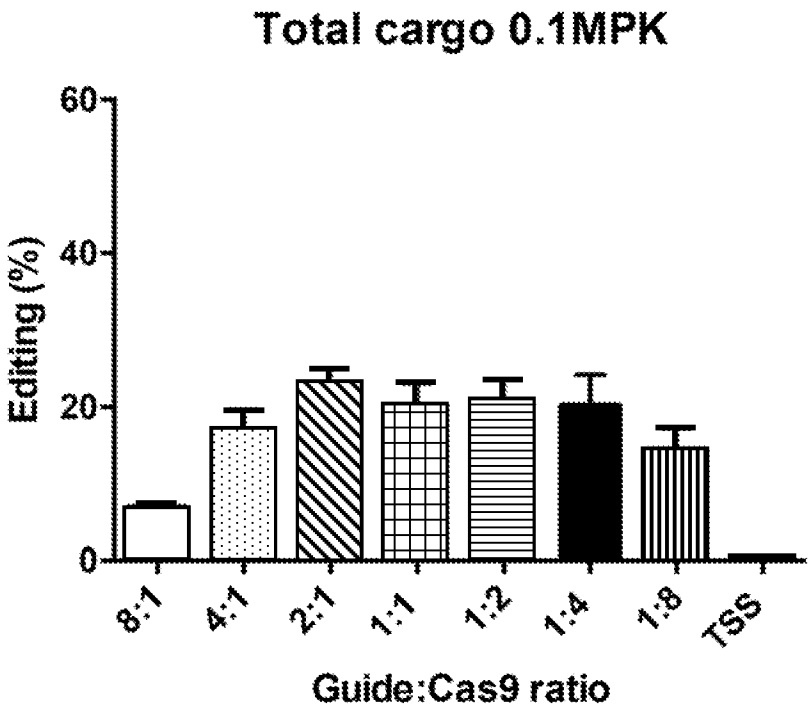

FIG. 19B

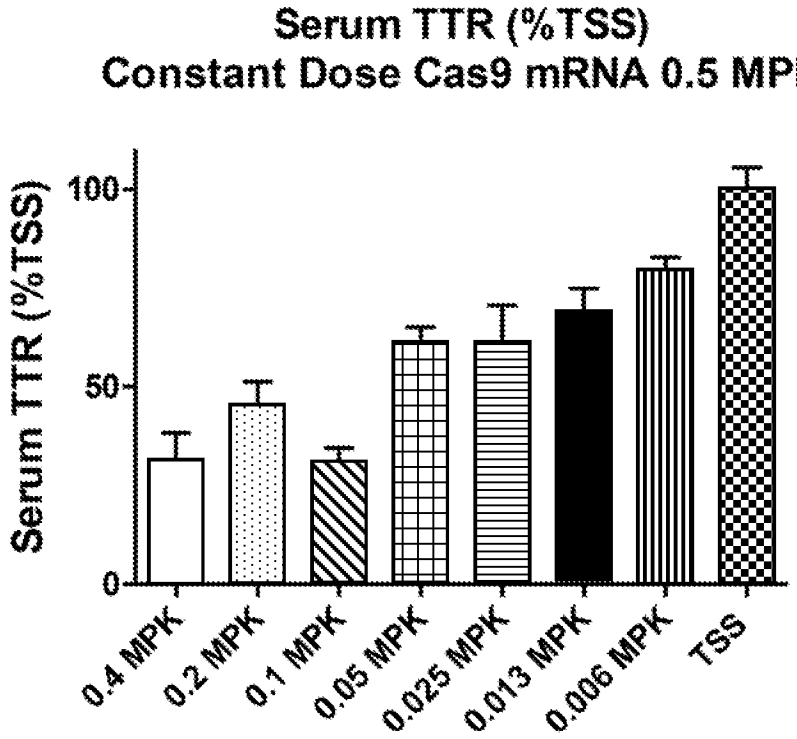

FIG. 19C

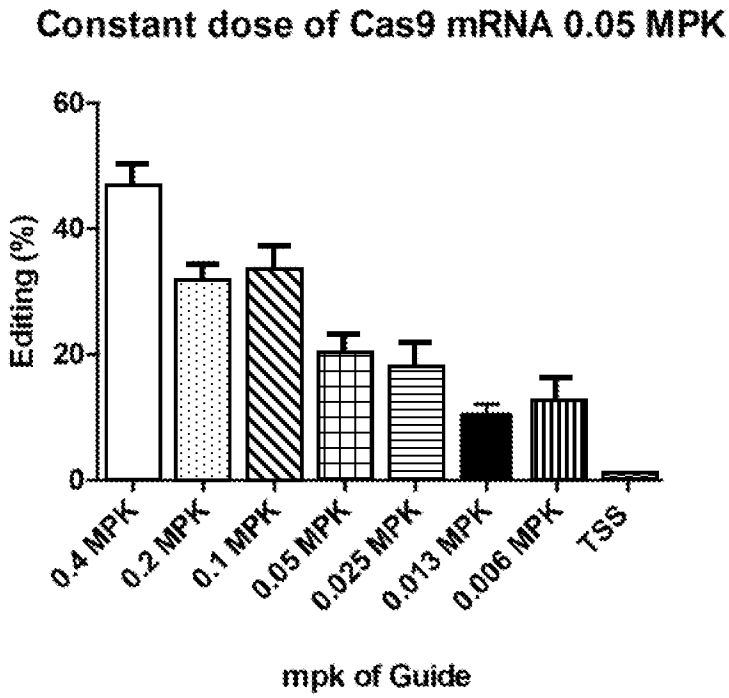

FIG. 19D

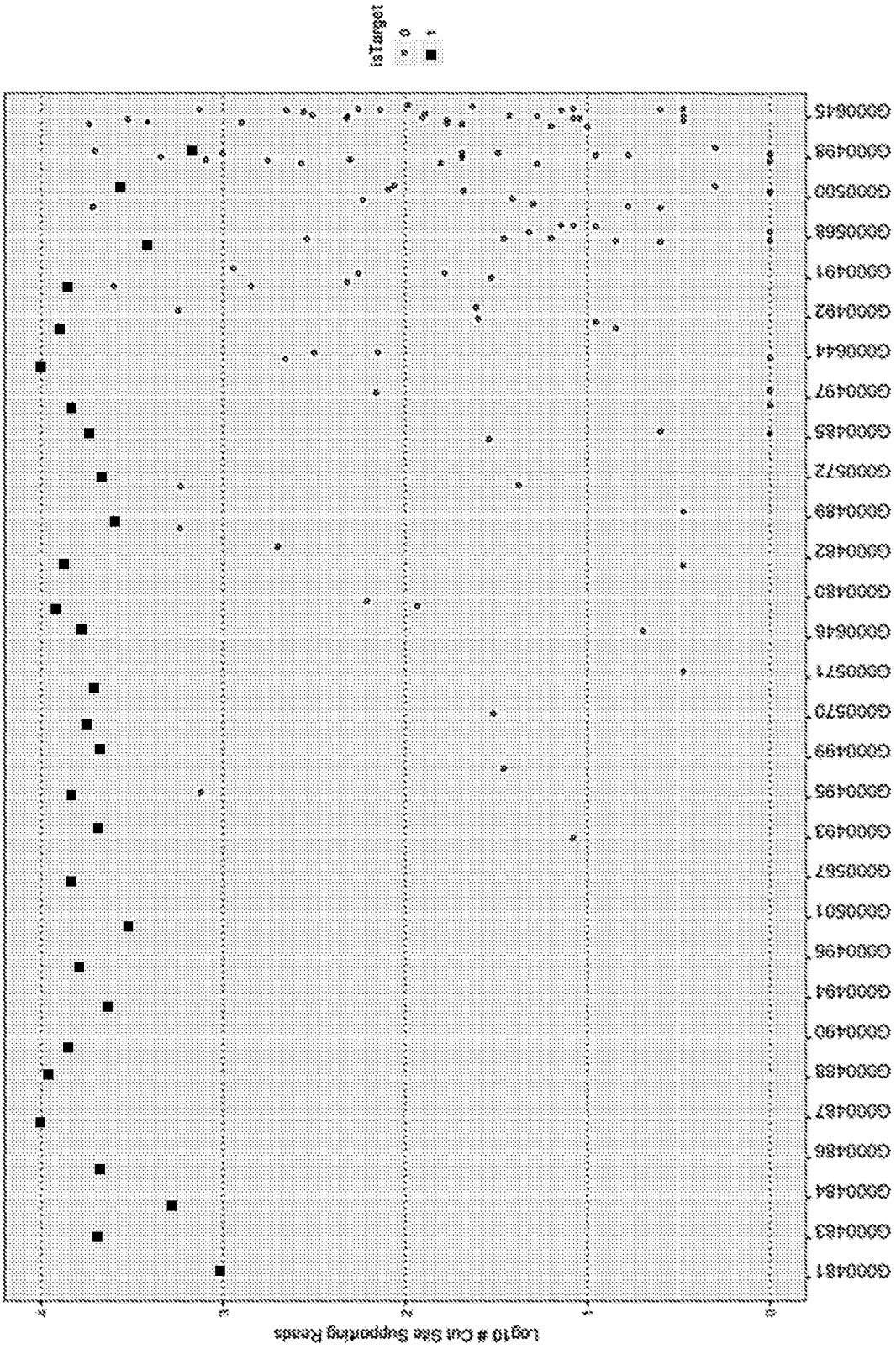
isTarget
0
1
log10 # Cut Site Supporting Reads
4
3
2
1
0
G0006451
G0004981
G0005001
G0005681
G0004911
G0004921
G0006441
G0004971
G0004851
G0005721
G0004891
G0004821
G0004801
G0006461
G0005711
G0005701
G0004991
G0004951
G0004931
G0005671
G0006011
G0004961
G0004941
G0004901
G0004681
G0004871
G0004861
G0004641
G0004631

FIG. 20

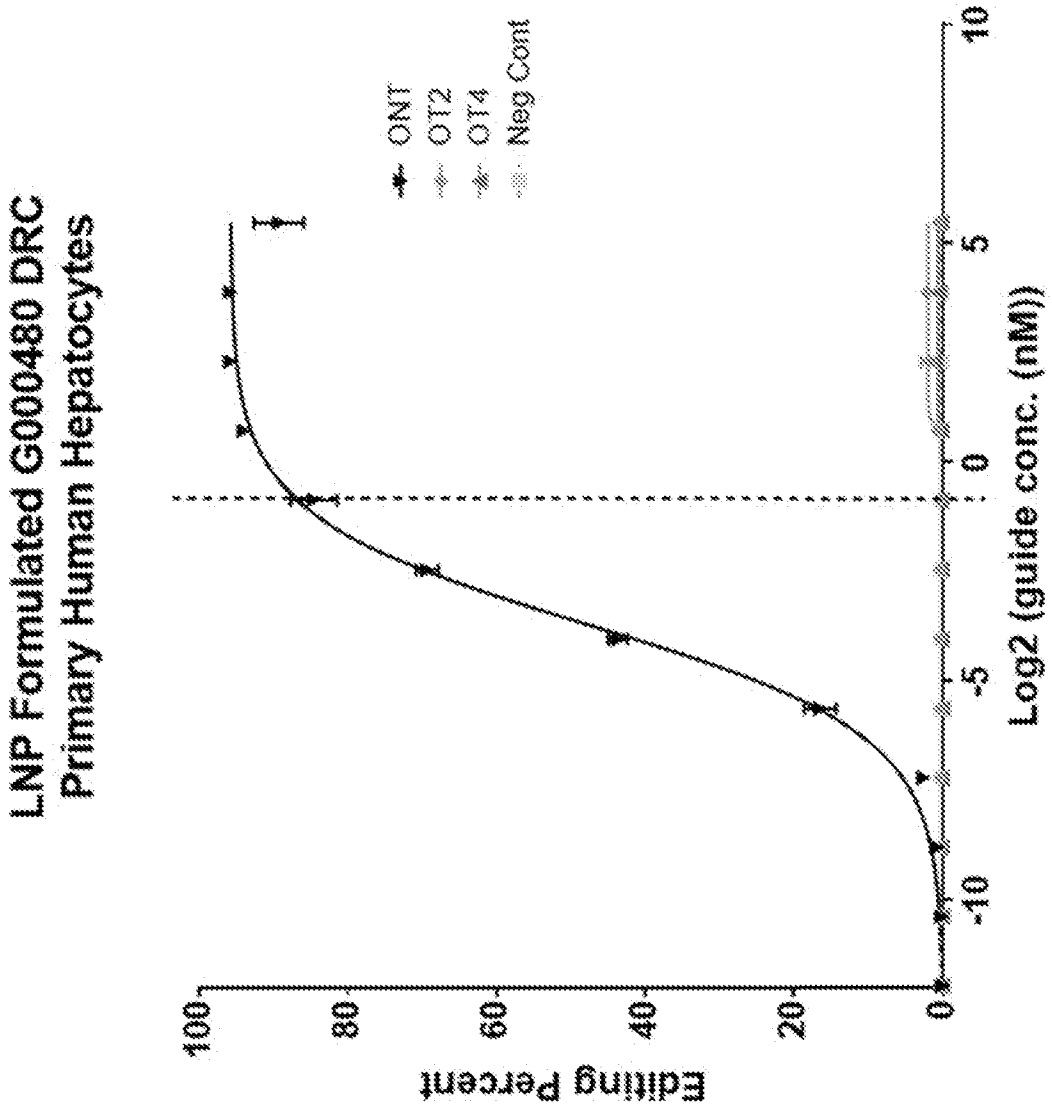
LNP Formulated G000480 DRC
Primary Human Hepatocytes
ONT
OT2
OT4
Neg Cont
Editing Percent
100
80
60
40
20
0
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 21A

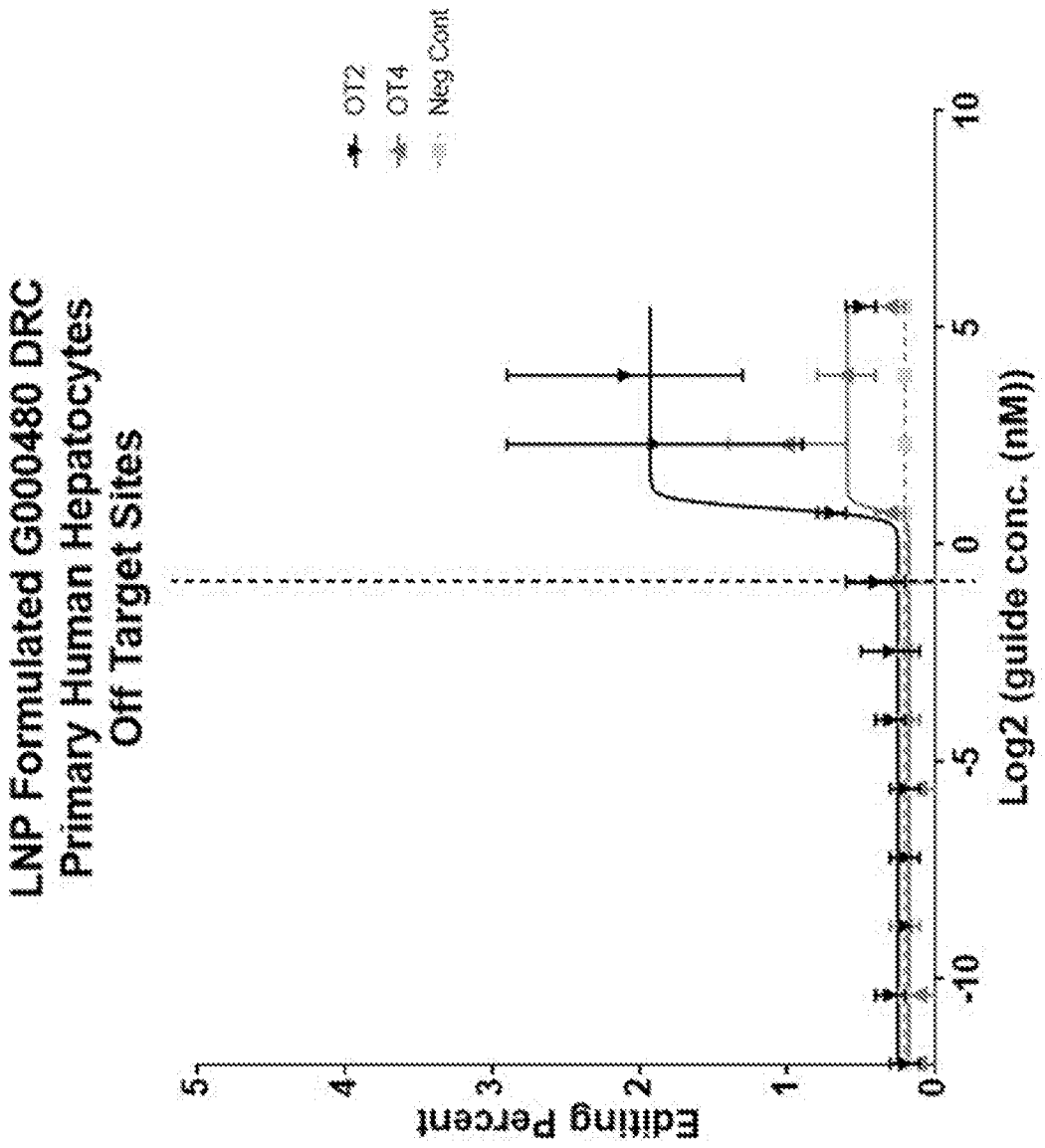
LNP Formulated G000480 DRC
Primary Human Hepatocytes
Off Target Sites
OT2
OT4
Neg Cont
Editing Percent
0
1
2
3
4
5
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 21B

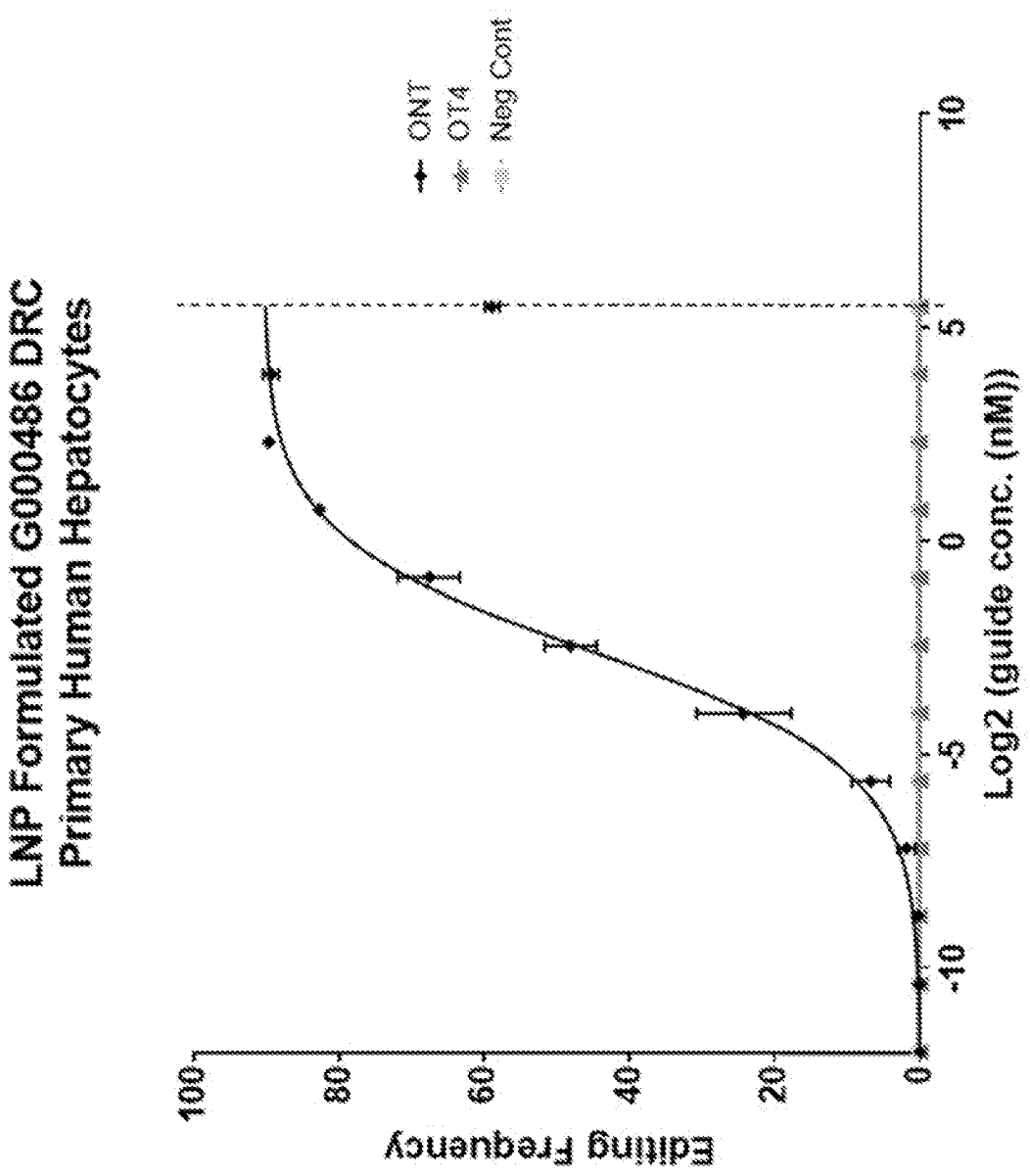
LNP Formulated G000486 DRC
Primary Human Hepatocytes
ONT
OT4
Neg Cont
Editing Frequency
0
20
40
60
80
100
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 22A

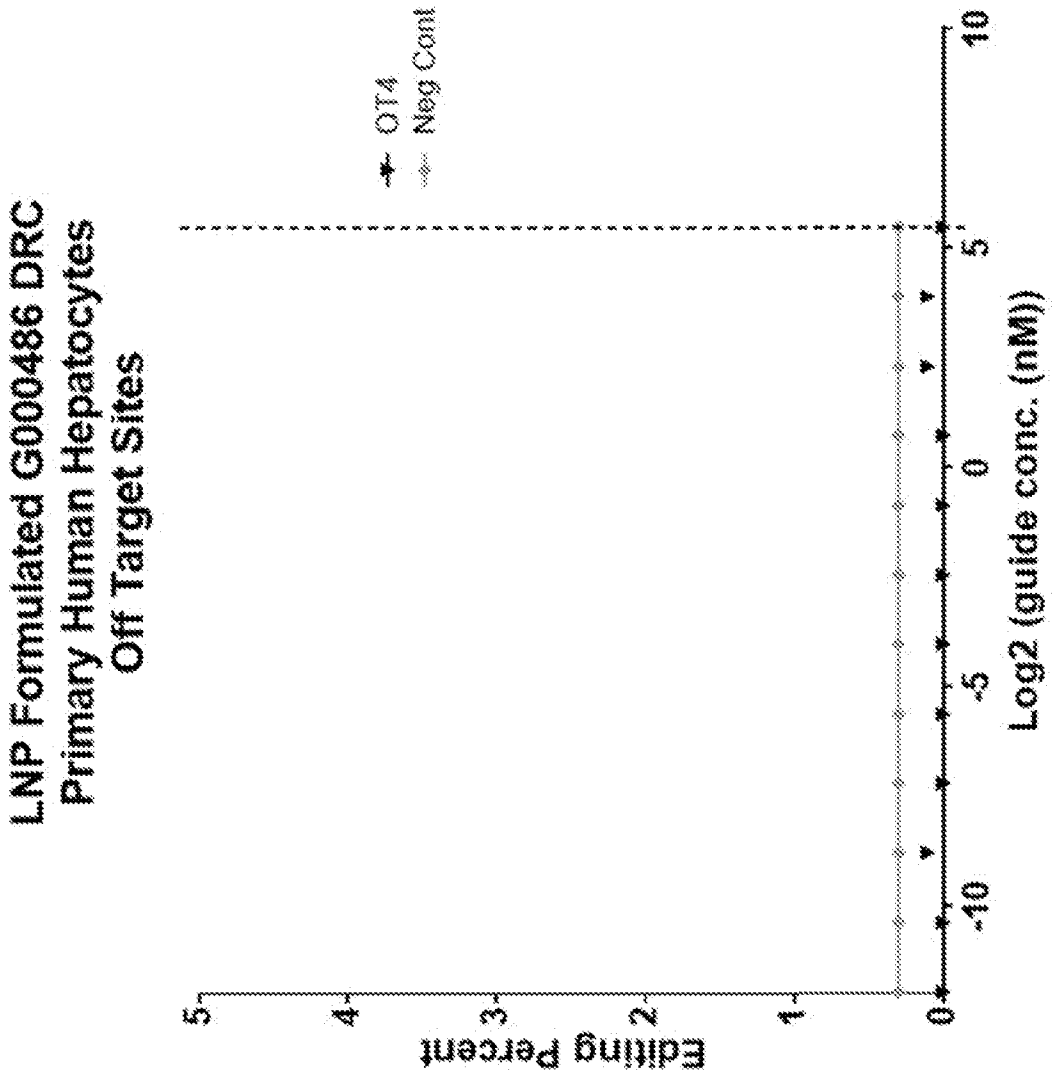
LNP Formulated G000486 DRC
Primary Human Hepatocytes
Off Target Sites
OT4
Neg Cont
Editing Percent
Log2 (guide conc. (nM))
0
1
2
3
4
5
-10
-5
0
5
10

FIG. 22B

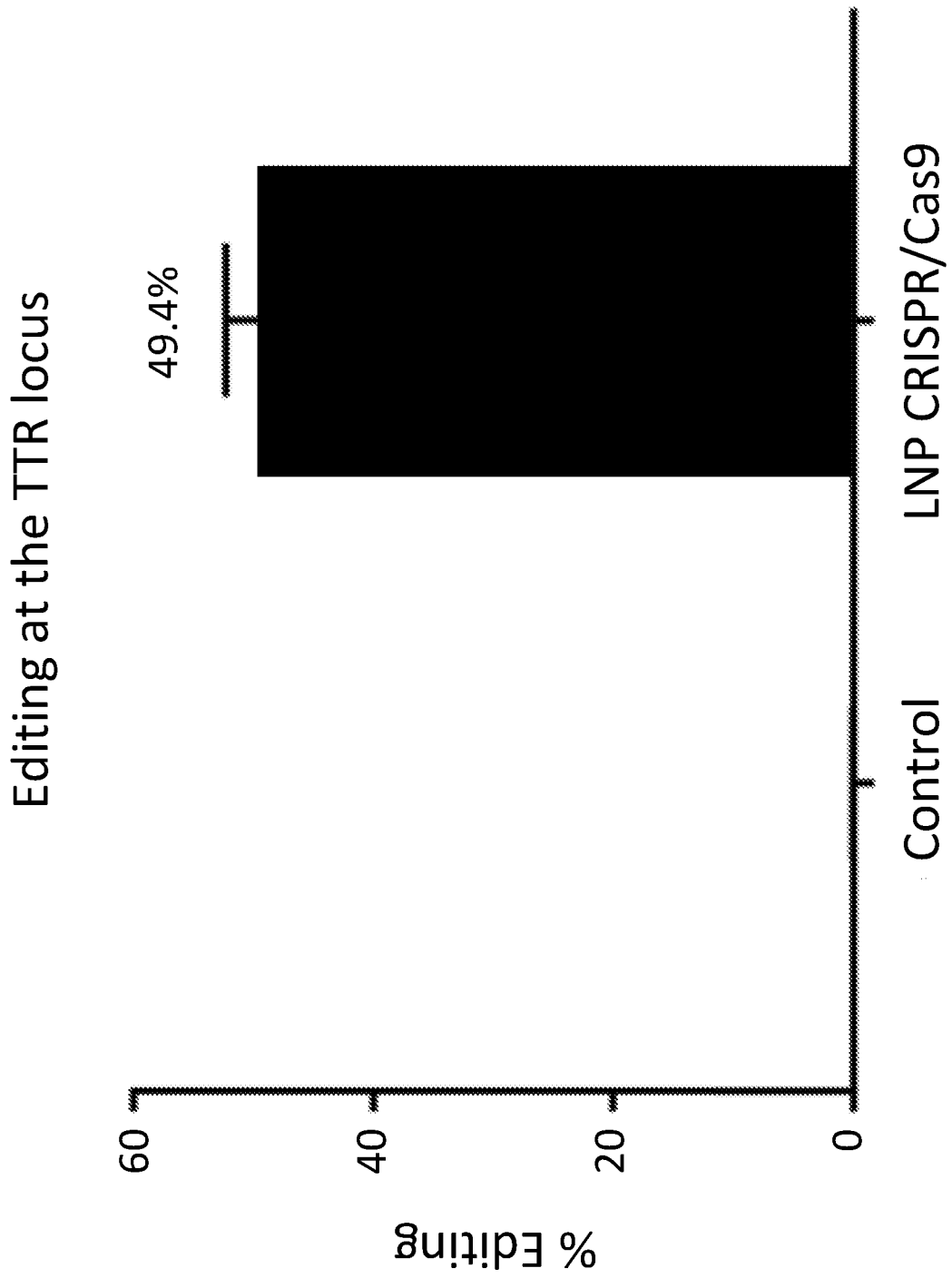
Editing at the TTR locus
49.4%
% Editing
60
40
20
0
Control
LNP CRISPR/Cas9

FIG. 23A

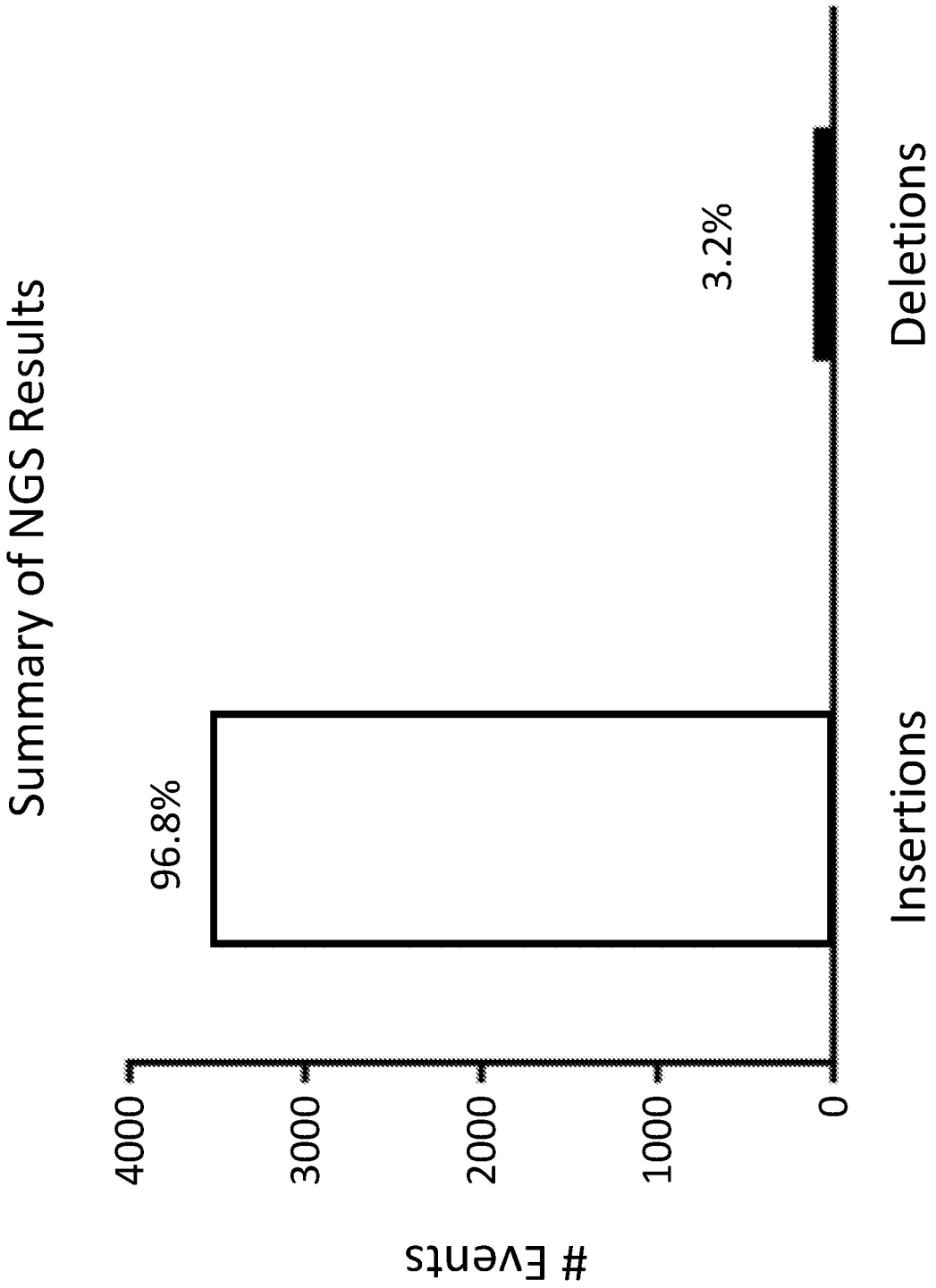
Summary of NGS Results
Events
4000
3000
2000
1000
0
96.8%
3.2%
Insertions
Deletions

FIG. 23B

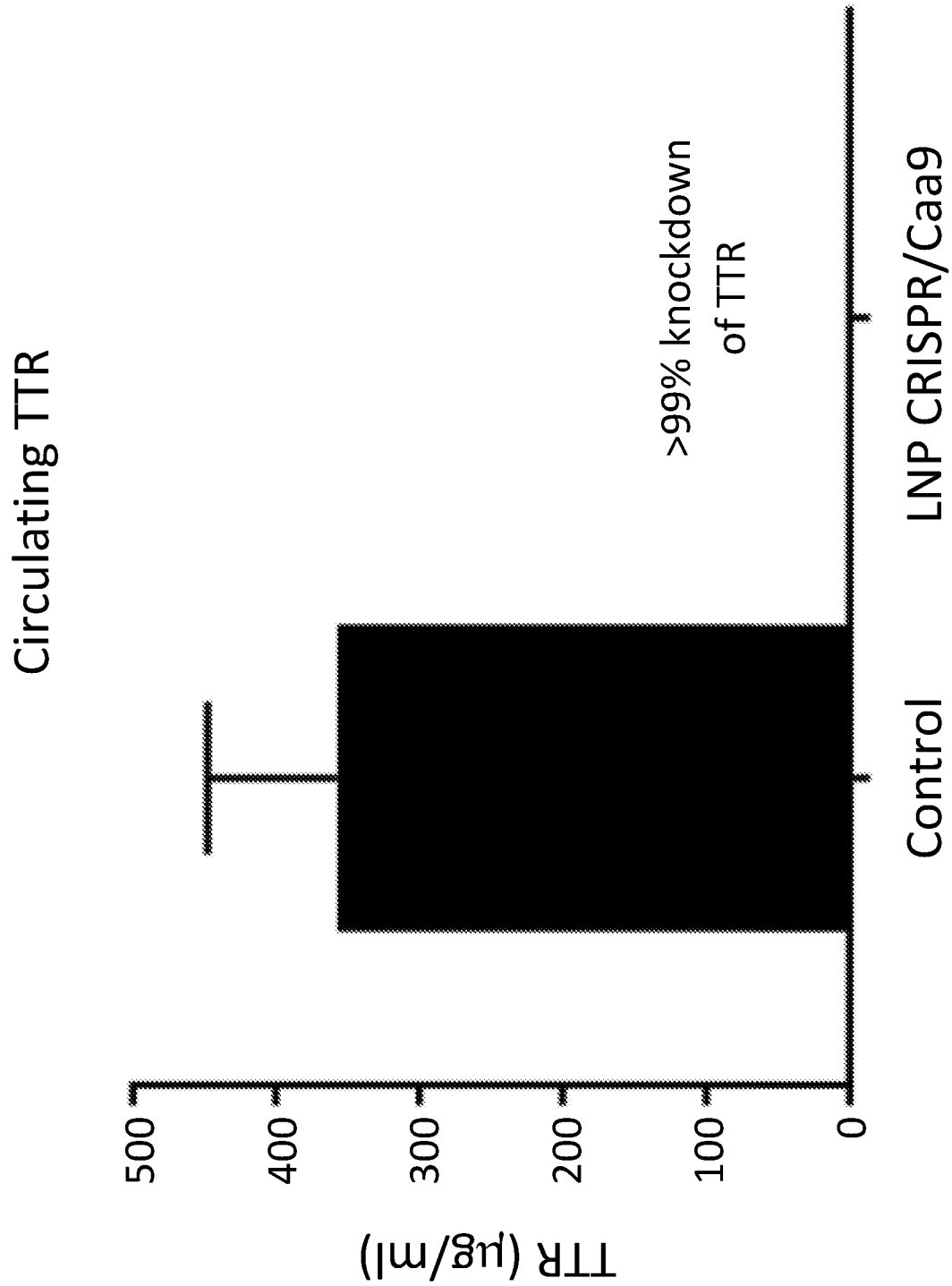
Circulating TTR
TTR (μg/ml)
500
400
300
200
100
0
>99% knockdown
of TTR
Control
LNP CRISPR/Caa9

FIG. 24A

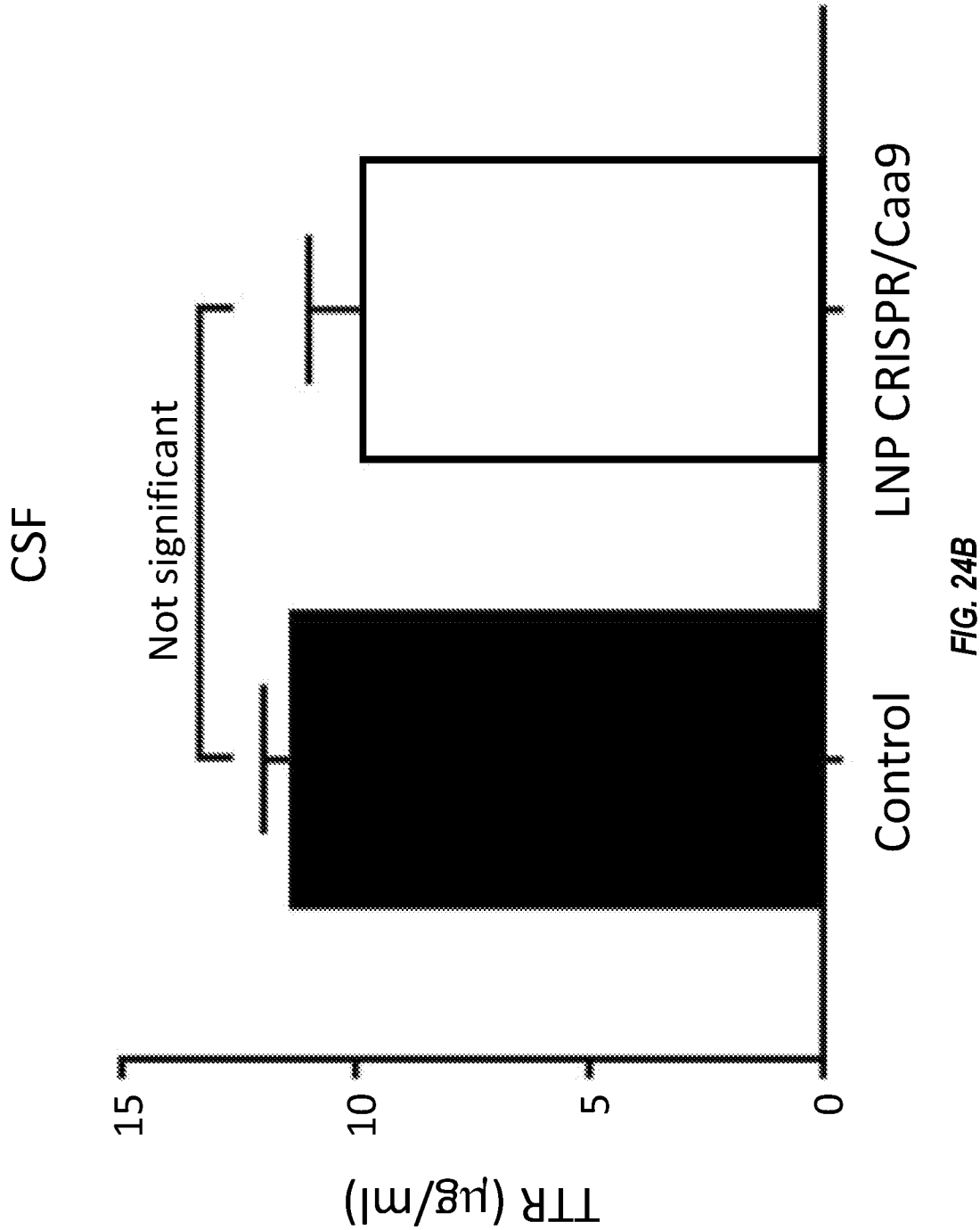
CSF
Not significant
TTR (μg/ml)
15
10
5
0
Control
LNP CRISPR/Caa9

FIG. 24B

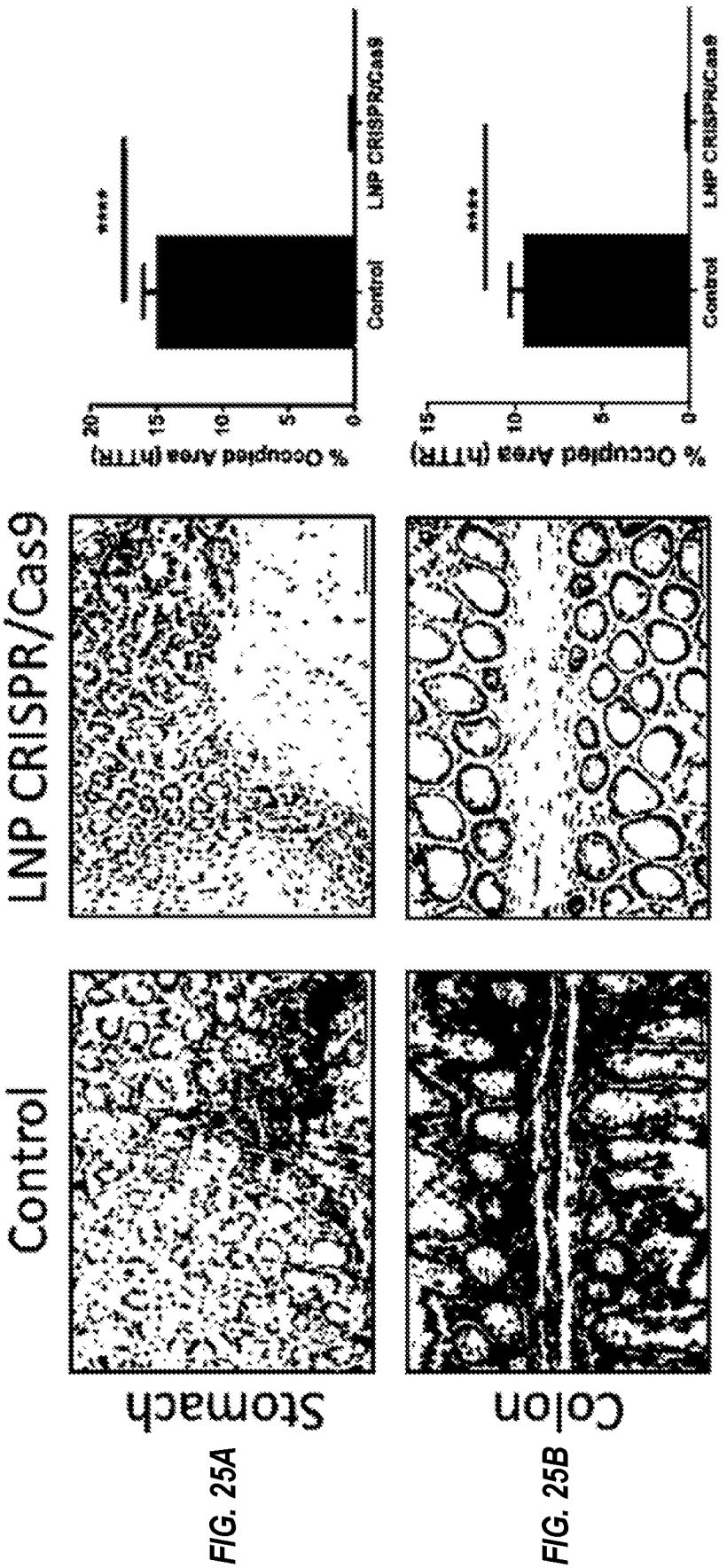

FIG. 25A

FIG. 25B

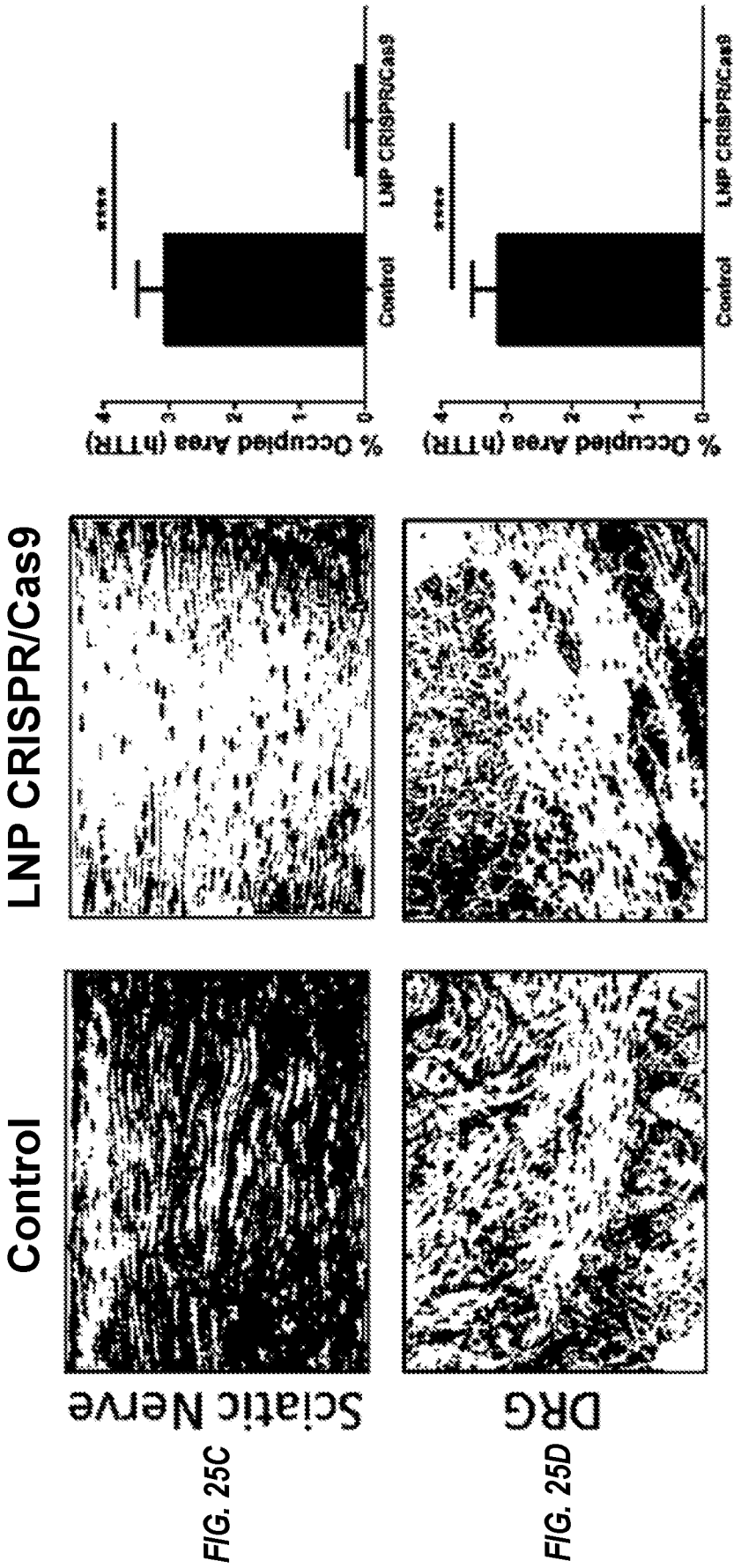

FIG. 25C

FIG. 25D

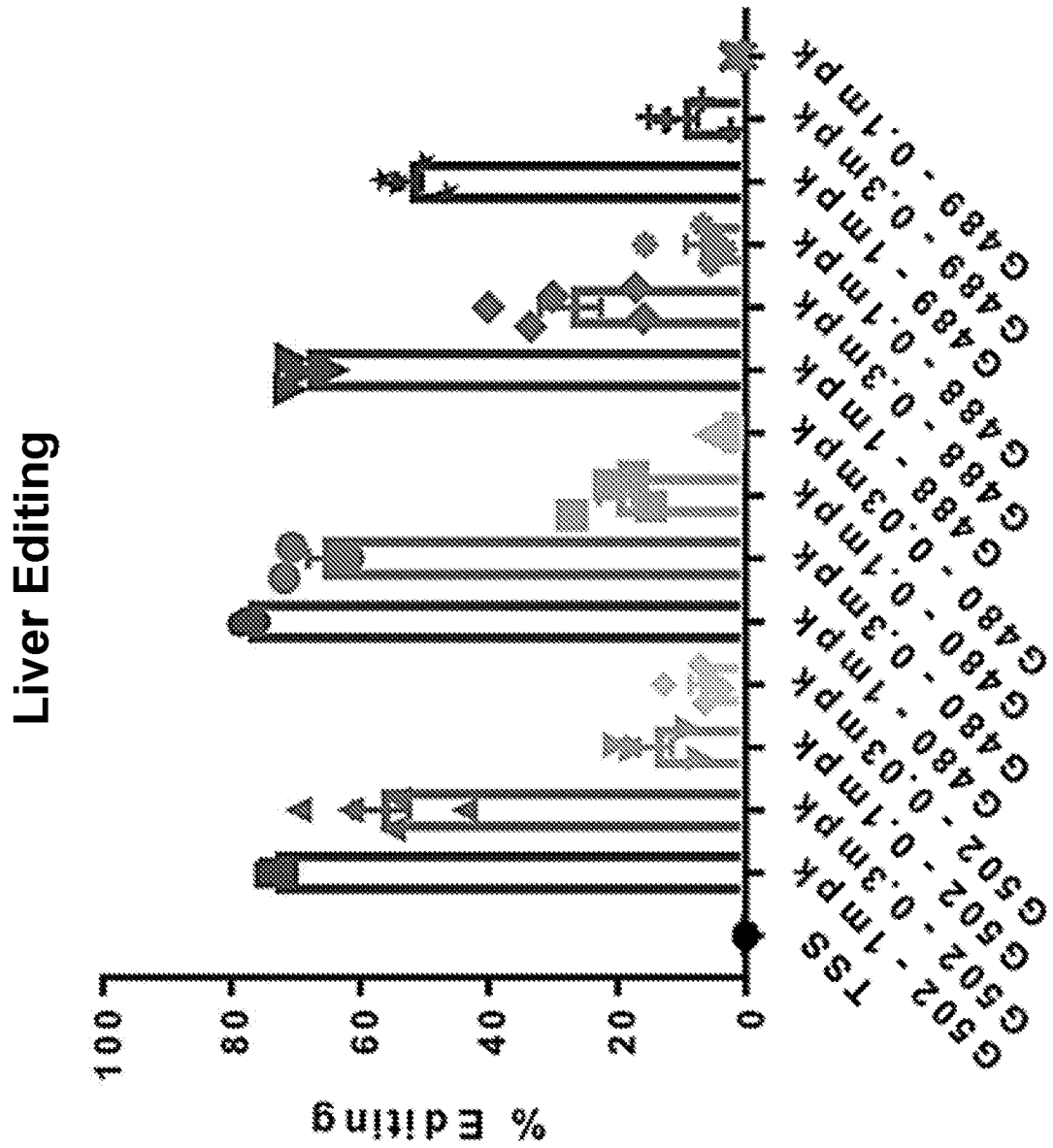
Liver Editing
% Editing
100
80
60
40
20
0
TSS - 1m pk
G502 - 0.3m pk
G502 - 0.1m pk
G502 - 0.03m pk
G502 - 1m pk
G480 - 0.3m pk
G480 - 0.1m pk
G480 - 0.03m pk
G480 - 1m pk
G488 - 0.3m pk
G488 - 0.1m pk
G488 - 0.03m pk
G488 - 1m pk
G489 - 0.3m pk
G489 - 0.1m pk
G489 - 1m pk

FIG. 26A

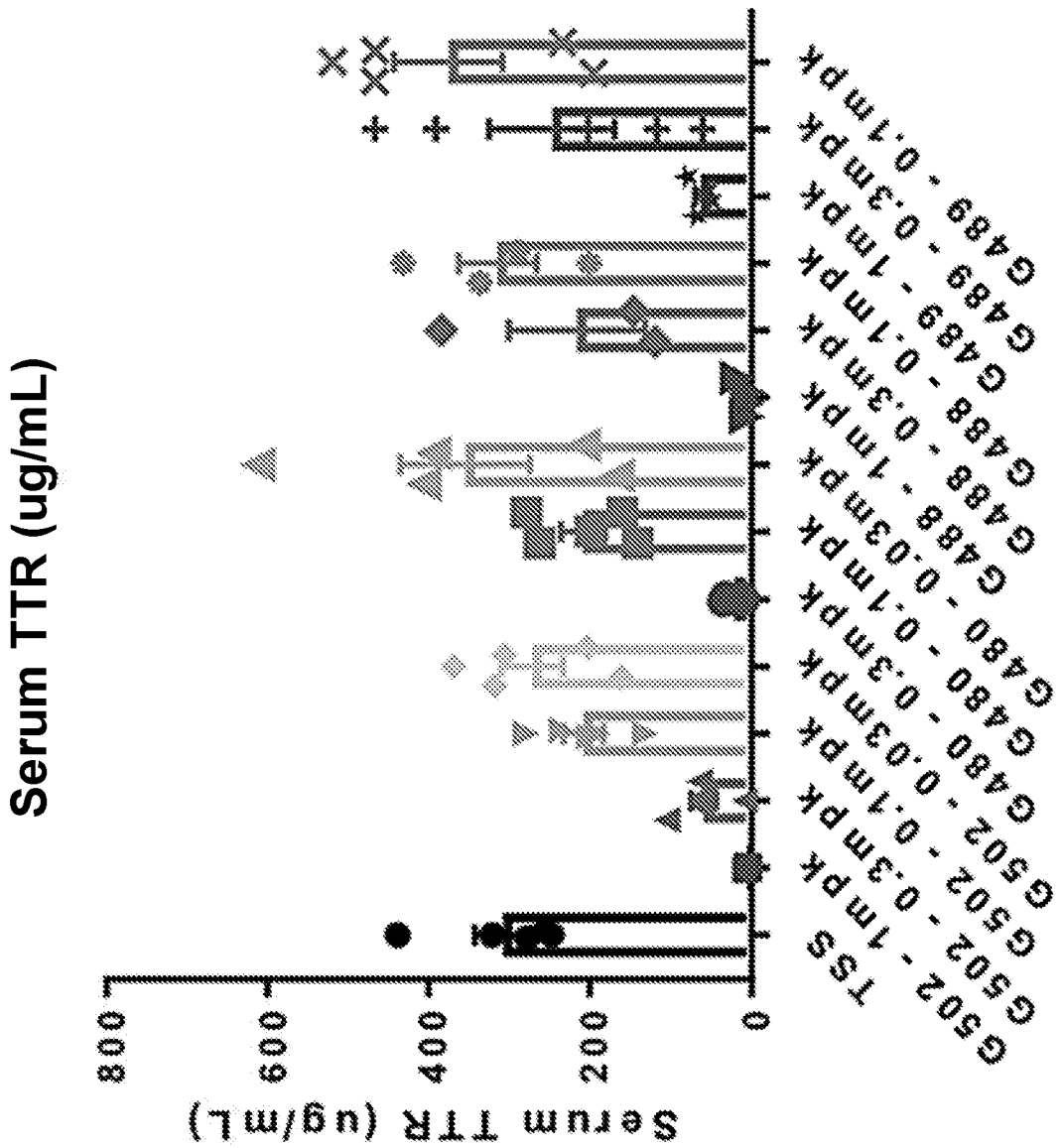
Serum TTR (ug/mL)
800
600
400
200
0
TSS - 1m pk
G502 - 0.3m pk
G502 - 0.1m pk
G502 - 0.03m pk
G480 - 0.3m pk
G480 - 0.1m pk
G480 - 0.03m pk
G488 - 1m pk
G488 - 0.3m pk
G488 - 0.1m pk
G489 - 1m pk
G489 - 0.3m pk
G489 - 0.1m pk

FIG. 26B

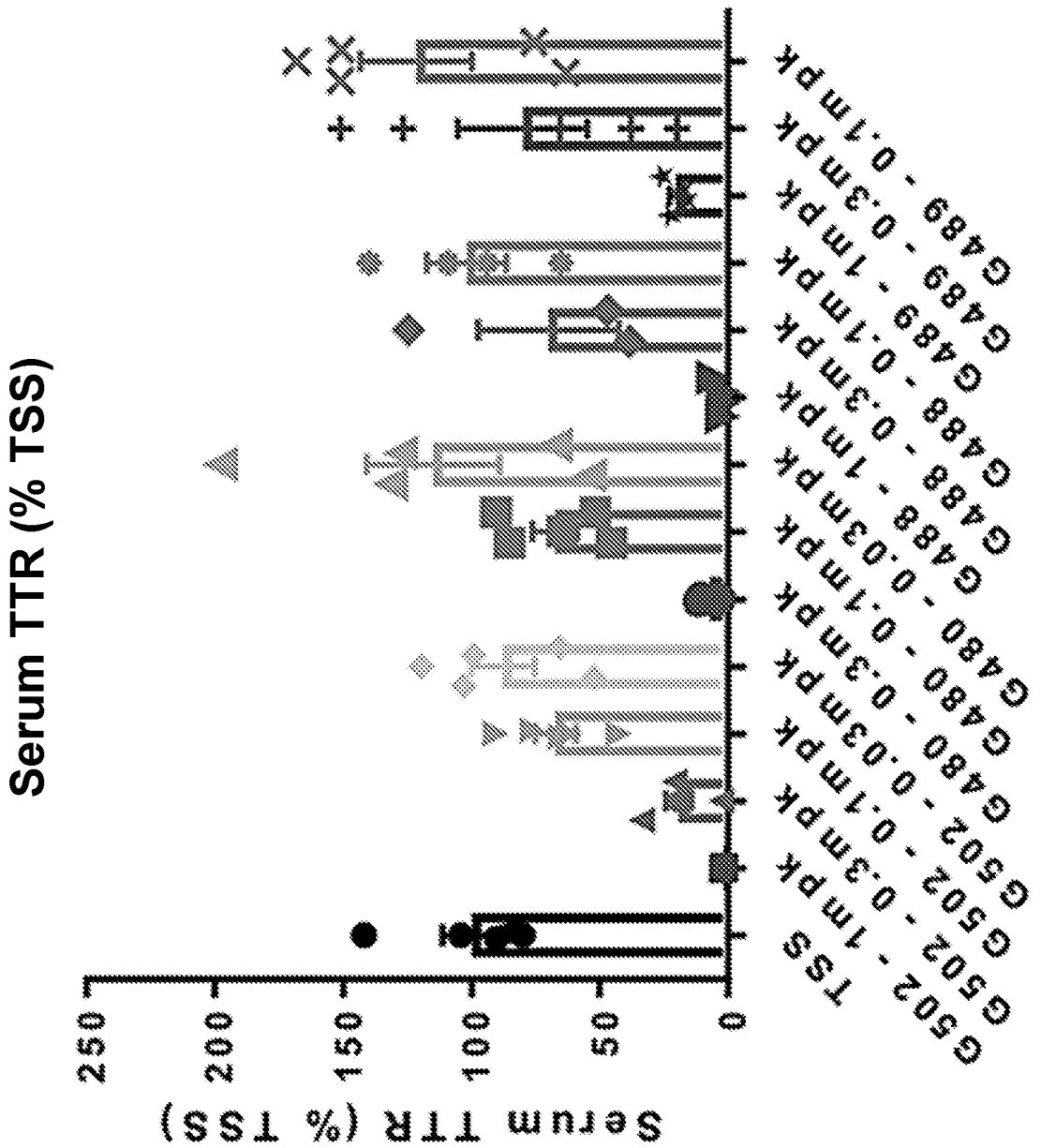
Serum TTR (% TSS)
250
200
150
100
50
0
TSS - 1m pk
G502 - 0.3m pk
G502 - 0.1m pk
G502 - 0.03m pk
G480 - 0.3m pk
G480 - 0.1m pk
G480 - 0.03m pk
G488 - 1m pk
G488 - 0.3m pk
G488 - 0.1m pk
G489 - 1m pk
G489 - 0.3m pk
G489 - 0.1m pk

FIG. 26C

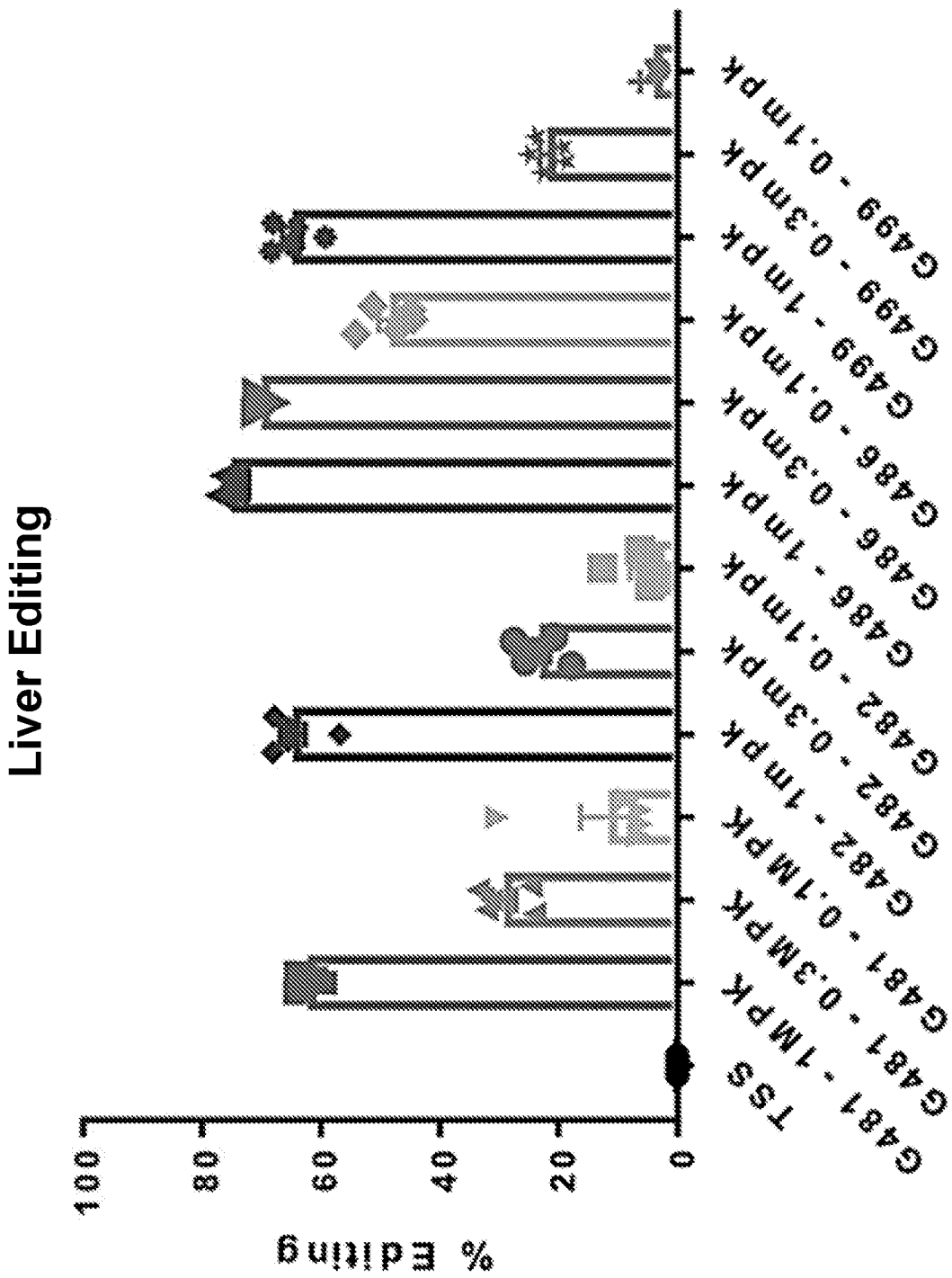
Liver Editing
% Editing
100
80
60
40
20
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G482 - 1MPK
G482 - 0.3MPK
G482 - 0.1MPK
G486 - 1MPK
G486 - 0.3MPK
G486 - 0.1MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

FIG. 27A

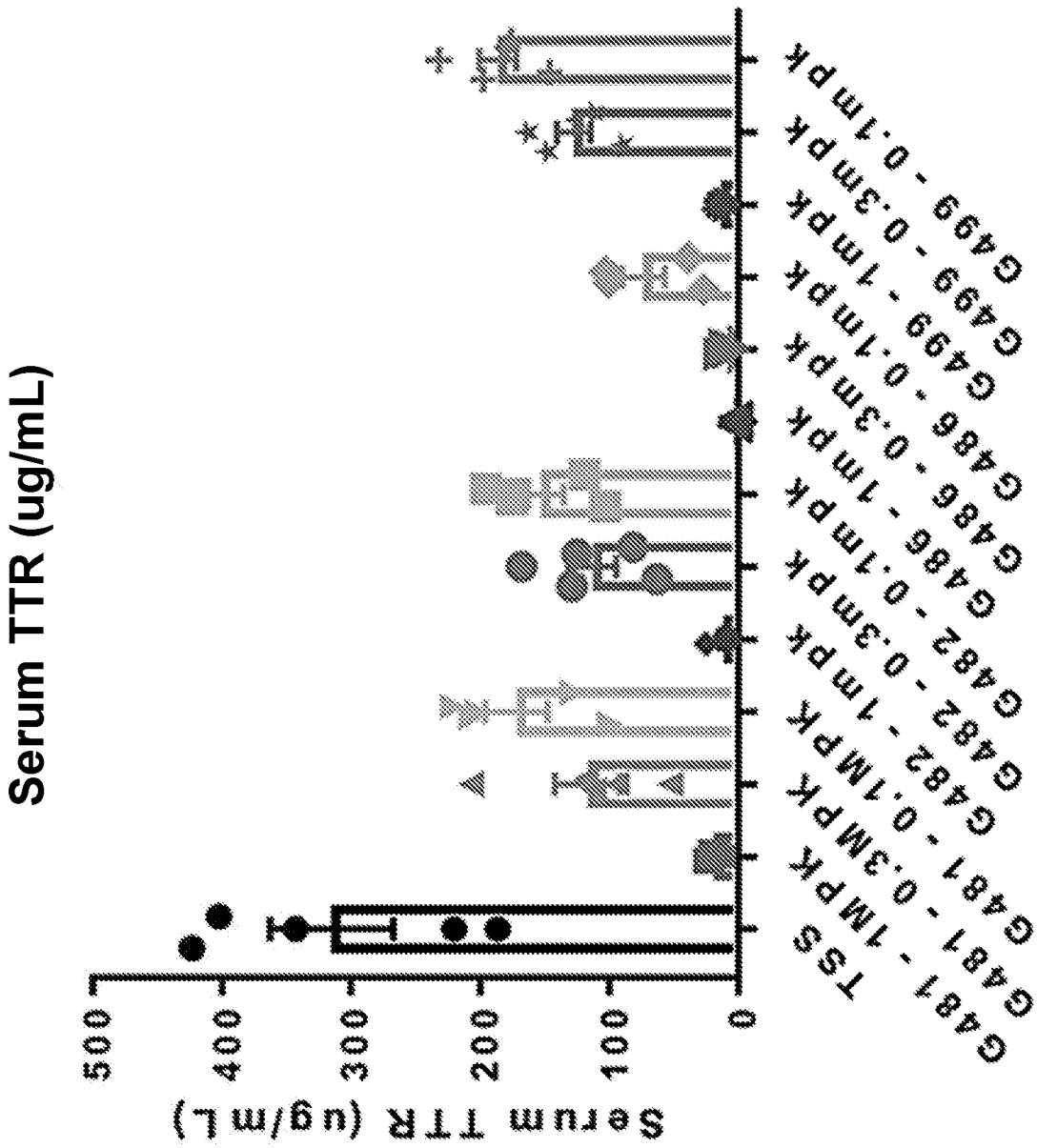
Serum TTR (ug/mL)
500
400
300
200
100
0
TSS - 1MPK
G481 - 0.3MPK
G481 - 1MPK
G482 - 0.3MPK
G482 - 1MPK
G486 - 0.1MPK
G486 - 0.3MPK
G486 - 1MPK
G499 - 0.1MPK
G499 - 0.3MPK
G499 - 1MPK
+
++

FIG. 27B

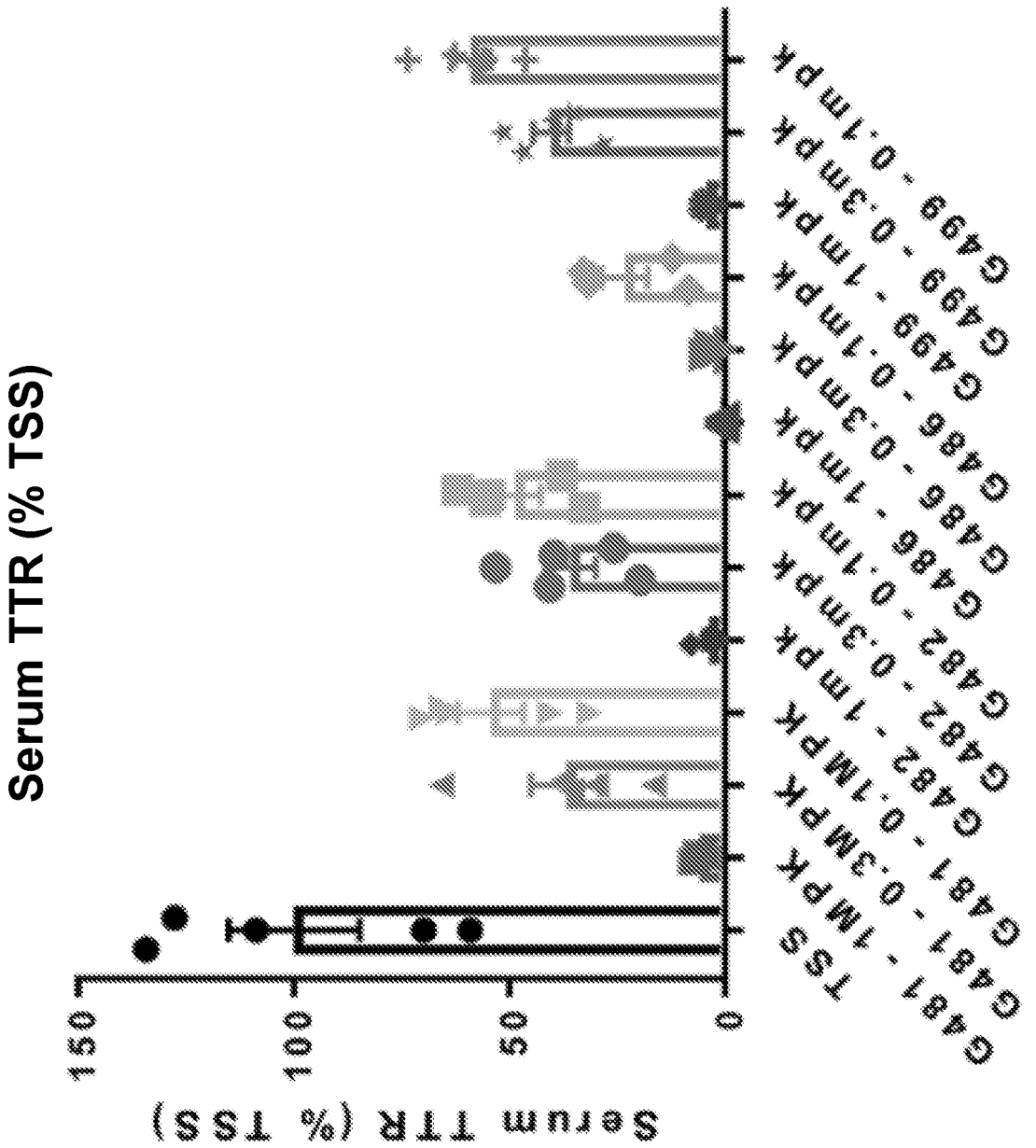
Serum TTR (% TSS)
150
100
50
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G482 - 1MPK
G482 - 0.3MPK
G482 - 0.1MPK
G486 - 1MPK
G486 - 0.3MPK
G486 - 0.1MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

FIG. 27C

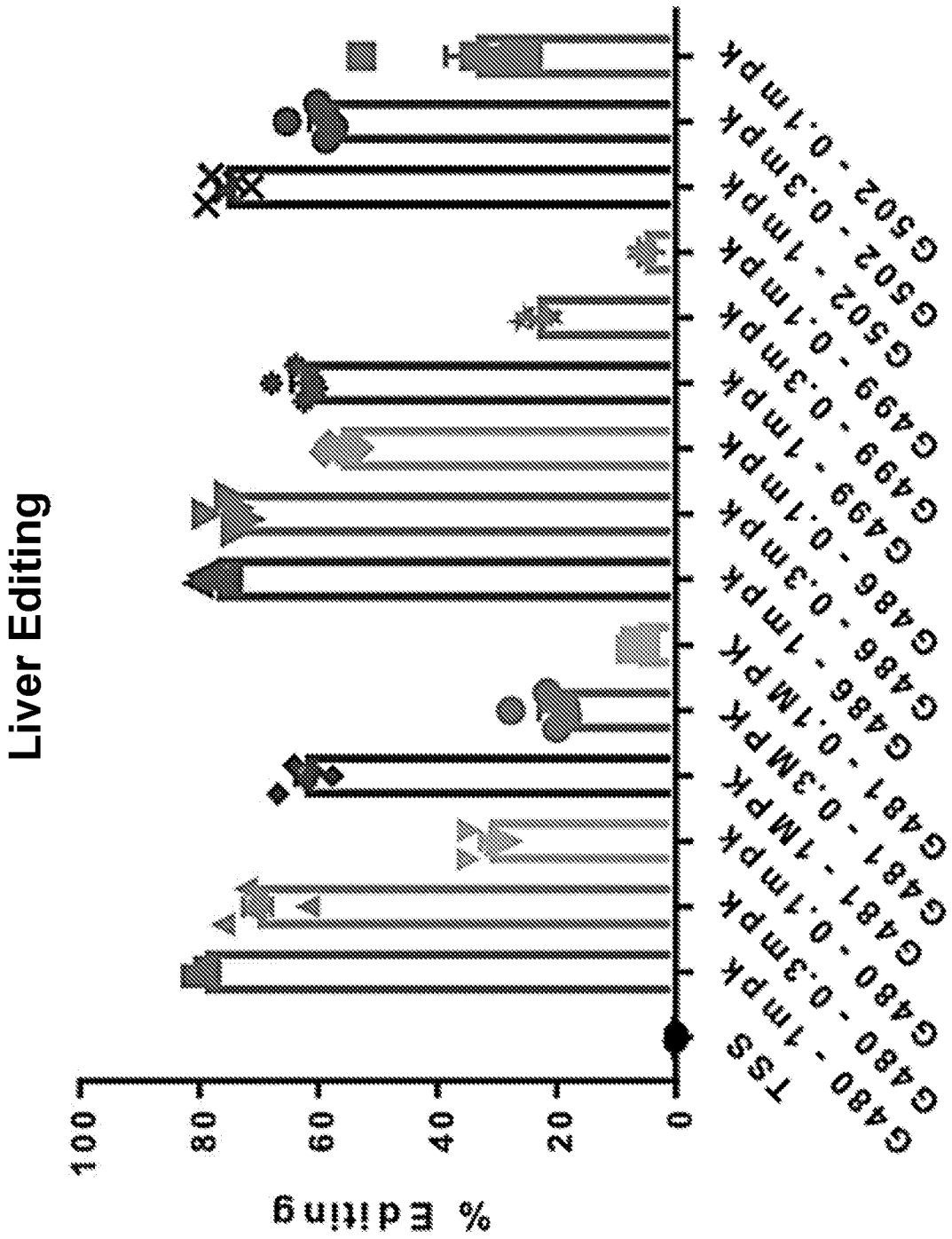
Liver Editing
% Editing
100
80
60
40
20
0
TSS - 1mpk
G480 - 0.3mpk
G480 - 0.1mpk
G481 - 1mpk
G481 - 0.3mpk
G481 - 0.1mpk
G486 - 1mpk
G486 - 0.3mpk
G486 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G499 - 0.1mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

FIG. 28A

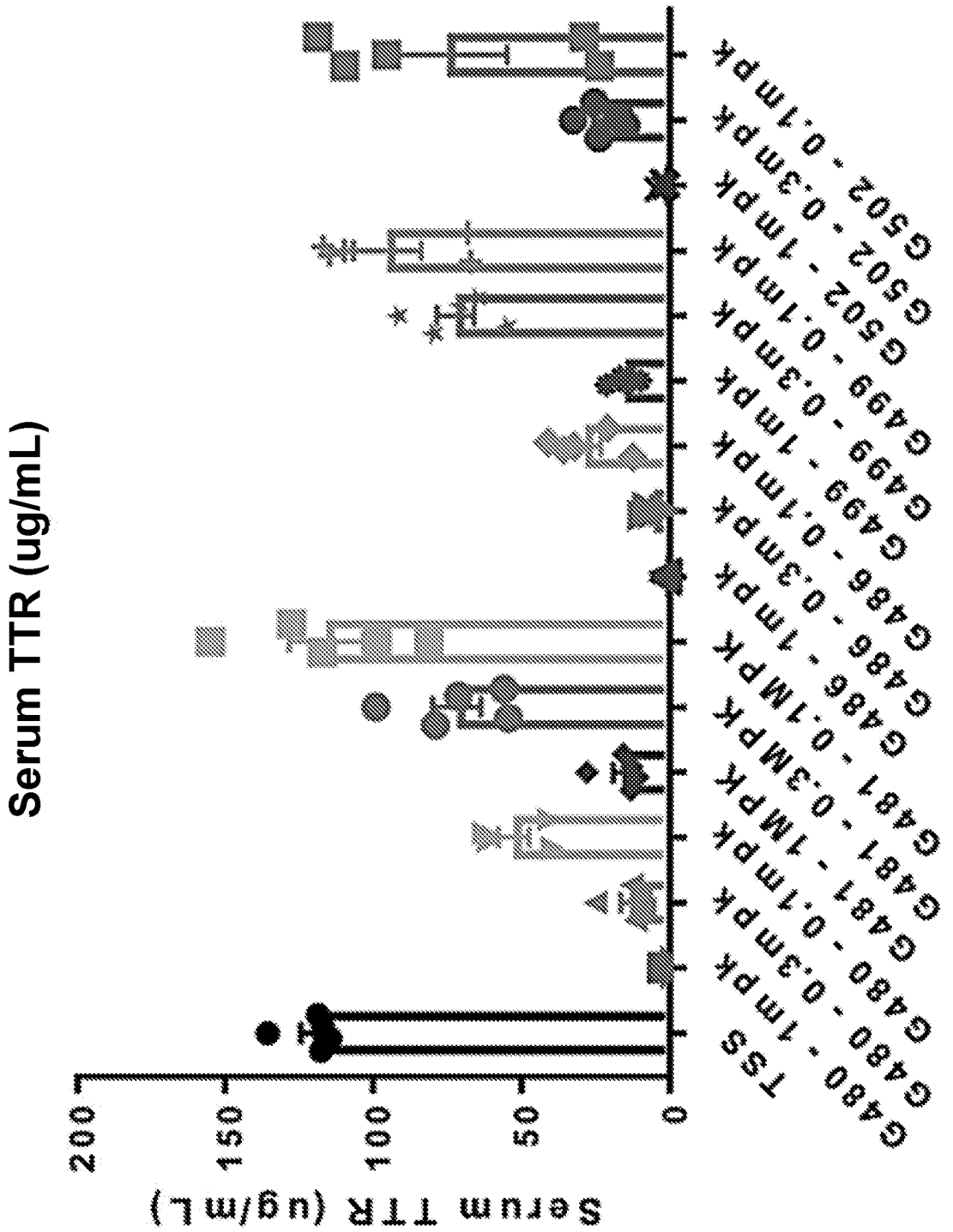
Serum TTR (ug/mL)
200
150
100
50
0
TSS - 1mpk
G480 - 0.3mpk
G480 - 0.1mpk
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G486 - 1MPK
G486 - 0.3mpk
G486 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G499 - 0.1mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

FIG. 28B

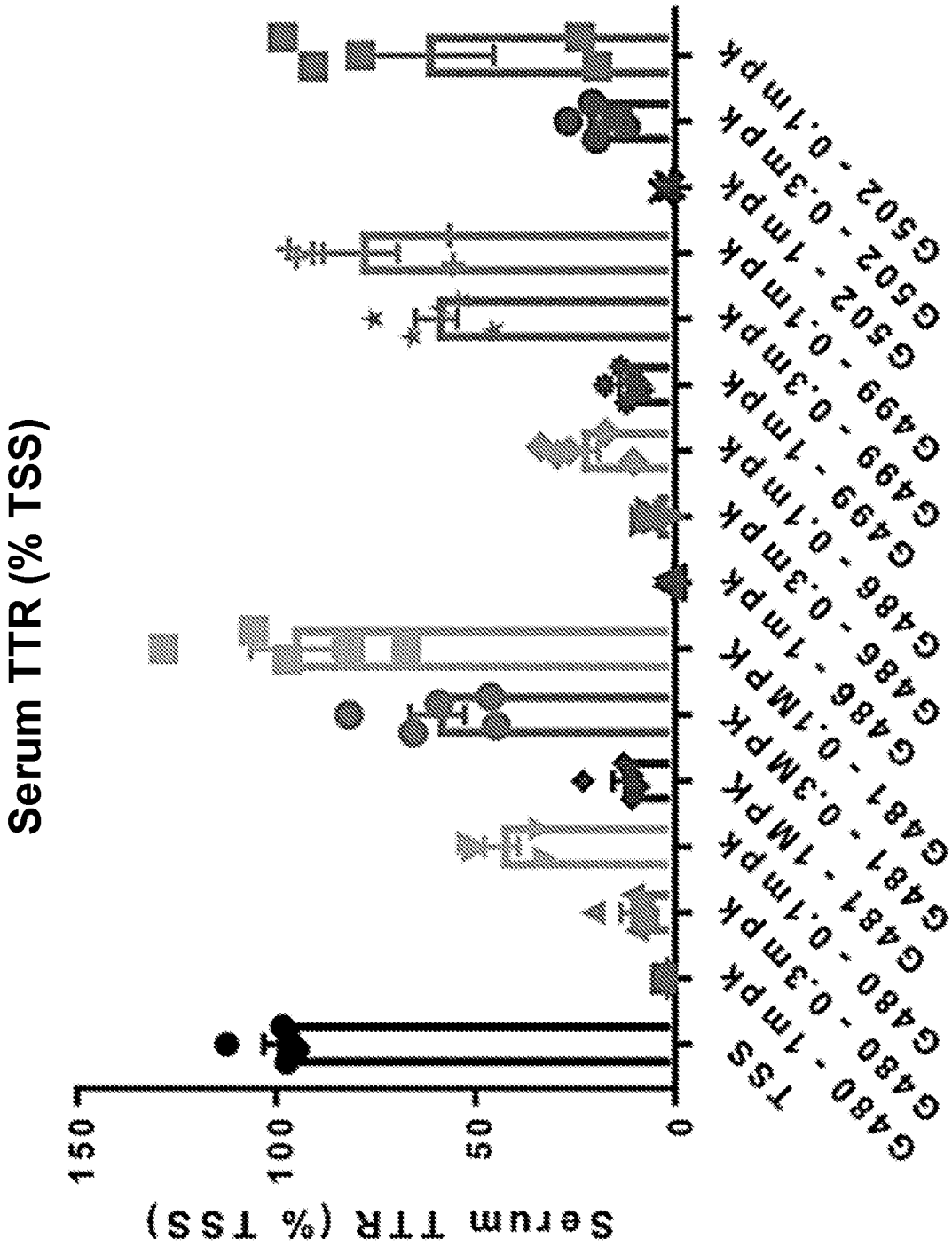
Serum TTR (% TSS)
150
100
50
0
TSS
G480 - 1mpk
G480 - 0.3mpk
G480 - 0.1mpk
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G486 - 1mpk
G486 - 0.3mpk
G486 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G499 - 0.1mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

FIG. 28C

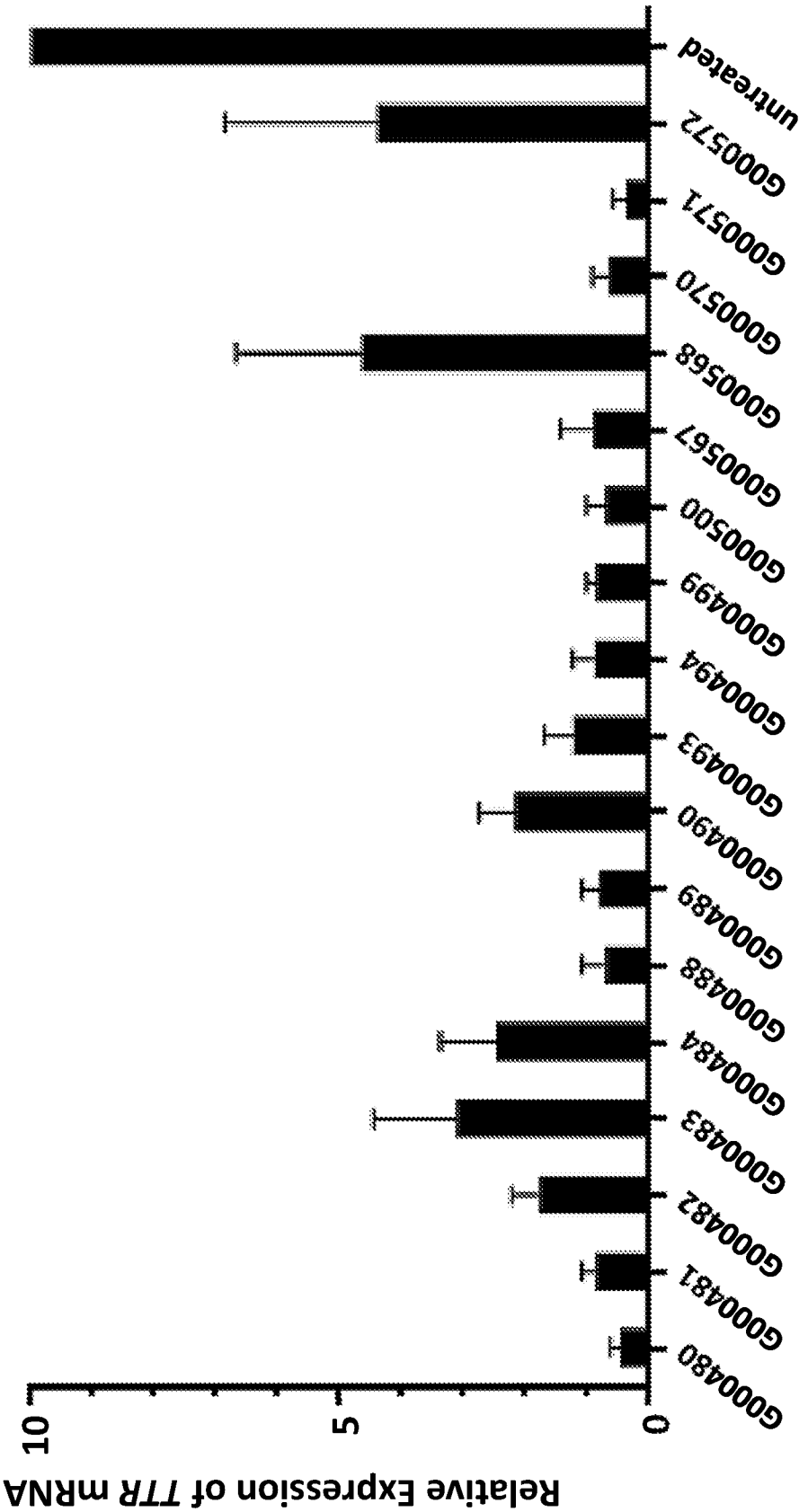
Relative Expression of TTR mRNA
10
5
0
G000480
G000481
G000482
G000483
G000484
G000488
G000489
G000490
G000493
G000494
G000499
G000500
G000567
G000568
G000570
G000571
G000572
untreated

FIG. 29

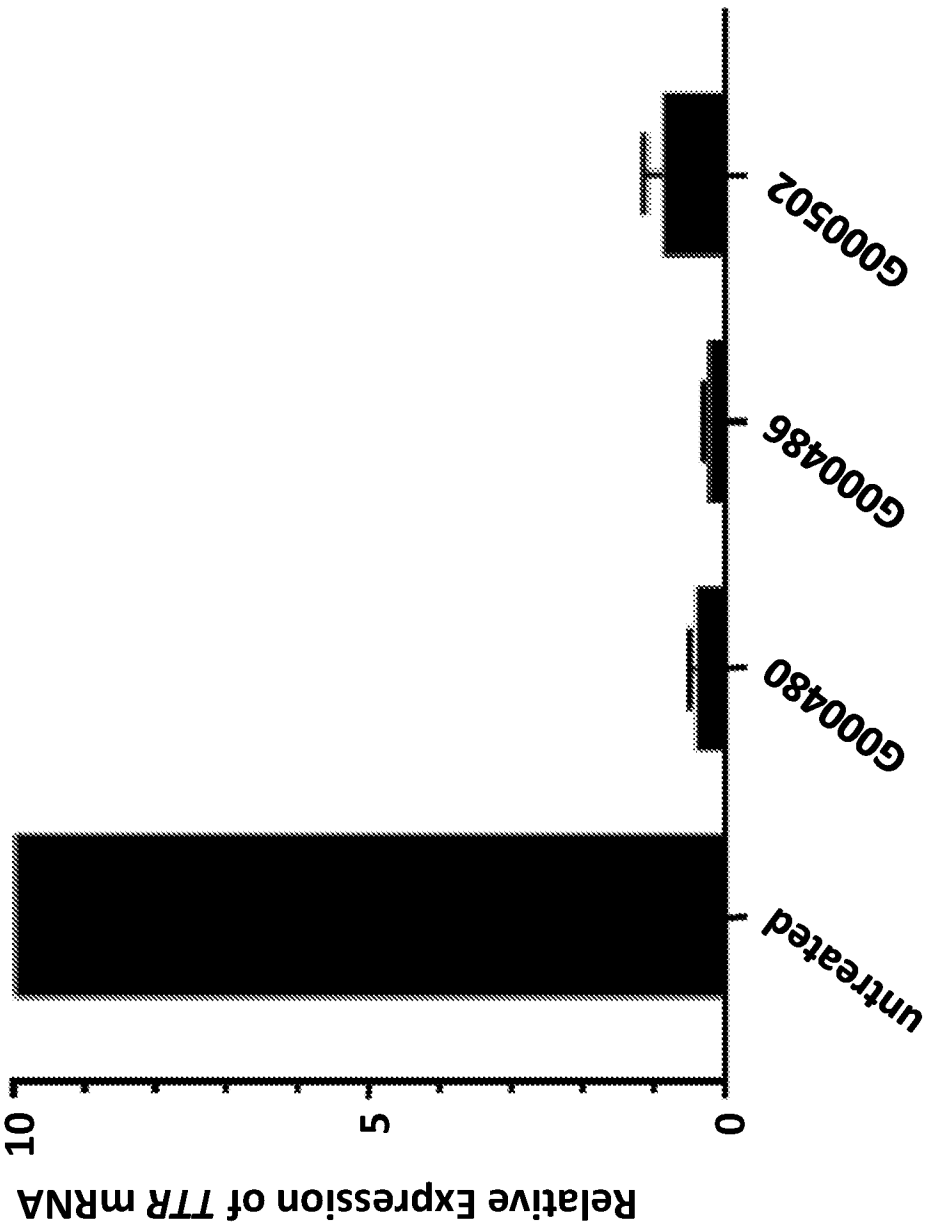
Relative Expression of TTR mRNA
10
5
0
untreated
G000480
G000486
G000502

FIG. 30

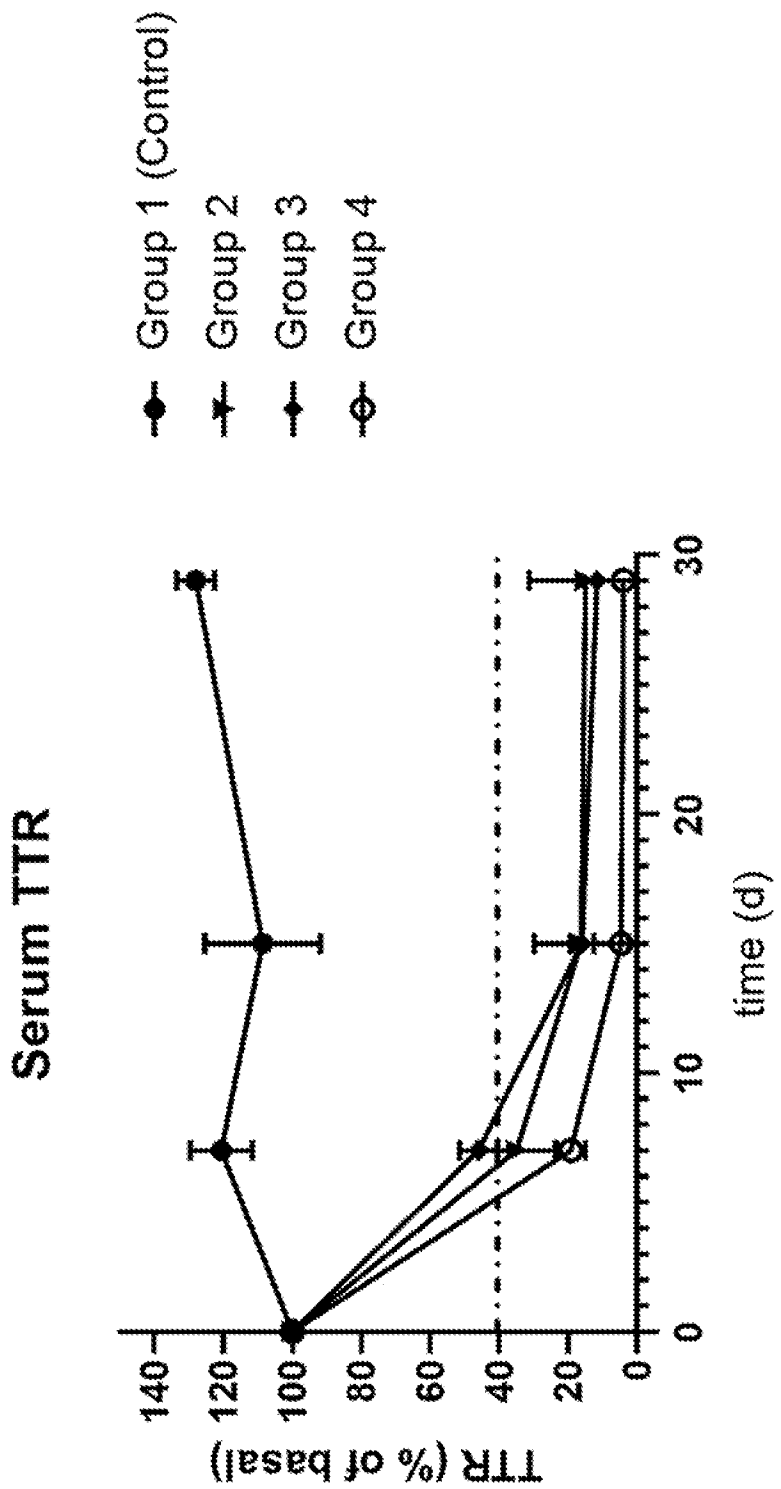
Serum TTR
Group 1 (Control)
Group 2
Group 3
Group 4
TTR (% of basal)
140
120
100
80
60
40
20
0
0
10
20
30
time (d)

FIG. 31A

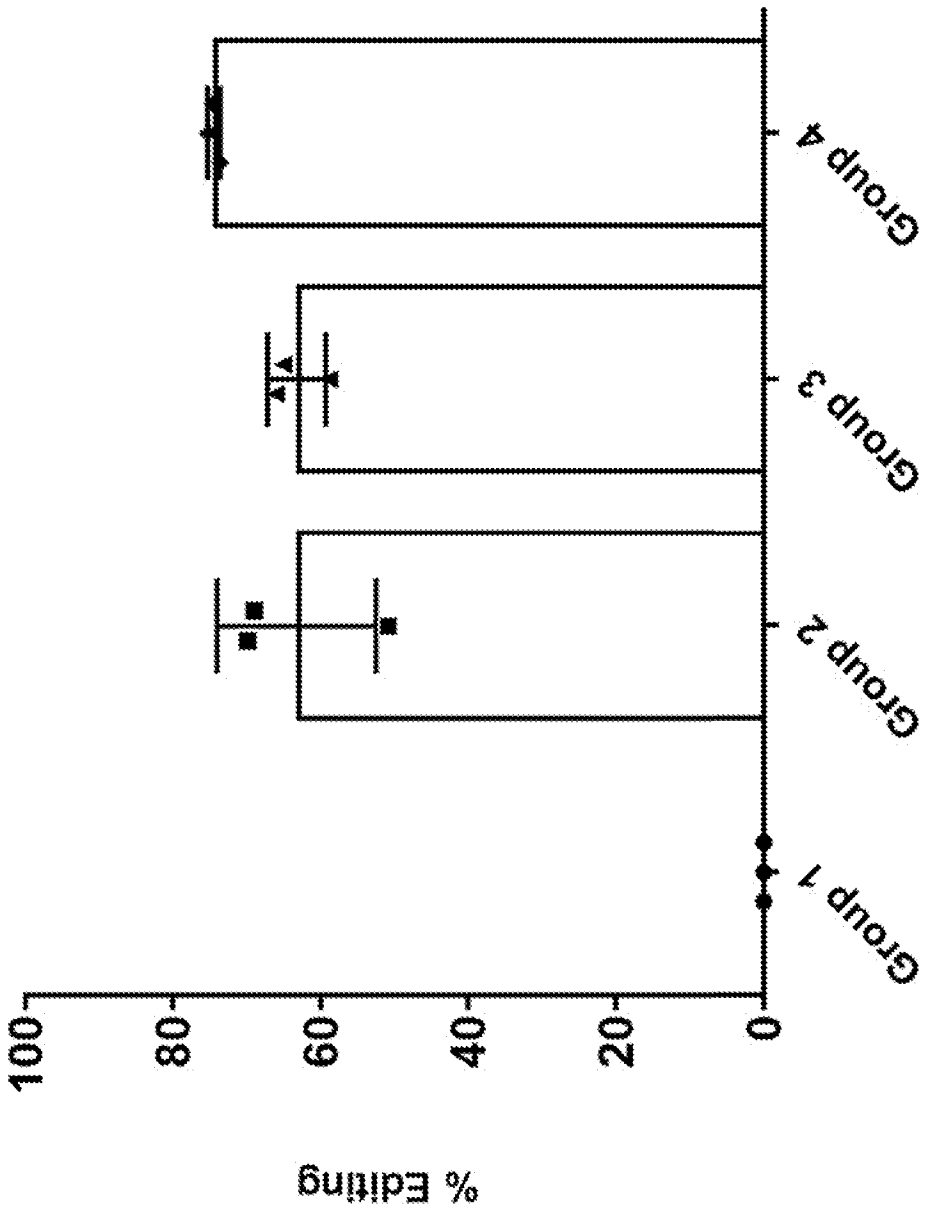

FIG. 31B

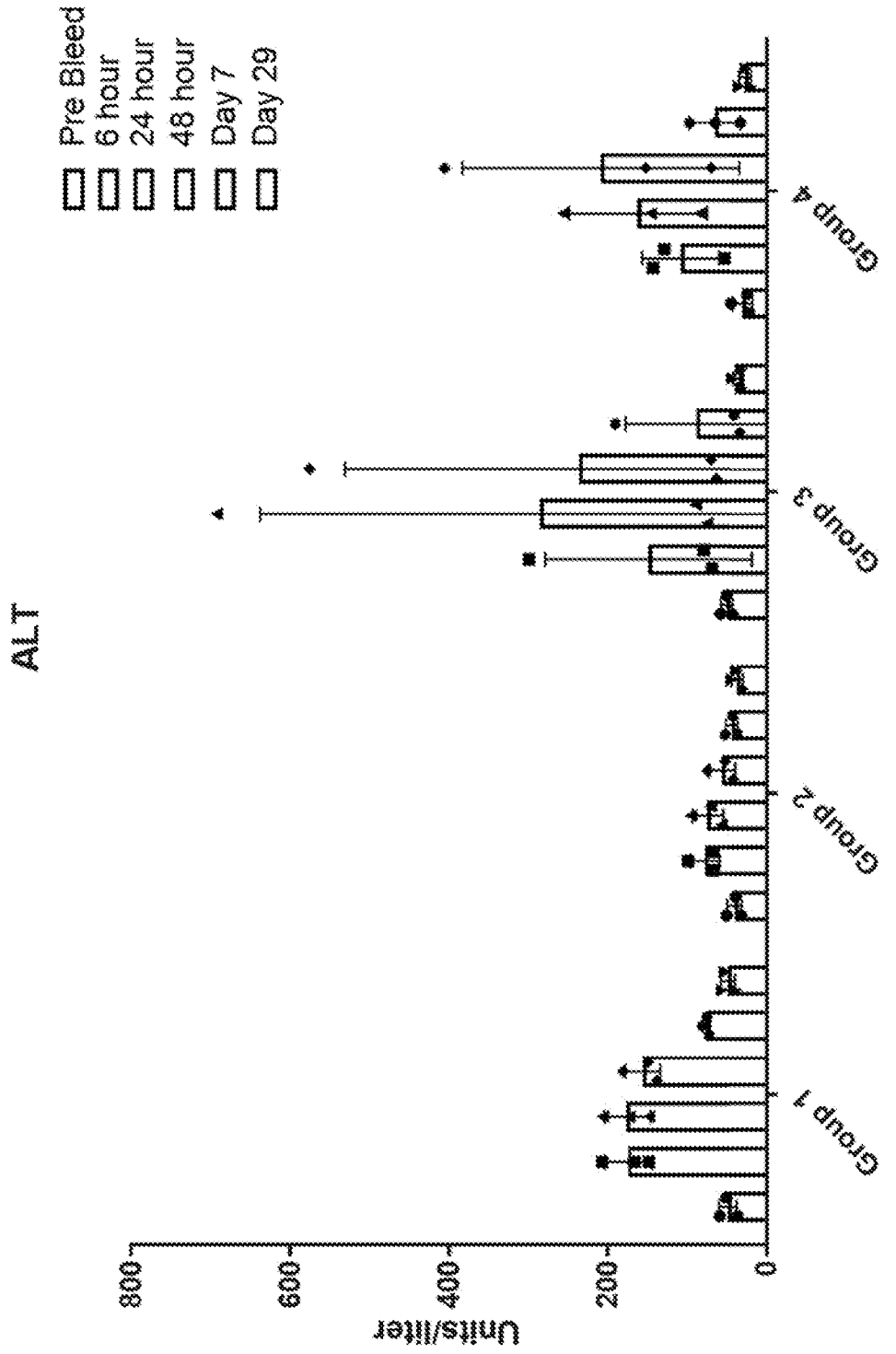
ALT
Pre Bleed
6 hour
24 hour
48 hour
Day 7
Day 29
Units/liter
800
600
400
200
0
Group 1
Group 2
Group 3
Group 4

FIG. 31C

SEQUENCE LISTING

<110> INTELLIA THERAPEUTICS, INC.

<120> COMPOSITIONS AND METHODS FOR TTR GENE EDITING AND TREATING ATTR AMYLOIDOSIS COMPRISING A CORTICOSTEROID OR USE THEREOF

<130> 01155-0029-00PCT

<150> US 62/825,676
<151> 2019-03-28

<150> US 62/825,637
<151> 2019-03-28

<160> 396

<170> PatentIn version 3.5

<210> 1
<211> 4411
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3 UTR of ALB

<400> 1
gggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc gccaccatgg acaagaagta cagcatcgga ctggacatcg gaacaaacag 120

cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag aaaagaagaa 780

cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg acgacgacct 900

ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg cagcaaagaa 960

cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc tgacactgct 1080

gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat tctacaagtt 1200

catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc accagatcca 1320

cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt tcctgaagga 1380

caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg tcggaccgct 1440

ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa caatcacacc 1500

gtggaacttc gaagaagtcg tcgacaaggg agcaagcgca cagagcttca tcgaaagaat 1560

gacaaacttc gacaagaacc tgccgaacga aaaggtcctg ccgaagcaca gcctgctgta 1620

cgaatacttc acagtctaca acgaactgac aaaggtcaag tacgtcacag aaggaatgag 1680

aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc tgttcaagac 1740

aaacagaaag gtcacagtca agcagctgaa ggaagactac ttcaagaaga tcgaatgctt 1800

cgacagcgtc gaaatcagcg gagtcgaaga cagattcaac gcaagcctgg gaacatacca 1860

cgacctgctg aagatcatca aggacaagga cttcctggac aacgaagaaa acgaagacat 1920

cctggaagac atcgtcctga cactgacact gttcgaagac agagaaatga tcgaagaaag 1980

actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga agagaagaag 2040

atacacagga tggggaagac tgagcagaaa gctgatcaac ggaatcagag acaagcagag 2100

cggaaagaca atcctggact tcctgaagag cgacggattc gcaaacagaa acttcatgca 2160

gctgatccac gacgacagcc tgacattcaa ggaagacatc cagaaggcac aggtcagcgg 2220

acagggagac agcctgcacg aacacatcgc aaacctggca ggaagcccgg caatcaagaa 2280

gggaatcctg cagacagtca aggtcgtcga cgaactggtc aaggtcatgg gaagacacaa 2340

gccggaaaac atcgtcatcg aaatggcaag agaaaaccag acaacacaga agggacagaa 2400

gaacagcaga gaaagaatga agagaatcga agaaggaatc aaggaactgg gaagccagat 2460

cctgaaggaa cacccggtcg aaaacacaca gctgcagaac gaaaagctgt acctgtacta 2520

cctgcagaac ggaagagaca tgtacgtcga ccaggaactg gacatcaaca gactgagcga 2580

ctacgacgtc gaccacatcg tcccgcagag cttcctgaag gacgacagca tcgacaacaa 2640

ggtcctgaca agaagcgaca agaacagagg aaagagcgac aacgtcccga gcgaagaagt 2700

cgtcaagaag atgaagaact actggagaca gctgctgaac gcaaagctga tcacacagag 2760

aaagttcgac aacctgacaa aggcagagag aggaggactg agcgaactgg acaaggcagg 2820

attcatcaag agacagctgg tcgaaacaag acagatcaca aagcacgtcg cacagatcct 2880

ggacagcaga atgaacacaa agtacgacga aaacgacaag ctgatcagag aagtcaaggt 2940

catcacactg aagagcaagc tggtcagcga cttcagaaag gacttccagt tctacaaggt 3000

cagagaaatc aacaactacc accacgcaca cgacgcatac ctgaacgcag tcgtcggaac 3060

agcactgatc aagaagtacc cgaagctgga aagcgaattc gtctacggag actacaaggt 3120

ctacgacgtc agaaagatga tcgcaaagag cgaacaggaa atcggaaagg caacagcaaa 3180

gtacttcttc tacagcaaca tcatgaactt cttcaagaca gaaatcacac tggcaaacgg 3240

agaaatcaga aagagaccgc tgatcgaaac aaacggagaa acaggagaaa tcgtctggga 3300

caagggaaga gacttcgcaa cagtcagaaa ggtcctgagc atgccgcagg tcaacatcgt 3360

caagaagaca gaagtccaga caggaggatt cagcaaggaa agcatcctgc cgaagagaaa 3420

cagcgacaag ctgatcgcaa gaaagaagga ctgggacccg aagaagtacg gaggattcga 3480

cagcccgaca gtcgcataca gcgtcctggt cgtcgcaaag gtcgaaaagg gaaagagcaa 3540

gaagctgaag agcgtcaagg aactgctggg aatcacaatc atggaaagaa gcagcttcga 3600

aaagaacccg atcgacttcc tggaagcaaa gggatacaag gaagtcaaga aggacctgat 3660

catcaagctg ccgaagtaca gcctgttcga actggaaaac ggaagaaaga gaatgctggc 3720

aagcgcagga gaactgcaga agggaaacga actggcactg ccgagcaagt acgtcaactt 3780

cctgtacctg gcaagccact acgaaaagct gaagggaagc ccggaagaca acgaacagaa 3840

gcagctgttc gtcgaacagc acaagcacta cctggacgaa atcatcgaac agatcagcga 3900

attcagcaag agagtcatcc tggcagacgc aaacctggac aaggtcctga gcgcatacaa 3960

caagcacaga gacaagccga tcagagaaca ggcagaaaac atcatccacc tgttcacact 4020

gacaaacctg ggagcaccgg cagcattcaa gtacttcgac acaacaatcg acagaaagag 4080

atacacaagc acaaaggaag tcctggacgc aacactgatc caccagagca tcacaggact 4140

gtacgaaaca agaatcgacc tgagccagct gggaggagac ggaggaggaa gcccgaagaa 4200

gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

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tcaattaata aaaaatggaa agaacctcga g 4411

<210> 2
<211> 4403
<212> DNA
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<220>
<223> Synthetic: Cas9 transcript comprising Cas9 ORF corresponding to SEQ ID NO: 205 using codons with generally high expression in humans

<400> 2
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gctggatatc ggaactaatt ccgtgggttg ggcagtgatc acggatgaat acaaagtgcc 180

gtccaagaag ttcaaggtcc tggggaacac cgatagacac agcatcaaga aaaatctcat 240

cggagccctg ctgtttgact ccggcgaaac cgcagaagcg acccggctca aacgtaccgc 300

gaggcgacgc tacacccggc ggaagaatcg catctgctat ctgcaagaga tcttttcgaa 360

cgaaatggca aaggtcgacg acagcttctt ccaccgcctg gaagaatctt tcctggtgga 420

ggaggacaag aagcatgaac ggcatcctat ctttggaaac atcgtcgacg aagtggcgta 480

ccacgaaaag tacccgacca tctaccatct gcggaagaag ttggttgact caactgacaa 540

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cctgatcgaa ggcgatctga accctgataa ctccgacgtg gataagcttt tcattcaact      660
ggtgcagacc tacaaccaac tgttcgaaga aaacccaatc aatgctagcg gcgtcgatgc      720
caaggccatc ctgtccgccc ggctgtcgaa gtcgcggcgc ctcgaaaacc tgatcgcaca      780
gctgccggga gagaaaaaga acggactttt cggcaacttg atcgctctct cactgggact      840
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taatgtgcca tcggaggagg tcgtgaagaa gatgaagaat tactggcggc agctcctgaa 2760

tgcgaagctg attacccaga gaaagtttga caatctcact aaagccgagc gcggcggact 2820

ctcagagctg gataaggctg gattcatcaa acggcagctg gtcgagactc ggcagattac 2880

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atcgatcctc ccaaagagaa atagcgacaa gctcattgca cgcaagaaag actgggaccc 3480

gaagaagtac ggaggattcg attcgccgac tgtcgcatac tccgtcctcg tggtggccaa 3540

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ccaccaaagc atcactggac tctacgaaac taggatcgat ctgtcgcagc tgggtggcga 4200

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taaaaaatgg aaagaacctc gag 4403

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<220>

<223> Synthetic: modified sgRNA sequence (N may be any natural or non-natural nucleotide)

<220>
<221> modified_base
<222> (1)..(3)
<223> 2'-O-Me nucleotide

<220>
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<222> (1)..(4)
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<220>
<221> misc_feature
<222> (1)..(20)
<223> n is a, c, g, or u

<220>
<221> modified_base
<222> (29)..(40)
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<220>
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<222> (97)..(100)
<223> PS linkage

<400> 3
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 4
<211> 105
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<220>
<223> Synthetic: 30/30/39 poly-A sequence

<400> 4
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa gcgaaaaaaa aaaaaaaaaa aaaaaaaaaa 60

aaaccgaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 105

<210> 5
<211> 20
<212> RNA
<213> Artificial Sequence

<220>
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<400> 5
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<210> 6
<211> 20
<212> RNA

<213> Artificial Sequence

<220>
<223> Synthetic: CR003336 gRNA targeting Human TTR (Exon 1)

<400> 6
ccuccucugc cuugcuggac 20

<210> 7
<211> 20
<212> RNA
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<220>
<223> Synthetic: CR003337 gRNA targeting Human TTR (Exon 1)

<400> 7
ccaguccagc aaggcagagg 20

<210> 8
<211> 20
<212> RNA
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<220>
<223> Synthetic: CR003338 gRNA targeting Human TTR (Exon 1)

<400> 8
auaccagucc agcaaggcag 20

<210> 9
<211> 20
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<220>
<223> Synthetic: CR003339 gRNA targeting Human TTR (Exon 1)

<400> 9
acacaaauac caguccagca 20

<210> 10
<211> 20
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<220>
<223> Synthetic: CR003340 gRNA targeting Human TTR (Exon 1)

<400> 10
uggacuggua uuugugucug 20

<210> 11
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<220>
<223> Synthetic: CR003341 gRNA targeting Human TTR (Exon 1)

<400> 11
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<210> 12
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<220>
<223> Synthetic: CR003342 gRNA targeting Human TTR (Exon 2)

<400> 12
cuucucuaca cccagggcac 20

<210> 13
<211> 20
<212> RNA
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<220>
<223> Synthetic: CR003343 gRNA targeting Human TTR (Exon 2)

<400> 13
cagaggacac uuggauucac 20

<210> 14
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<220>
<223> Synthetic: CR003344 gRNA targeting Human TTR (Exon 2)

<400> 14
uuugaccauc agaggacacu 20

<210> 15
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<220>
<223> Synthetic: CR003345 gRNA targeting Human TTR (Exon 2)

<400> 15
ucuagaacuu ugaccaucag 20

<210> 16
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<220>
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<210> 17
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<220>
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<400> 17

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<210> 18
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<220>
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<400> 18
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<210> 19
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<220>
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<210> 20
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<220>
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<210> 21
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<220>
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<400> 21
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<210> 22
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<220>
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<400> 22
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<210> 23
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<212> RNA
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<220>

<223> Synthetic: CR003353 gRNA targeting Human TTR (Exon 2)

<400> 23
aaaggcugcu gaugacaccu 20

<210> 24
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<220>
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<400> 24
accugggagc cauuugccuc 20

<210> 25
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<220>
<223> Synthetic: CR003355 gRNA targeting Human TTR (Exon 2)

<400> 25
cccagaggca aauggcuccc 20

<210> 26
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<220>
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<400> 26
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<210> 27
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<220>
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<400> 27
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<210> 28
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<220>
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<400> 28
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<210> 29
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<213> Artificial Sequence

<220>
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<400> 29
agugagucug gagagcugca 20

<210> 30
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<220>
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<400> 30
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<210> 31
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<220>
<223> Synthetic: CR003361 gRNA targeting Human TTR (Exon 3)

<400> 31
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<210> 32
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<220>
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<400> 32
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<210> 33
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<220>
<223> Synthetic: CR003363 gRNA targeting Human TTR (Exon 3)

<400> 33
acugaggagg aauuuguaga 20

<210> 34
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<220>
<223> Synthetic: CR003364 gRNA targeting Human TTR (Exon 3)

<400> 34
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<210> 35
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<220>
<223> Synthetic: CR003365 gRNA targeting Human TTR (Exon 3)

<400> 35
uguagaaggg auauacaaag 20

<210> 36
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<220>
<223> Synthetic: CR003366 gRNA targeting Human TTR (Exon 3)

<400> 36
aaauagacac caaaucuuac 20

<210> 37
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<220>
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<400> 37
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<210> 38
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<220>
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<400> 38
aagugccuuc caguaagauu 20

<210> 39
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<220>
<223> Synthetic: CR003369 gRNA targeting Human TTR (Exon 3)

<400> 39
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<210> 40
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<220>
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<400> 40

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<210> 41
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<400> 41
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<210> 42
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<400> 42
uacucaccuc ugcaugcuca 20

<210> 43
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<220>
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<400> 43
guauucacag ccaacgacuc 20

<210> 44
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<220>
<223> Synthetic: CR003374 gRNA targeting Human TTR (Exon 4)

<400> 44
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<210> 45
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<212> RNA
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<220>
<223> Synthetic: CR003375 gRNA targeting Human TTR (Exon 4)

<400> 45
aaugguguag cggcgggggc 20

<210> 46
<211> 20
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<220>

<223> Synthetic: CR003376 gRNA targeting Human TTR (Exon 4)

<400> 46
cggcaauggu guagcggcgg 20

<210> 47
<211> 20
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: CR003377 gRNA targeting Human TTR (Exon 4)

<400> 47
gcggcaaugg uguagcggcg 20

<210> 48
<211> 20
<212> RNA
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<220>
<223> Synthetic: CR003378 gRNA targeting Human TTR (Exon 4)

<400> 48
ggcggcaaug guguagcggc 20

<210> 49
<211> 20
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: CR003379 gRNA targeting Human TTR (Exon 4)

<400> 49
gggcggcaau gguguagcgg 20

<210> 50
<211> 20
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<220>
<223> Synthetic: CR003380 gRNA targeting Human TTR (Exon 4)

<400> 50
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<210> 51
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<220>
<223> Synthetic: CR003381 gRNA targeting Human TTR (Exon 4)

<400> 51
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<210> 52
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<212> RNA

<213> Artificial Sequence

<220>
<223> Synthetic: CR003382 gRNA targeting Human TTR (Exon 4)

<400> 52
ggaguagggg cucagcaggg 20

<210> 53
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<220>
<223> Synthetic: CR003383 gRNA targeting Human TTR (Exon 4)

<400> 53
auaggaguag gggcucagca 20

<210> 54
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<220>
<223> Synthetic: CR003384 gRNA targeting Human TTR (Exon 4)

<400> 54
aauaggagua ggggcucagc 20

<210> 55
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<212> RNA
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<220>
<223> Synthetic: CR003385 gRNA targeting Human TTR (Exon 4)

<400> 55
ccccuacucc uauuccacca 20

<210> 56
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<212> RNA
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<220>
<223> Synthetic: CR003386 gRNA targeting Human TTR (Exon 4)

<400> 56
ccguggugga auaggaguag 20

<210> 57
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<220>
<223> Synthetic: CR003387 gRNA targeting Human TTR (Exon 4)

<400> 57
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<210> 58
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<220>
<223> Synthetic: CR003388 gRNA targeting Human TTR (Exon 4)

<400> 58
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<210> 59
<211> 20
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<220>
<223> Synthetic: CR003389 gRNA targeting Human TTR (Exon 4)

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<210> 60
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<220>
<223> Synthetic: CR003390 gRNA targeting Human TTR (Exon 4)

<400> 60
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<210> 61
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<220>
<223> Synthetic: CR003391 gRNA targeting Human TTR (Exon 4)

<400> 61
ggcugucguc accaauccca 20

<210> 62
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<220>
<223> Synthetic: CR003392 gRNA targeting Human TTR (Exon 4)

<400> 62
agucccucau uccuugggau 20

<210> 63
<211> 20
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<220>
<223> Synthetic: CR005298 gRNA targeting Human TTR (Exon 1)

<400> 63

uccacucauu cuuggcagga 20

<210> 64
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<220>
<223> Synthetic: CR005299 gRNA targeting Human TTR (Exon 4)

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<210> 65
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<210> 66
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<220>
<223> Synthetic: CR005301 gRNA targeting Human TTR (Exon 1)

<400> 66
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<210> 67
<211> 20
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<220>
<223> Synthetic: CR005302 gRNA targeting Human TTR (Exon 2)

<400> 67
acgugucuuc ucuacaccca 20

<210> 68
<211> 20
<212> RNA
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<220>
<223> Synthetic: CR005303 gRNA targeting Human TTR (Exon 2)

<400> 68
ugaauccaag uguccucuga 20

<210> 69
<211> 20
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<220>

<223> Synthetic: CR005304 gRNA targeting Human TTR (Exon 2)

<400> 69
ggccgugcau guguucagaa 20

<210> 70
<211> 20
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<220>
<223> Synthetic: CR005305 gRNA targeting Human TTR (Exon 3)

<400> 70
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<210> 71
<211> 20
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<220>
<223> Synthetic: CR005306 gRNA targeting Human TTR (Exon 3)

<400> 71
aaaucuuacu ggaaggcacu 20

<210> 72
<211> 20
<212> RNA
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<220>
<223> Synthetic: CR005307 gRNA targeting Human TTR (Exon 4)

<400> 72
ugucugucuu cucucauagg 20

<210> 73
<211> 20
<212> RNA
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<220>
<223> Synthetic: CR000689 gRNA targeting Cyno TTR

<400> 73
acacaaauac caguccagcg 20

<210> 74
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<212> RNA
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<220>
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<400> 74
aaaggcugcu gaugagaccu 20

<210> 75
<211> 20
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<213> Artificial Sequence

<220>
<223> Synthetic: CR005365 gRNA targeting Cyno TTR

<400> 75
cauugacagc aggacugccu 20

<210> 76
<211> 20
<212> RNA
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<220>
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auaccagucc agcgaggcag 20

<210> 77
<211> 20
<212> RNA
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<220>
<223> Synthetic: CR005367 gRNA targeting Cyno TTR

<400> 77
ccaguccagc gaggcagagg 20

<210> 78
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ccuccucugc cucgcuggac 20

<210> 79
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<220>
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<400> 79
aaaguucuag augccguccg 20

<210> 80
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<220>
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<400> 80
acuugucuuc ucuauaccca 20

<210> 81
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<220>
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<400> 81
aagugacuuc caguaagauu 20

<210> 82
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<220>
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aaaaggcugc ugaugagacc 20

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<210> 88
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<213> Artificial Sequence

<220>
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<400> 88
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 89
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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<220>
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 91
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<213> Artificial Sequence

<220>
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<223> PS linkage

<400> 92
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 93
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<400> 94
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 95
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<220>
<223> Synthetic: G000488 sgRNA modified sequence targeting Human TTR

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<400> 95
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 96
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<220>
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<400> 96
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 97
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<220>
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<400> 97
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 98
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<400> 98
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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<400> 112
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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<400> 113
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 114
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<400> 114
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<400> 115
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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<223> 2'-O-Me nucleotide

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<223> PS linkage

<400> 119
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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<400> 121
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 123
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<400> 123
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 124
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<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: G000282 sgRNA modified sequence targeting Mouse TTR

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<223> 2'-O-Me nucleotide

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<222> (1)..(4)
<223> PS linkage

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<223> 2'-O-Me nucleotide

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<222> (69)..(100)
<223> 2'-O-Me nucleotide

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<221> modified_base
<222> (97)..(100)
<223> PS linkage

<400> 124
uuacagccac gucuacagca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 125
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<212> RNA
<213> Artificial Sequence

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<223> Synthetic: exemplary nucleotide sequence

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ggcaccgagu cggugcuuuu   80

<210> 126
<211> 22
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: exemplary nucleotide sequence

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<210> 201
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 DNA coding sequence 2

<400> 201
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac aggatgggga 1980

agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

gacttcctga agagcgacgg attcgcaaac agaaacttca tgcagctgat ccacgacgac 2100

agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

cacgaacaca tcgcaaacct ggcaggaagc ccggcaatca agaagggaat cctgcagaca 2220

gtcaaggtcg tcgacgaact ggtcaaggtc atgggaagac acaagccgga aaacatcgtc 2280

atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

ctggtcgaaa caagacagat cacaaagcac gtcgcacaga tcctggacag cagaatgaac 2820

acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

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cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

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<220>
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atcacggatg aatacaaagt gccgtccaag aagttcaagg tcctggggaa caccgataga 120

cacagcatca agaaaaatct catcggagcc ctgctgtttg actccggcga aaccgcagaa 180

gcgacccggc tcaaacgtac cgcgaggcga cgctacaccc ggcggaagaa tcgcatctgc 240

tatctgcaag agatcttttc gaacgaaatg gcaaaggtcg acgacagctt cttccaccgc 300

ctggaagaat ctttcctggt ggaggaggac aagaagcatg aacggcatcc tatctttgga 360

aacatcgtcg acgaagtggc gtaccacgaa aagtacccga ccatctacca tctgcggaag 420

aagttggttg actcaactga caaggccgac ctcagattga tctacttggc cctcgcccat 480

atgatcaaat tccgcggaca cttcctgatc gaaggcgatc tgaaccctga taactccgac 540

gtggataagc ttttcattca actggtgcag acctacaacc aactgttcga agaaaaccca 600

atcaatgcta gcggcgtcga tgccaaggcc atcctgtccg cccggctgtc gaagtcgcgg 660

cgcctcgaaa acctgatcgc acagctgccg ggagagaaaa agaacggact tttcggcaac 720

ttgatcgctc tctcactggg actcactccc aatttcaagt ccaattttga cctggccgag 780

gacgcgaagc tgcaactctc aaaggacacc tacgacgacg acttggacaa tttgctggca 840

caaattggcg atcagtacgc ggatctgttc cttgccgcta agaacctttc ggacgcaatc 900

ttgctgtccg atatcctgcg cgtgaacacc gaaataacca aagcgccgct tagcgcctcg 960

atgattaagc ggtacgacga gcatcaccag gatctcacgc tgctcaaagc gctcgtgaga 1020

cagcaactgc ctgaaaagta caaggagatc ttcttcgacc agtccaagaa tgggtacgca 1080

gggtacatcg atggaggcgc tagccaggaa gagttctata agttcatcaa gccaatcctg 1140

gaaaagatgg acggaaccga agaactgctg gtcaagctga acagggagga tctgctccgg 1200

aaacagagaa cctttgacaa cggatccatt ccccaccaga tccatctggg tgagctgcac 1260

gccatcttgc ggcgccagga ggacttttac ccattcctca aggacaaccg ggaaaagatc 1320

gagaaaattc tgacgttccg catcccgtat tacgtgggcc cactggcgcg cggcaattcg 1380

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Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile

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Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
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Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
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Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
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Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
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Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
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Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
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Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
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Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590
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Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
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Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
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Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
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Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
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Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
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Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
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Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
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Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
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Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
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Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975


Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990


Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
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Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
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Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
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Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
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Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
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Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
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Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
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Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
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Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
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Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
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Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
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Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
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Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
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Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
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Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
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His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
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Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
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Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
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Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
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Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
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Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
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Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
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gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

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aucaaggaca aggacuuccu ugacaacgag gagaacgagg acauccugga agauaucguc 1860

cugaccuuga cccuuuucga ggaucgcgag augaucgagg agaggcuuaa gaccuacgcu 1920

caucucuucg acgauaaggu caugaaacaa cucaagcgcc gccgguacac ugguuggggc 1980

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gauuuccuca aaucggaugg cuucgcuaau cguaacuuca ugcaauugau ccacgacgac 2100

agccugaccu uuaaggagga cauccaaaaa gcacaagugu ccggacaggg agacucacuc 2160

caugaacaca ucgcgaaucu ggccgguucg ccggcgauua agaagggaau ucugcaaacu 2220

gugaaggugg ucgacgagcu ggugaagguc augggacggc acaaaccgga gaauaucgug 2280

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ccagccgccu ucaaguacuu cgauacuacu aucgaucgca aaagauacac guccaccaag 4020

gaaguucugg acgcgacccu gauccaccaa agcaucacug gacucuacga aacuaggauc 4080

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uga 4143

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

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Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430
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Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
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705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990
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Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020


Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035


Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050


Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065


Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080


Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095


Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110


Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125


Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140


Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155


Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170


Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185


Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200


Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215


Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230


Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245
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Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
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His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                 1270                 1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                 1285                 1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                 1300                 1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                 1315                 1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                 1330                 1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                 1345                 1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa      180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc      240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga      300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga      360

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agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac      720
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ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

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Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
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                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605
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Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880
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Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
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Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
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Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
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Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
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Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
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Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
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Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
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Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
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Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
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Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
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Gly Gly  Gly Ser Pro Lys Lys  Lys Arg Lys Val
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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga 300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

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cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

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aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

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aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa gguc 4134

<210> 212
<211> 4134
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 bare coding sequence

<400> 212
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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccuggggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

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aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

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ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

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gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

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gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa gguc 4134

<210> 213
<211> 1368
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence of Cas9 (without NLS)

<400> 213

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

```
Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
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    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655
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Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925
```

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Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185
```

```
Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200


Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215


Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230


Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245


Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260


His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275


Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290


Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305


Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320


Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335


Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365
```

<210> 214
<211> 4107
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 mRNA ORF encoding SEQ ID NO: 13 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> 214

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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa      180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc      240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga      300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga      360
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aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

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gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

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aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

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gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

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aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

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acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacuag 4107

<210> 215
<211> 4101
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 coding sequence encoding SEQ ID NO: 13 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 215
gacaagaagu acagcaucgg acuggacauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccuggggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga c 4101

<210> 216
<211> 1368
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence of Cas9 nickase (without NLS)

<400> 216

```
Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1               5                   10                  15


Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45


Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60


Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80


Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95


Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110


His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125


His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140


Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160


Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175


Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190


Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205


Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220


Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
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225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510
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Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780
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Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800


Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815


Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830


Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845


Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860


Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880


Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895


Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910


Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925


Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940


Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960


Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975


Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990


Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020


Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035


Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050
```

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Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
```

1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

<210> 217
<211> 4107
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase mRNA ORF encoding SEQ ID NO: 16 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> 217
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aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga 120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga 300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacuag 4107

<210> 218
<211> 4101
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase coding sequence encoding SEQ ID NO: 16 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 218
gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggaccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga c 4101

<210> 219
<211> 1368
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence of dCas9 (without NLS)

<400> 219

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

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Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350
```

```
Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
```

625 630 635 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
900 905 910

```
Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925


Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940


Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960


Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975


Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990


Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020


Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035


Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050


Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065


Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080


Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095


Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110


Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125


Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140


Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155


Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170
```

```
Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185


Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200


Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215


Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230


Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245


Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260


His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275


Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290


Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305


Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320


Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335


Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365
```

<210> 220
<211> 4107
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 mRNA ORF encoding SEQ ID NO: 19 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> 220

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aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga      120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa      180
```

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga 300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

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gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgacgca 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacuag 4107

<210> 221
<211> 4113
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 coding sequence encoding SEQ ID NO: 19 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 221
gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccuggggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgacgcaauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaggagga agc 4113

<210> 222
<211> 1392
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence of Cas9 with two nuclear localization signals as the C-terminal amino acids

<400> 222

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

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Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480
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Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
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        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035
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Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290
```

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Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                 1300                 1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                 1315                 1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                 1330                 1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                 1345                 1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                 1360                 1365

Gly Ser  Gly Ser Pro Lys Lys  Lys Arg Lys Val Asp  Gly Ser Pro
    1370                 1375                 1380

Lys Lys  Lys Arg Lys Val Asp  Ser Gly
    1385                 1390
```

<210> 223
<211> 4179
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 mRNA ORF encoding SEQ ID NO: 22 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> 223

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auggacaaga aguacagcau cggacuggac aucggaacaa acagcgucgg augggcaguc       60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga      120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa      180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc      240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga      300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga      360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag      420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac      480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac      540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg      600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga      660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac      720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa      780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca      840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc      900
```

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggaagc ggaagcccga agaagaagag aaaggucgac 4140

ggaagcccga agaagaagag aaaggucgac agcggauag 4179

<210> 224
<211> 4173
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 coding sequence encoding SEQ ID NO: 23 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 224
gacaagaagu acagcaucgg acuggacauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaagcgga agcccgaaga agaagagaaa ggucgacgga 4140

agcccgaaga agaagagaaa ggucgacagc gga 4173

<210> 225
<211> 1392
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence of Cas9 nickase with two nuclear localization signals as the C-terminal amino acids

<400> 225

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1 5 10 15

```
Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285
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Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560
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Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
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        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110
```

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Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365
```

Gly Ser Gly Ser Pro Lys Lys Lys Arg Lys Val Asp Gly Ser Pro
    1370   1375   1380

Lys Lys Lys Arg Lys Val Asp Ser Gly
    1385   1390

<210> 226
<211> 4179
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase mRNA ORF encoding SEQ ID NO: 25 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> 226
auggacaaga aguacagcau cggacuggca aucggaacaa acagcgucgg augggcaguc   60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga   120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa   180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc   240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga   300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga   360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag   420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac   480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac   540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg   600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga   660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac   720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa   780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca   840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc   900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc   960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga   1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca   1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug   1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga   1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac   1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc   1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc   1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa   1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag   1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggaagc ggaagcccga agaagaagag aaaggucgac 4140

ggaagcccga agaagaagag aaaggucgac agcggauag 4179

<210> 227
<211> 4173
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase coding sequence encoding SEQ ID NO: 25 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 227
gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggaccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccuggggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaagcgga agcccgaaga agaagagaaa ggucgacgga 4140

agcccgaaga agaagagaaa ggucgacagc gga 4173

<210> 228
<211> 1392
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence of dCas9 with two nuclear localization signals as the C-terminal amino acids

<400> 228

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1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

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Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
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        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640
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His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910
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Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925


Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940


Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960


Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975


Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990


Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020


Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035


Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050


Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065


Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080


Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095


Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110


Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125


Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140


Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155


Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170
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Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185


Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200


Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215


Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230


Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245


Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260


His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275


Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290


Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305


Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320


Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335


Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365


Gly Ser  Gly Ser Pro Lys Lys  Lys Arg Lys Val Asp  Gly Ser Pro
    1370                1375                1380


Lys Lys  Lys Arg Lys Val Asp  Ser Gly
    1385                1390
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<210> 229
<211> 4179
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 mRNA ORF encoding SEQ ID NO: 28 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> 229
auggacaaga aguacagcau cggacuggca aucggaacaa acagcgucgg augggcaguc 60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga 120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga 300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

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aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgacgca 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggaagc ggaagcccga agaagaagag aaaggucgac 4140

ggaagcccga agaagaagag aaaggucgac agcggauag 4179

<210> 230
<211> 4173
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 coding sequence encoding SEQ ID NO: 28 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 230
gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgacgcaauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaagcgga agcccgaaga agaagagaaa ggucgacgga 4140

agcccgaaga agaagagaaa ggucgacagc gga 4173

<210> 231
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: T7 promoter

<400> 231
taatacgact cactata 17

<210> 232
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Human beta-globin 5 UTR

<400> 232
acatttgctt ctgacacaac tgtgttcact agcaacctca aacagacacc 50

<210> 233
<211> 132
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Human beta-globin 3 UTR

<400> 233
gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac 60

taaactgggg gatattatga agggccttga gcatctggat tctgcctaat aaaaaacatt 120

tattttcatt gc 132

<210> 234
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Human alpha-globin 5 UTR

<400> 234
cataaaccct ggcgcgctcg cggcccggca ctcttctggt ccccacagac tcagagagaa 60

cccacc 66

<210> 235
<211> 110
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Human alpha-globin 3 UTR

<400> 235
gctggagcct cggtggccat gcttcttgcc ccttgggcct ccccccagcc cctcctcccc 60

ttcctgcacc cgtacccccg tggtctttga ataaagtctg agtgggcggc 110

<210> 236
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Xenopus laevis beta-globin 5 UTR

<400> 236
aagctcagaa taaacgctca actttggcc 29

<210> 237
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Xenopus laevis beta-globin 3 UTR

<400> 237
accagcctca agaacacccg aatggagtct ctaagctaca taataccaac ttacacttta 60

caaaatgttg tcccccaaaa tgtagccatt cgtatctgct cctaataaaa agaaagtttc 120

ttcacattct 130

<210> 238
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Bovine Growth Hormone 5 UTR

<400> 238
cagggtcctg tggacagctc accagct 27

<210> 239
<211> 102
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Bovine Growth Hormone 3 UTR

<400> 239
ttgccagcca tctgttgttt gcccctcccc cgtgccttcc ttgaccctgg aaggtgccac 60

tcccactgtc ctttcctaat aaaatgagga attgcatcg ca 102

<210> 240
<211> 93
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Mus musculus hemoglobin alpha, adult chain 1 (Hba-a1),
       3UTR

<400> 240
gctgccttct gcggggcttg ccttctggcc atgcccttct tctctccctt gcacctgtac   60

ctcttggtct ttgaataaag cctgagtagg aag   93

<210> 241
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: HSD17B4 5 UTR

<400> 241
tcccgcagtc ggcgtccagc ggctctgctt gttcgtgtgt gtgtcgttgc aggccttatt   60

c   61

<210> 242
<211> 100
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: G282 guide RNA targeting TTR

<220>
<221> modified_base
<222> (1)..(3)
<223> 2'-O-Me nucleotide

<220>
<221> modified_base
<222> (1)..(4)
<223> PS linkage

<220>
<221> modified_base
<222> (29)..(40)
<223> 2'-O-Me nucleotide

<220>
<221> modified_base
<222> (69)..(100)
<223> 2'-O-Me nucleotide

<220>
<221> modified_base
<222> (97)..(100)
<223> PS linkage

<400> 242
uuacagccac gucuacagca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc   60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 243
<211> 4411
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3 UTR of ALB

<400> 243
gggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc gccaccatgg acaagaagta cagcatcgga ctggacatcg gaacaaacag 120

cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag aaaagaagaa 780

cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg acgacgacct 900

ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg cagcaaagaa 960

cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc tgacactgct 1080

gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat tctacaagtt 1200

catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc accagatcca 1320

cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt tcctgaagga 1380

caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg tcggaccgct 1440

ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa caatcacacc 1500

gtggaacttc gaagaagtcg tcgacaaggg agcaagcgca cagagcttca tcgaaagaat 1560

gacaaacttc gacaagaacc tgccgaacga aaaggtcctg ccgaagcaca gcctgctgta 1620

cgaatacttc acagtctaca acgaactgac aaaggtcaag tacgtcacag aaggaatgag 1680

aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc tgttcaagac 1740

aaacagaaag gtcacagtca agcagctgaa ggaagactac ttcaagaaga tcgaatgctt 1800

cgacagcgtc gaaatcagcg gagtcgaaga cagattcaac gcaagcctgg gaacatacca 1860

cgacctgctg aagatcatca aggacaagga cttcctggac aacgaagaaa acgaagacat 1920

cctggaagac atcgtcctga cactgacact gttcgaagac agagaaatga tcgaagaaag 1980

actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga agagaagaag 2040

atacacagga tggggaagac tgagcagaaa gctgatcaac ggaatcagag acaagcagag 2100

cggaaagaca atcctggact tcctgaagag cgacggattc gcaaacagaa acttcatgca 2160

gctgatccac gacgacagcc tgacattcaa ggaagacatc cagaaggcac aggtcagcgg 2220

acagggagac agcctgcacg aacacatcgc aaacctggca ggaagcccgg caatcaagaa 2280

gggaatcctg cagacagtca aggtcgtcga cgaactggtc aaggtcatgg gaagacacaa 2340

gccggaaaac atcgtcatcg aaatggcaag agaaaaccag acaacacaga agggacagaa 2400

gaacagcaga gaaagaatga agagaatcga agaaggaatc aaggaactgg gaagccagat 2460

cctgaaggaa cacccggtcg aaaacacaca gctgcagaac gaaaagctgt acctgtacta 2520

cctgcagaac ggaagagaca tgtacgtcga ccaggaactg gacatcaaca gactgagcga 2580

ctacgacgtc gaccacatcg tcccgcagag cttcctgaag gacgacagca tcgacaacaa 2640

ggtcctgaca agaagcgaca agaacagagg aaagagcgac aacgtcccga gcgaagaagt 2700

cgtcaagaag atgaagaact actggagaca gctgctgaac gcaaagctga tcacacagag 2760

aaagttcgac aacctgacaa aggcagagag aggaggactg agcgaactgg acaaggcagg 2820

attcatcaag agacagctgg tcgaaacaag acagatcaca aagcacgtcg cacagatcct 2880

ggacagcaga atgaacacaa agtacgacga aaacgacaag ctgatcagag aagtcaaggt 2940

catcacactg aagagcaagc tggtcagcga cttcagaaag gacttccagt tctacaaggt 3000

cagagaaatc aacaactacc accacgcaca cgacgcatac ctgaacgcag tcgtcggaac 3060

agcactgatc aagaagtacc cgaagctgga aagcgaattc gtctacggag actacaaggt 3120

ctacgacgtc agaaagatga tcgcaaagag cgaacaggaa atcggaaagg caacagcaaa 3180

gtacttcttc tacagcaaca tcatgaactt cttcaagaca gaaatcacac tggcaaacgg 3240

agaaatcaga aagagaccgc tgatcgaaac aaacggagaa acaggagaaa tcgtctggga 3300

caagggaaga gacttcgcaa cagtcagaaa ggtcctgagc atgccgcagg tcaacatcgt 3360

caagaagaca gaagtccaga caggaggatt cagcaaggaa agcatcctgc cgaagagaaa 3420

cagcgacaag ctgatcgcaa gaaagaagga ctgggacccg aagaagtacg gaggattcga 3480

cagcccgaca gtcgcataca gcgtcctggt cgtcgcaaag gtcgaaaagg gaaagagcaa 3540

gaagctgaag agcgtcaagg aactgctggg aatcacaatc atggaaagaa gcagcttcga 3600

aaagaacccg atcgacttcc tggaagcaaa gggatacaag gaagtcaaga aggacctgat 3660

catcaagctg ccgaagtaca gcctgttcga actggaaaac ggaagaaaga gaatgctggc 3720

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gcagctgttc gtcgaacagc acaagcacta cctggacgaa atcatcgaac agatcagcga 3900

attcagcaag agagtcatcc tggcagacgc aaacctggac aaggtcctga gcgcatacaa 3960

caagcacaga gacaagccga tcagagaaca ggcagaaaac atcatccacc tgttcacact 4020

gacaaacctg ggagcaccgg cagcattcaa gtacttcgac acaacaatcg acagaaagag 4080

atacacaagc acaaaggaag tcctggacgc aacactgatc caccagagca tcacaggact 4140

gtacgaaaca agaatcgacc tgagccagct gggaggagac ggaggaggaa gcccgaagaa 4200

gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt tctctgtgct 4380

tcaattaata aaaaatggaa agaacctcga g 4411

<210> 244
<211> 4405
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 4, and 3 UTR of ALB

<400> 244
gggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg 120

atgggcagtc atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa 180

cacagacaga cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga 240

aacagcagaa gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa 300

cagaatctgc tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt 360

cttccacaga ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc 420

gatcttcgga aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca 480

cctgagaaag aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc 540

actggcacac atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga 600

caacagcgac gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga 660

agaaaacccg atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag 720

caagagcaga agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact 780

gttcggaaac ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga 840

cctggcagaa gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa 900

cctgctggca cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag 960

cgacgcaatc ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct 1020

gagcgcaagc atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc 1080

actggtcaga cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa 1140

cggatacgca ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa 1200

gccgatcctg gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga 1260

cctgctgaga aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg 1320

agaactgcac gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag 1380

agaaaagatc gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag 1440

aggaaacagc agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa 1500

cttcgaagaa gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa 1560

cttcgacaag aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata 1620

cttcacagtc tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc 1680

ggcattcctg agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag 1740

aaaggtcaca gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag 1800

cgtcgaaatc agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct 1860

gctgaagatc atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga 1920

agacatcgtc ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa 1980

gacatacgca cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac 2040

aggatgggga agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa 2100

gacaatcctg gacttcctga agagcgacgg attcgcaaac agaaacttca tgcagctgat 2160

ccacgacgac agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg 2220

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cctgcagaca gtcaaggtcg tcgacgaact ggtcaaggtc atgggaagac acaagccgga 2340

aaacatcgtc atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag 2400

cagagaaaga atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa 2460

ggaacacccg gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca 2520

gaacggaaga gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga 2580

cgtcgaccac atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct 2640

gacaagaagc gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa 2700

gaagatgaag aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt 2760

cgacaacctg acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat 2820

caagagacag ctggtcgaaa caagacagat cacaaagcac gtcgcacaga tcctggacag 2880

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aatcaacaac taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact 3060

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caagagagtc atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca 3960

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aagcacaaag gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga 4140

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gaaaatgaag atcaatagct tattcatctc ttttttctttt tcgttggtgt aaagccaaca 4320

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<210> 246

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<210> 248

<400> 248
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<210> 249

<400> 249
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<210> 250
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF with splice junctions removed; 12.75% U content

<400> 250
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cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccaccgg 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

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ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

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agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

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aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac aggatgggga 1980

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aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

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atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

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ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcccga agaagaagag aaaggtctag 4140

<210> 251
<211> 4411
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 50, Kozak sequence, and 3 UTR of ALB

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cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

cagcttcttc caccggctgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

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cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

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cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

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gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

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catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

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cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt tcctgaagga 1380

caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg tcggaccgct 1440

ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa caatcacacc 1500

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gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

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tcaattaata aaaaatggaa agaacctcga g 4411

<210> 252
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF with minimal uridine codons frequently used in humans in general; 12.75% U content

<400> 252
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cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccaccagac tgaagagaac cgccagaaga agatacacca gaagaaagaa cagaatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccacaga 300

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aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgagaaag 420

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accaagtacg acgagaacga caagctgatc agagaggtga aggtgatcac cctgaagagc 2880

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taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgagaaag 3060

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gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gaccagaatc 4080

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<210> 253
<211> 4411
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding
to SEQ ID NO: 52, Kozak sequence, and 3 UTR of ALB

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cggcgagacc gccgaggcca ccagactgaa gagaaccgcc agaagaagat acaccagaag 300

aaagaacaga atctgctacc tgcaggagat cttcagcaac gagatggcca aggtggacga 360

cagcttcttc cacagactgg aggagagctt cctggtggag gaggacaaga agcacgagag 420

acaccccatc ttcggcaaca tcgtggacga ggtggcctac cacgagaagt accccaccat 480

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gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

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tcaattaata aaaaatggaa agaacctcga g 4411

<210> 254
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF with minimal uridine codons infrequently used in humans in general; 12.75% U content

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cacagcataa aaaaaaacct aataggggcg ctactattcg acagcgggga aacggcggaa 180

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gacgcgaaac tacaactaag caaagacacg tacgacgacg acctagacaa cctactagcg 840

caaatagggg accaatacgc ggacctattc ctagcggcga aaaacctaag cgacgcgata 900

ctactaagcg acatactacg agtaaacacg gaaataacga aagcgccgct aagcgcgagc 960

atgataaaac gatacgacga acaccaccaa gacctaacgc tactaaaagc gctagtacga 1020

caacaactac cggaaaaata caaagaaata ttcttcgacc aaagcaaaaa cgggtacgcg 1080

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cgactaagcc gaaaactaat aaacgggata cgagacaaac aaagcgggaa aacgatacta     2040

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<210> 255
<211> 4411
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 54, Kozak sequence, and 3 UTR of ALB

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aaaactaaaa agcgtaaaag aactactagg gataacgata atggaacgaa gcagcttcga 3600

aaaaaacccg atagacttcc tagaagcgaa agggtacaaa gaagtaaaaa aagacctaat 3660

aataaaacta ccgaaataca gcctattcga actagaaaac gggcgaaaac gaatgctagc 3720

gagcgcgggg gaactacaaa aagggaacga actagcgcta ccgagcaaat acgtaaactt 3780

cctataccta gcgagccact acgaaaaact aaaagggagc ccggaagaca acgaacaaaa 3840

acaactattc gtagaacaac acaaacacta cctagacgaa ataatagaac aaataagcga 3900

attcagcaaa cgagtaatac tagcggacgc gaacctagac aaagtactaa gcgcgtacaa 3960

caaacaccga gacaaaccga tacgagaaca agcggaaaac ataatacacc tattcacgct 4020

aacgaaccta ggggcgccgg cggcgttcaa atacttcgac acgacgatag accgaaaacg 4080

atacacgagc acgaaagaag tactagacgc gacgctaata caccaaagca taacggggct 4140

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aaaacgaaaa gtatgactag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt tctctgtgct 4380

tcaattaata aaaaatggaa agaacctcga g 4411

<210> 256
<211> 4411
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with AGG as first three nucleotides for use with CleanCapTM, 5 UTR of HSD, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3 UTR of ALB

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attcggatcc gccaccatgg acaagaagta cagcatcgga ctggacatcg gaacaaacag 120

cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag aaaagaagaa 780

cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg acgacgacct 900

ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg cagcaaagaa 960

cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc tgacactgct 1080

gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat tctacaagtt 1200

catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc accagatcca 1320

cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt tcctgaagga 1380

caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg tcggaccgct 1440

ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa caatcacacc 1500

gtggaacttc gaagaagtcg tcgacaaggg agcaagcgca cagagcttca tcgaaagaat 1560

gacaaacttc gacaagaacc tgccgaacga aaaggtcctg ccgaagcaca gcctgctgta 1620

cgaatacttc acagtctaca acgaactgac aaaggtcaag tacgtcacag aaggaatgag 1680

aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc tgttcaagac 1740

aaacagaaag gtcacagtca agcagctgaa ggaagactac ttcaagaaga tcgaatgctt 1800

cgacagcgtc gaaatcagcg gagtcgaaga cagattcaac gcaagcctgg gaacatacca 1860

cgacctgctg aagatcatca aggacaagga cttcctggac aacgaagaaa acgaagacat 1920

cctggaagac atcgtcctga cactgacact gttcgaagac agagaaatga tcgaagaaag 1980

actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga agagaagaag 2040

atacacagga tggggaagac tgagcagaaa gctgatcaac ggaatcagag acaagcagag 2100

cggaaagaca atcctggact tcctgaagag cgacggattc gcaaacagaa acttcatgca 2160

gctgatccac gacgacagcc tgacattcaa ggaagacatc cagaaggcac aggtcagcgg 2220

acagggagac agcctgcacg aacacatcgc aaacctggca ggaagcccgg caatcaagaa 2280

gggaatcctg cagacagtca aggtcgtcga cgaactggtc aaggtcatgg gaagacacaa 2340

gccggaaaac atcgtcatcg aaatggcaag agaaaaccag acaacacaga agggacagaa 2400

gaacagcaga gaaagaatga agagaatcga agaaggaatc aaggaactgg gaagccagat 2460

cctgaaggaa cacccggtcg aaaacacaca gctgcagaac gaaaagctgt acctgtacta 2520

cctgcagaac ggaagagaca tgtacgtcga ccaggaactg gacatcaaca gactgagcga 2580

ctacgacgtc gaccacatcg tcccgcagag cttcctgaag gacgacagca tcgacaacaa 2640

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aaagttcgac aacctgacaa aggcagagag aggaggactg agcgaactgg acaaggcagg 2820

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ggacagcaga atgaacacaa agtacgacga aaacgacaag ctgatcagag aagtcaaggt 2940

catcacactg aagagcaagc tggtcagcga cttcagaaag gacttccagt tctacaaggt 3000

cagagaaatc aacaactacc accacgcaca cgacgcatac ctgaacgcag tcgtcggaac 3060

agcactgatc aagaagtacc cgaagctgga aagcgaattc gtctacggag actacaaggt 3120

ctacgacgtc agaaagatga tcgcaaagag cgaacaggaa atcggaaagg caacagcaaa 3180

gtacttcttc tacagcaaca tcatgaactt cttcaagaca gaaatcacac tggcaaacgg 3240

agaaatcaga aagagaccgc tgatcgaaac aaacggagaa acaggagaaa tcgtctggga 3300

caagggaaga gacttcgcaa cagtcagaaa ggtcctgagc atgccgcagg tcaacatcgt 3360

caagaagaca gaagtccaga caggaggatt cagcaaggaa agcatcctgc cgaagagaaa 3420

cagcgacaag ctgatcgcaa gaaagaagga ctgggacccg aagaagtacg gaggattcga 3480

cagcccgaca gtcgcataca gcgtcctggt cgtcgcaaag gtcgaaaagg gaaagagcaa 3540

gaagctgaag agcgtcaagg aactgctggg aatcacaatc atggaaagaa gcagcttcga 3600

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gtacgaaaca agaatcgacc tgagccagct gggaggagac ggaggaggaa gcccgaagaa 4200

gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt tctctgtgct 4380

tcaattaata aaaaatggaa agaacctcga g 4411

<210> 257
<211> 4481
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR from CMV, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3 UTR of ALB

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cgatccagcc tccgcggccg ggaacggtgc attggaacgc ggattccccg tgccaagagt 120

gactcaccgt ccttgacacg gccaccatgg acaagaagta cagcatcgga ctggacatcg 180

gaacaaacag cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt 240

tcaaggtcct gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc 300

tgttcgacag cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat 360

acacaagaag aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa 420

aggtcgacga cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga 480

agcacgaaag acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt 540

acccgacaat ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga 600

gactgatcta cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag 660

gagacctgaa cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat 720

acaaccagct gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc 780

tgagcgcaag actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag 840

aaaagaagaa cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact 900

tcaagagcaa cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg 960

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cagcaaagaa cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa 1080

tcacaaaggc accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc 1140

tgacactgct gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct 1200

tcgaccagag caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat 1260

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agctgaacag agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc 1380

accagatcca cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt 1440

tcctgaagga caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg 1500

tcggaccgct ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa 1560

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caatcaagaa gggaatcctg cagacagtca aggtcgtcga cgaactggtc aaggtcatgg 2400

gaagacacaa gccggaaaac atcgtcatcg aaatggcaag agaaaaccag acaacacaga 2460

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caacagcaaa gtacttcttc tacagcaaca tcatgaactt cttcaagaca gaaatcacac 3300

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gaaagagcaa gaagctgaag agcgtcaagg aactgctggg aatcacaatc atggaaagaa 3660

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aggacctgat catcaagctg ccgaagtaca gcctgttcga actggaaaac ggaagaaaga 3780

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tggtgtaaag ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt 4440

tctctgtgct tcaattaata aaaaatggaa agaacctcga g 4481

<210> 258
<211> 4348
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR from HBB, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3 UTR of HBB

<400> 258
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ccaccatgga caagaagtac agcatcggac tggacatcgg aacaaacagc gtcggatggg 120

cagtcatcac agacgaatac aaggtcccga gcaagaagtt caaggtcctg ggaaacacag 180

acagacacag catcaagaag aacctgatcg gagcactgct gttcgacagc ggagaaacag 240

cagaagcaac aagactgaag agaacagcaa gaagaagata cacaagaaga aagaacagaa 300

tctgctacct gcaggaaatc ttcagcaacg aaatggcaaa ggtcgacgac agcttcttcc 360

acagactgga agaaagcttc ctggtcgaag aagacaagaa gcacgaaaga cacccgatct 420

tcggaaacat cgtcgacgaa gtcgcatacc acgaaaagta cccgacaatc taccacctga 480

gaaagaagct ggtcgacagc acagacaagg cagacctgag actgatctac ctggcactgg 540

cacacatgat caagttcaga ggacacttcc tgatcgaagg agacctgaac ccggacaaca 600

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acccgatcaa cgcaagcgga gtcgacgcaa aggcaatcct gagcgcaaga ctgagcaaga 720

gcagaagact ggaaaacctg atcgcacagc tgccgggaga aaagaagaac ggactgttcg 780

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aaagaatgaa gagaatcgaa gaaggaatca aggaactggg aagccagatc ctgaaggaac 2460

acccggtcga aaacacacag ctgcagaacg aaaagctgta cctgtactac ctgcagaacg 2520

gaagagacat gtacgtcgac caggaactgg acatcaacag actgagcgac tacgacgtcg 2580

accacatcgt cccgcagagc ttcctgaagg acgacagcat cgacaacaag gtcctgacaa 2640

gaagcgacaa gaacagagga aagagcgaca acgtcccgag cgaagaagtc gtcaagaaga 2700

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acctgacaaa ggcagagaga ggaggactga gcgaactgga caaggcagga ttcatcaaga 2820

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gaaagatgat cgcaaagagc gaacaggaaa tcggaaaggc aacagcaaag tacttcttct 3180

acagcaacat catgaacttc ttcaagacag aaatcacact ggcaaacgga gaaatcagaa 3240

agagaccgct gatcgaaaca aacggagaaa caggagaaat cgtctgggac aagggaagag 3300

acttcgcaac agtcagaaag gtcctgagca tgccgcaggt caacatcgtc aagaagacag 3360

aagtccagac aggaggattc agcaaggaaa gcatcctgcc gaagagaaac agcgacaagc 3420

tgatcgcaag aaagaaggac tgggacccga agaagtacgg aggattcgac agcccgacag 3480

tcgcatacag cgtcctggtc gtcgcaaagg tcgaaaaggg aaagagcaag aagctgaaga 3540

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<210> 259
<211> 4325
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 transcript with 5 UTR from XBG, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3 UTR of XBG

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aggtcccgag caagaagttc aaggtcctgg gaaacacaga cagacacagc atcaagaaga 180

acctgatcgg agcactgctg ttcgacagcg gagaaacagc agaagcaaca agactgaaga 240

gaacagcaag aagaagatac acaagaagaa agaacagaat ctgctacctg caggaaatct 300

tcagcaacga aatggcaaag gtcgacgaca gcttcttcca cagactggaa gaaagcttcc 360

tggtcgaaga agacaagaag cacgaaagac acccgatctt cggaaacatc gtcgacgaag 420

tcgcatacca cgaaaagtac ccgacaatct accacctgag aaagaagctg gtcgacagca 480

cagacaaggc agacctgaga ctgatctacc tggcactggc acacatgatc aagttcagag 540

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tcgag 4325

<210> 260
<211> 4325
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<213> Artificial Sequence

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<223> Synthetic: Cas9 transcript with AGG as first three nucleotides for use with CleanCapTM, 5 UTR from XBG, ORF corresponding to SEQ ID NO: 4, Kozak sequence, and 3 UTR of XBG

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gaacagcaag aagaagatac acaagaagaa agaacagaat ctgctacctg caggaaatct 300

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tggtcgaaga agacaagaag cacgaaagac acccgatctt cggaaacatc gtcgacgaag 420

tcgcatacca cgaaaagtac ccgacaatct accacctgag aaagaagctg gtcgacagca 480

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<223> Synthetic: Cas9 transcript with AGG as first three nucleotides
for use with CleanCapTM, 5 UTR from HSD, ORF corresponding to

SEQ ID NO: 4, Kozak sequence, and 3 UTR of ALB

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catcaagctg ccgaagtaca gcctgttcga actggaaaac ggaagaaaga gaatgctggc 3720

aagcgcagga gaactgcaga agggaaacga actggcactg ccgagcaagt acgtcaactt 3780

cctgtacctg gcaagccact acgaaaagct gaagggaagc ccggaagaca acgaacagaa 3840

gcagctgttc gtcgaacagc acaagcacta cctggacgaa atcatcgaac agatcagcga 3900

attcagcaag agagtcatcc tggcagacgc aaacctggac aaggtcctga gcgcatacaa 3960

caagcacaga gacaagccga tcagagaaca ggcagaaaac atcatccacc tgttcacact 4020

gacaaacctg ggagcaccgg cagcattcaa gtacttcgac acaacaatcg acagaaagag 4080

atacacaagc acaaaggaag tcctggacgc aacactgatc caccagagca tcacaggact 4140

gtacgaaaca agaatcgacc tgagccagct gggaggagac ggaggaggaa gcccgaagaa 4200

gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt tctctgtgct 4380

tcaattaata aaaaatggaa agaacctcga g 4411

<210> 262
<211> 105
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: 30/30/39 poly-A sequence

<400> 262
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa gcgaaaaaaa aaaaaaaaaa aaaaaaaaaa 60

aaaccgaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 105

<210> 263
<211> 93
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: poly-A 100 sequence

<400> 263
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 60

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa 93

<210> 264

<400> 264
000

<210> 265
<211> 3312
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: ORF encoding Neisseria meningitidis Cas9

<400> 265
atggcagcat tcaagccgaa ctcgatcaac tacatcctgg gactggacat cggaatcgca 60

tcggtcggat gggcaatggt cgaaatcgac gaagaagaaa acccgatcag actgatcgac 120

ctgggagtca gagtcttcga aagagcagaa gtcccgaaga caggagactc gctggcaatg 180

gcaagaagac tggcaagatc ggtcagaaga ctgacaagaa gaagagcaca cagactgctg 240

agaacaagaa gactgctgaa gagagaagga gtcctgcagg cagcaaactt cgacgaaaac 300

ggactgatca agtcgctgcc gaacacaccg tggcagctga gagcagcagc actggacaga 360

aagctgacac cgctggaatg gtcggcagtc ctgctgcacc tgatcaagca cagaggatac 420

ctgtcgcaga gaaagaacga aggagaaaca gcagacaagg aactgggagc actgctgaag 480

ggagtcgcag gaaacgcaca cgcactgcag acaggagact tcagaacacc ggcagaactg 540

gcactgaaca agttcgaaaa ggaatcggga cacatcagaa accagagatc ggactactcg 600

cacacattct cgagaaagga cctgcaggca gaactgatcc tgctgttcga aaagcagaag 660

gaattcggaa acccgcacgt ctcgggagga ctgaaggaag gaatcgaaac actgctgatg 720

acacagagac cggcactgtc gggagacgca gtccagaaga tgctgggaca ctgcacattc 780

gaaccggcag aaccgaaggc agcaaagaac acatacacag cagaaagatt catctggctg 840

acaaagctga acaacctgag aatcctggaa cagggatcgg aaagaccgct gacagacaca 900

gaaagagcaa cactgatgga cgaaccgtac agaaagtcga agctgacata cgcacaggca 960

agaaagctgc tgggactgga agacacagca ttcttcaagg gactgagata cggaaaggac 1020

aacgcagaag catcgacact gatggaaatg aaggcatacc acgcaatctc gagagcactg 1080

gaaaaggaag gactgaagga caagaagtcg ccgctgaacc tgtcgccgga actgcaggac 1140

gaaatcggaa cagcattctc gctgttcaag acagacgaag acatcacagg aagactgaag 1200

gacagaatcc agccggaaat cctggaagca ctgctgaagc acatctcgtt cgacaagttc 1260

gtccagatct cgctgaaggc actgagaaga atcgtcccgc tgatggaaca gggaaagaga 1320

tacgacgaag catgcgcaga aatctacgga gaccactacg gaaagaagaa cacagaagaa 1380

aagatctacc tgccgccgat cccggcagac gaaatcagaa acccggtcgt cctgagagca 1440

ctgtcgcagg caagaaaggt catcaacgga gtcgtcagaa gatacggatc gccggcaaga 1500

atccacatcg aaacagcaag agaagtcgga aagtcgttca aggacagaaa ggaaatcgaa 1560

aagagacagg aagaaaacag aaaggacaga gaaaaggcag cagcaaagtt cagagaatac 1620

ttcccgaact tcgtcggaga accgaagtcg aaggacatcc tgaagctgag actgtacgaa 1680

cagcagcacg gaaagtgcct gtactcggga aaggaaatca acctgggaag actgaacgaa 1740

aagggatacg tcgaaatcga ccacgcactg ccgttctcga gaacatggga cgactcgttc 1800

aacaacaagg tcctggtcct gggatcggaa aaccagaaca agggaaacca gacaccgtac 1860

gaatacttca acggaaagga caactcgaga gaatggcagg aattcaaggc aagagtcgaa 1920

acatcgagat tcccgagatc gaagaagcag agaatcctgc tgcagaagtt cgacgaagac 1980

ggattcaagg aaagaaacct gaacgacaca agatacgtca acagattcct gtgccagttc 2040

gtcgcagaca gaatgagact gacaggaaag ggaaagaaga gagtcttcgc atcgaacgga 2100

cagatcacaa acctgctgag aggattctgg ggactgagaa aggtcagagc agaaaacgac 2160

agacaccacg cactggacgc agtcgtcgtc gcatgctcga cagtcgcaat gcagcagaag 2220

atcacaagat tcgtcagata caaggaaatg aacgcattcg acggaaagac aatcgacaag 2280

gaaacaggag aagtcctgca ccagaagaca cacttcccgc agccgtggga attcttcgca 2340

caggaagtca tgatcagagt cttcggaaag ccggacggaa agccggaatt cgaagaagca 2400

gacacactgg aaaagctgag aacactgctg gcagaaaagc tgtcgtcgag accggaagca 2460

gtccacgaat acgtcacacc gctgttcgtc tcgagagcac cgaacagaaa gatgtcggga 2520

cagggacaca tggaaacagt caagtcggca aagagactgg acgaaggagt ctcggtcctg 2580

agagtcccgc tgacacagct gaagctgaag gacctggaaa agatggtcaa cagagaaaga 2640

gaaccgaagc tgtacgaagc actgaaggca agactggaag cacacaagga cgacccggca 2700

aaggcattcg cagaaccgtt ctacaagtac gacaaggcag gaaacagaac acagcaggtc 2760

aaggcagtca gagtcgaaca ggtccagaag acaggagtct gggtcagaaa ccacaacgga 2820

atcgcagaca acgcaacaat ggtcagagta gacgtcttcg aaaagggaga caagtactac 2880

ctggtcccga tctactcgtg gcaggtcgca aagggaatcc tgccggacag agcagtcgtc 2940

cagggaaagg acgaagaaga ctggcagctg atcgacgact cgttcaactt caagttctcg 3000

ctgcacccga acgacctggt cgaagtcatc acaaagaagg caagaatgtt cggatacttc 3060

gcatcgtgcc acagaggaac aggaaacatc aacatcagaa tccacgacct ggaccacaag 3120

atcggaaaga acggaatcct ggaaggaatc ggagtcaaga cagcactgtc gttccagaag 3180

taccagatcg acgaactggg aaaggaaatc agaccgtgca gactgaagaa gagaccgccg 3240

gtcagatccg gaaagagaac agcagacgga tcggaattcg aatcgccgaa gaagaagaga 3300

aaggtcgaat ga 3312

<210> 266
<211> 3306
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: ORF encoding Neisseria meningitidis Cas9 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 266
gcagcattca agccgaactc gatcaactac atcctgggac tggacatcgg aatcgcatcg 60

gtcggatggg caatggtcga aatcgacgaa gaagaaaacc cgatcagact gatcgacctg 120

ggagtcagag tcttcgaaag agcagaagtc ccgaagacag gagactcgct ggcaatggca 180

agaagactgg caagatcggt cagaagactg acaagaagaa gagcacacag actgctgaga 240

acaagaagac tgctgaagag agaaggagtc ctgcaggcag caaacttcga cgaaaacgga 300

ctgatcaagt cgctgccgaa cacaccgtgg cagctgagag cagcagcact ggacagaaag 360

ctgacaccgc tggaatggtc ggcagtcctg ctgcacctga tcaagcacag aggatacctg 420

tcgcagagaa agaacgaagg agaaacagca gacaaggaac tgggagcact gctgaaggga 480

gtcgcaggaa acgcacacgc actgcagaca ggagacttca gaacaccggc agaactggca 540

ctgaacaagt tcgaaaagga atcgggacac atcagaaacc agagatcgga ctactcgcac 600

acattctcga gaaaggacct gcaggcagaa ctgatcctgc tgttcgaaaa gcagaaggaa 660

ttcggaaacc cgcacgtctc gggaggactg aaggaaggaa tcgaaacact gctgatgaca 720

cagagaccgg cactgtcggg agacgcagtc cagaagatgc tgggacactg cacattcgaa 780

ccggcagaac cgaaggcagc aaagaacaca tacacagcag aaagattcat ctggctgaca 840

aagctgaaca acctgagaat cctggaacag ggatcggaaa gaccgctgac agacacagaa 900

agagcaacac tgatggacga accgtacaga aagtcgaagc tgacatacgc acaggcaaga 960

aagctgctgg gactggaaga cacagcattc ttcaagggac tgagatacgg aaaggacaac 1020

gcagaagcat cgacactgat ggaaatgaag gcataccacg caatctcgag agcactggaa 1080

aaggaaggac tgaaggacaa gaagtcgccg ctgaacctgt cgccggaact gcaggacgaa 1140

atcggaacag cattctcgct gttcaagaca gacgaagaca tcacaggaag actgaaggac 1200

agaatccagc cggaaatcct ggaagcactg ctgaagcaca tctcgttcga caagttcgtc 1260

cagatctcgc tgaaggcact gagaagaatc gtcccgctga tggaacaggg aaagagatac 1320

gacgaagcat gcgcagaaat ctacggagac cactacggaa agaagaacac agaagaaaag 1380

atctacctgc cgccgatccc ggcagacgaa atcagaaacc cggtcgtcct gagagcactg 1440

tcgcaggcaa gaaaggtcat caacggagtc gtcagaagat acggatcgcc ggcaagaatc 1500

cacatcgaaa cagcaagaga agtcggaaag tcgttcaagg acagaaagga aatcgaaaag 1560

agacaggaag aaaacagaaa ggacagagaa aaggcagcag caaagttcag agaatacttc 1620

ccgaacttcg tcggagaacc gaagtcgaag gacatcctga agctgagact gtacgaacag 1680

cagcacggaa agtgcctgta ctcgggaaag gaaatcaacc tgggaagact gaacgaaaag 1740

ggatacgtcg aaatcgacca cgcactgccg ttctcgagaa catgggacga ctcgttcaac 1800

aacaaggtcc tggtcctggg atcggaaaac cagaacaagg gaaaccagac accgtacgaa 1860

tacttcaacg gaaaggacaa ctcgagagaa tggcaggaat tcaaggcaag agtcgaaaca 1920

tcgagattcc cgagatcgaa gaagcagaga atcctgctgc agaagttcga cgaagacgga 1980

ttcaaggaaa gaaacctgaa cgacacaaga tacgtcaaca gattcctgtg ccagttcgtc 2040

gcagacagaa tgagactgac aggaaaggga aagaagagag tcttcgcatc gaacggacag 2100

atcacaaacc tgctgagagg attctgggga ctgagaaagg tcagagcaga aaacgacaga 2160

caccacgcac tggacgcagt cgtcgtcgca tgctcgacag tcgcaatgca gcagaagatc 2220

acaagattcg tcagatacaa ggaaatgaac gcattcgacg gaaagacaat cgacaaggaa 2280

acaggagaag tcctgcacca gaagacacac ttcccgcagc cgtgggaatt cttcgcacag 2340

gaagtcatga tcagagtctt cggaaagccg gacggaaagc cggaattcga agaagcagac 2400

acactggaaa agctgagaac actgctggca gaaaagctgt cgtcgagacc ggaagcagtc 2460

cacgaatacg tcacaccgct gttcgtctcg agagcaccga acagaaagat gtcgggacag 2520

ggacacatgg aaacagtcaa gtcggcaaag agactggacg aaggagtctc ggtcctgaga 2580

gtcccgctga cacagctgaa gctgaaggac ctggaaaaga tggtcaacag agaaagagaa 2640

ccgaagctgt acgaagcact gaaggcaaga ctggaagcac acaaggacga cccggcaaag 2700

gcattcgcag aaccgttcta caagtacgac aaggcaggaa acagaacaca gcaggtcaag 2760

gcagtcagag tcgaacaggt ccagaagaca ggagtctggg tcagaaacca caacggaatc 2820

gcagacaacg caacaatggt cagagtagac gtcttcgaaa agggagacaa gtactacctg 2880

gtcccgatct actcgtggca ggtcgcaaag ggaatcctgc cggacagagc agtcgtccag 2940

ggaaaggacg aagaagactg gcagctgatc gacgactcgt tcaacttcaa gttctcgctg 3000

cacccgaacg acctggtcga agtcatcaca aagaaggcaa gaatgttcgg atacttcgca 3060

tcgtgccaca gaggaacagg aaacatcaac atcagaatcc acgacctgga ccacaagatc 3120

ggaaagaacg gaatcctgga aggaatcgga gtcaagacag cactgtcgtt ccagaagtac 3180

cagatcgacg aactgggaaa ggaaatcaga ccgtgcagac tgaagaagag accgccggtc 3240

agatccggaa agagaacagc agacggatcg gaattcgaat cgccgaagaa gaagagaaag 3300

gtcgaa 3306

<210> 267
<211> 3636
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Transcript comprising SEQ ID NO: 65 (encoding Neisseria meningitidis Cas9)

<400> 267
gggagaccca agctggctag cgtttaaact taagcttgga tccgccacca tggcagcatt 60

caagccgaac tcgatcaact acatcctggg actggacatc ggaatcgcat cggtcggatg 120

ggcaatggtc gaaatcgacg aagaagaaaa cccgatcaga ctgatcgacc tgggagtcag 180

agtcttcgaa agagcagaag tcccgaagac aggagactcg ctggcaatgg caagaagact 240

ggcaagatcg gtcagaagac tgacaagaag aagagcacac agactgctga gaacaagaag 300

actgctgaag agagaaggag tcctgcaggc agcaaacttc gacgaaaacg gactgatcaa 360

gtcgctgccg aacacaccgt ggcagctgag agcagcagca ctggacagaa agctgacacc 420

gctggaatgg tcggcagtcc tgctgcacct gatcaagcac agaggatacc tgtcgcagag 480

aaagaacgaa ggagaaacag cagacaagga actgggagca ctgctgaagg gagtcgcagg 540

aaacgcacac gcactgcaga caggagactt cagaacaccg gcagaactgg cactgaacaa 600

gttcgaaaag gaatcgggac acatcagaaa ccagagatcg gactactcgc acacattctc 660

gagaaaggac ctgcaggcag aactgatcct gctgttcgaa aagcagaagg aattcggaaa 720

cccgcacgtc tcgggaggac tgaaggaagg aatcgaaaca ctgctgatga cacagagacc 780

ggcactgtcg ggagacgcag tccagaagat gctgggacac tgcacattcg aaccggcaga 840

accgaaggca gcaaagaaca catacacagc agaaagattc atctggctga caaagctgaa 900

caacctgaga atcctggaac agggatcgga aagaccgctg acagacacag aaagagcaac 960

actgatggac gaaccgtaca gaaagtcgaa gctgacatac gcacaggcaa gaaagctgct 1020

gggactggaa gacacagcat tcttcaaggg actgagatac ggaaaggaca acgcagaagc 1080

atcgacactg atggaaatga aggcatacca cgcaatctcg agagcactgg aaaaggaagg 1140

actgaaggac aagaagtcgc cgctgaacct gtcgccggaa ctgcaggacg aaatcggaac 1200

agcattctcg ctgttcaaga cagacgaaga catcacagga agactgaagg acagaatcca 1260

gccggaaatc ctggaagcac tgctgaagca catctcgttc gacaagttcg tccagatctc 1320

gctgaaggca ctgagaagaa tcgtcccgct gatggaacag ggaaagagat acgacgaagc 1380

atgcgcagaa atctacggag accactacgg aaagaagaac acagaagaaa agatctacct 1440

gccgccgatc ccggcagacg aaatcagaaa cccggtcgtc ctgagagcac tgtcgcaggc 1500

aagaaaggtc atcaacggag tcgtcagaag atacggatcg ccggcaagaa tccacatcga 1560

aacagcaaga gaagtcggaa agtcgttcaa ggacagaaag gaaatcgaaa agagacagga 1620

agaaaacaga aaggacagag aaaaggcagc agcaaagttc agagaatact tcccgaactt 1680

cgtcggagaa ccgaagtcga aggacatcct gaagctgaga ctgtacgaac agcagcacgg 1740

aaagtgcctg tactcgggaa aggaaatcaa cctgggaaga ctgaacgaaa agggatacgt 1800

cgaaatcgac cacgcactgc cgttctcgag aacatgggac gactcgttca acaacaaggt 1860

cctggtcctg ggatcggaaa accagaacaa gggaaaccag acaccgtacg aatacttcaa 1920

cggaaaggac aactcgagag aatggcagga attcaaggca agagtcgaaa catcgagatt 1980

cccgagatcg aagaagcaga gaatcctgct gcagaagttc gacgaagacg gattcaagga 2040

aagaaacctg aacgacacaa gatacgtcaa cagattcctg tgccagttcg tcgcagacag 2100

aatgagactg acaggaaagg gaaagaagag agtcttcgca tcgaacggac agatcacaaa 2160

cctgctgaga ggattctggg gactgagaaa ggtcagagca gaaaacgaca gacaccacgc 2220

actggacgca gtcgtcgtcg catgctcgac agtcgcaatg cagcagaaga tcacaagatt 2280

cgtcagatac aaggaaatga acgcattcga cggaaagaca atcgacaagg aaacaggaga 2340

agtcctgcac cagaagacac acttcccgca gccgtgggaa ttcttcgcac aggaagtcat 2400

gatcagagtc ttcggaaagc cggacggaaa gccggaattc gaagaagcag acacactgga 2460

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cgtcacaccg ctgttcgtct cgagagcacc gaacagaaag atgtcgggac agggacacat 2580

ggaaacagtc aagtcggcaa agagactgga cgaaggagtc tcggtcctga gagtcccgct 2640

gacacagctg aagctgaagg acctggaaaa gatggtcaac agagaaagag aaccgaagct 2700

gtacgaagca ctgaaggcaa gactggaagc acacaaggac gacccggcaa aggcattcgc 2760

agaaccgttc tacaagtacg acaaggcagg aaacagaaca cagcaggtca aggcagtcag 2820

agtcgaacag gtccagaaga caggagtctg ggtcagaaac cacaacggaa tcgcagacaa 2880

cgcaacaatg gtcagagtag acgtcttcga aaagggagac aagtactacc tggtcccgat 2940

ctactcgtgg caggtcgcaa agggaatcct gccggacaga gcagtcgtcc agggaaagga 3000

cgaagaagac tggcagctga tcgacgactc gttcaacttc aagttctcgc tgcacccgaa 3060

cgacctggtc gaagtcatca caaagaaggc aagaatgttc ggatacttcg catcgtgcca 3120

cagaggaaca ggaaacatca acatcagaat ccacgacctg gaccacaaga tcggaaagaa 3180

cggaatcctg gaaggaatcg gagtcaagac agcactgtcg ttccagaagt accagatcga 3240

cgaactggga aaggaaatca gaccgtgcag actgaagaag agaccgccgg tcagatccgg 3300

aaagagaaca gcagacggat cggaattcga atcgccgaag aagaagagaa aggtcgaatg 3360

atagctagct cgagtctaga gggcccgttt aaacccgctg atcagcctcg actgtgcctt 3420

ctagttgcca gccatctgtt gtttgcccct cccccgtgcc ttccttgacc ctggaaggtg 3480

ccactcccac tgtcctttcc taataaaatg aggaaattgc atcgcattgt ctgagtaggt 3540

gtcattctat tctgggggggt ggggtggggc aggacagcaa gggggaggat tgggaagaca 3600

atagcaggca tgctggggat gcggtgggct ctatgg 3636

<210> 268
<211> 1103
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence of Neisseria meningitidis Cas9

<400> 268

Met Ala Ala Phe Lys Pro Asn Ser Ile Asn Tyr Ile Leu Gly Leu Asp
1 5 10 15

Ile Gly Ile Ala Ser Val Gly Trp Ala Met Val Glu Ile Asp Glu Glu
20 25 30

Glu Asn Pro Ile Arg Leu Ile Asp Leu Gly Val Arg Val Phe Glu Arg
35 40 45

Ala Glu Val Pro Lys Thr Gly Asp Ser Leu Ala Met Ala Arg Arg Leu
50 55 60

Ala Arg Ser Val Arg Arg Leu Thr Arg Arg Arg Ala His Arg Leu Leu
65 70 75 80

Arg Thr Arg Arg Leu Leu Lys Arg Glu Gly Val Leu Gln Ala Ala Asn
85 90 95

Phe Asp Glu Asn Gly Leu Ile Lys Ser Leu Pro Asn Thr Pro Trp Gln
100 105 110

Leu Arg Ala Ala Ala Leu Asp Arg Lys Leu Thr Pro Leu Glu Trp Ser
115 120 125

Ala Val Leu Leu His Leu Ile Lys His Arg Gly Tyr Leu Ser Gln Arg
130 135 140

Lys Asn Glu Gly Glu Thr Ala Asp Lys Glu Leu Gly Ala Leu Leu Lys
145 150 155 160

Gly Val Ala Gly Asn Ala His Ala Leu Gln Thr Gly Asp Phe Arg Thr
165 170 175

Pro Ala Glu Leu Ala Leu Asn Lys Phe Glu Lys Glu Ser Gly His Ile
180 185 190

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Arg Asn Gln Arg Ser Asp Tyr Ser His Thr Phe Ser Arg Lys Asp Leu
        195                 200                 205

Gln Ala Glu Leu Ile Leu Leu Phe Glu Lys Gln Lys Glu Phe Gly Asn
    210                 215                 220

Pro His Val Ser Gly Gly Leu Lys Glu Gly Ile Glu Thr Leu Leu Met
225                 230                 235                 240

Thr Gln Arg Pro Ala Leu Ser Gly Asp Ala Val Gln Lys Met Leu Gly
                245                 250                 255

His Cys Thr Phe Glu Pro Ala Glu Pro Lys Ala Ala Lys Asn Thr Tyr
            260                 265                 270

Thr Ala Glu Arg Phe Ile Trp Leu Thr Lys Leu Asn Asn Leu Arg Ile
        275                 280                 285

Leu Glu Gln Gly Ser Glu Arg Pro Leu Thr Asp Thr Glu Arg Ala Thr
    290                 295                 300

Leu Met Asp Glu Pro Tyr Arg Lys Ser Lys Leu Thr Tyr Ala Gln Ala
305                 310                 315                 320

Arg Lys Leu Leu Gly Leu Glu Asp Thr Ala Phe Phe Lys Gly Leu Arg
                325                 330                 335

Tyr Gly Lys Asp Asn Ala Glu Ala Ser Thr Leu Met Glu Met Lys Ala
            340                 345                 350

Tyr His Ala Ile Ser Arg Ala Leu Glu Lys Glu Gly Leu Lys Asp Lys
        355                 360                 365

Lys Ser Pro Leu Asn Leu Ser Pro Glu Leu Gln Asp Glu Ile Gly Thr
    370                 375                 380

Ala Phe Ser Leu Phe Lys Thr Asp Glu Asp Ile Thr Gly Arg Leu Lys
385                 390                 395                 400

Asp Arg Ile Gln Pro Glu Ile Leu Glu Ala Leu Leu Lys His Ile Ser
                405                 410                 415

Phe Asp Lys Phe Val Gln Ile Ser Leu Lys Ala Leu Arg Arg Ile Val
            420                 425                 430

Pro Leu Met Glu Gln Gly Lys Arg Tyr Asp Glu Ala Cys Ala Glu Ile
        435                 440                 445

Tyr Gly Asp His Tyr Gly Lys Lys Asn Thr Glu Glu Lys Ile Tyr Leu
    450                 455                 460
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Pro Pro Ile Pro Ala Asp Glu Ile Arg Asn Pro Val Val Leu Arg Ala
465                 470                 475                 480

Leu Ser Gln Ala Arg Lys Val Ile Asn Gly Val Val Arg Arg Tyr Gly
                485                 490                 495

Ser Pro Ala Arg Ile His Ile Glu Thr Ala Arg Glu Val Gly Lys Ser
            500                 505                 510

Phe Lys Asp Arg Lys Glu Ile Glu Lys Arg Gln Glu Glu Asn Arg Lys
        515                 520                 525

Asp Arg Glu Lys Ala Ala Ala Lys Phe Arg Glu Tyr Phe Pro Asn Phe
    530                 535                 540

Val Gly Glu Pro Lys Ser Lys Asp Ile Leu Lys Leu Arg Leu Tyr Glu
545                 550                 555                 560

Gln Gln His Gly Lys Cys Leu Tyr Ser Gly Lys Glu Ile Asn Leu Gly
                565                 570                 575

Arg Leu Asn Glu Lys Gly Tyr Val Glu Ile Asp His Ala Leu Pro Phe
            580                 585                 590

Ser Arg Thr Trp Asp Asp Ser Phe Asn Asn Lys Val Leu Val Leu Gly
        595                 600                 605

Ser Glu Asn Gln Asn Lys Gly Asn Gln Thr Pro Tyr Glu Tyr Phe Asn
    610                 615                 620

Gly Lys Asp Asn Ser Arg Glu Trp Gln Glu Phe Lys Ala Arg Val Glu
625                 630                 635                 640

Thr Ser Arg Phe Pro Arg Ser Lys Lys Gln Arg Ile Leu Leu Gln Lys
                645                 650                 655

Phe Asp Glu Asp Gly Phe Lys Glu Arg Asn Leu Asn Asp Thr Arg Tyr
            660                 665                 670

Val Asn Arg Phe Leu Cys Gln Phe Val Ala Asp Arg Met Arg Leu Thr
        675                 680                 685

Gly Lys Gly Lys Lys Arg Val Phe Ala Ser Asn Gly Gln Ile Thr Asn
    690                 695                 700

Leu Leu Arg Gly Phe Trp Gly Leu Arg Lys Val Arg Ala Glu Asn Asp
705                 710                 715                 720

Arg His His Ala Leu Asp Ala Val Val Val Ala Cys Ser Thr Val Ala
                725                 730                 735

Met Gln Gln Lys Ile Thr Arg Phe Val Arg Tyr Lys Glu Met Asn Ala
```

```
            740                 745                 750

Phe Asp Gly Lys Thr Ile Asp Lys Glu Thr Gly Glu Val Leu His Gln
        755                 760                 765

Lys Thr His Phe Pro Gln Pro Trp Glu Phe Phe Ala Gln Glu Val Met
    770                 775                 780

Ile Arg Val Phe Gly Lys Pro Asp Gly Lys Pro Glu Phe Glu Glu Ala
785                 790                 795                 800

Asp Thr Leu Glu Lys Leu Arg Thr Leu Leu Ala Glu Lys Leu Ser Ser
                805                 810                 815

Arg Pro Glu Ala Val His Glu Tyr Val Thr Pro Leu Phe Val Ser Arg
            820                 825                 830

Ala Pro Asn Arg Lys Met Ser Gly Gln Gly His Met Glu Thr Val Lys
        835                 840                 845

Ser Ala Lys Arg Leu Asp Glu Gly Val Ser Val Leu Arg Val Pro Leu
    850                 855                 860

Thr Gln Leu Lys Leu Lys Asp Leu Glu Lys Met Val Asn Arg Glu Arg
865                 870                 875                 880

Glu Pro Lys Leu Tyr Glu Ala Leu Lys Ala Arg Leu Glu Ala His Lys
                885                 890                 895

Asp Asp Pro Ala Lys Ala Phe Ala Glu Pro Phe Tyr Lys Tyr Asp Lys
            900                 905                 910

Ala Gly Asn Arg Thr Gln Gln Val Lys Ala Val Arg Val Glu Gln Val
        915                 920                 925

Gln Lys Thr Gly Val Trp Val Arg Asn His Asn Gly Ile Ala Asp Asn
    930                 935                 940

Ala Thr Met Val Arg Val Asp Val Phe Glu Lys Gly Asp Lys Tyr Tyr
945                 950                 955                 960

Leu Val Pro Ile Tyr Ser Trp Gln Val Ala Lys Gly Ile Leu Pro Asp
                965                 970                 975

Arg Ala Val Val Gln Gly Lys Asp Glu Glu Asp Trp Gln Leu Ile Asp
            980                 985                 990

Asp Ser Phe Asn Phe Lys Phe Ser  Leu His Pro Asn Asp  Leu Val Glu
        995                 1000                1005

Val Ile  Thr Lys Lys Ala Arg  Met Phe Gly Tyr Phe  Ala Ser Cys
    1010                1015                1020
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His Arg  Gly Thr Gly Asn Ile  Asn Ile Arg Ile His  Asp Leu Asp
    1025                1030                1035


His Lys  Ile Gly Lys Asn Gly  Ile Leu Glu Gly Ile  Gly Val Lys
    1040                1045                1050


Thr Ala  Leu Ser Phe Gln Lys  Tyr Gln Ile Asp Glu  Leu Gly Lys
    1055                1060                1065


Glu Ile  Arg Pro Cys Arg Leu  Lys Lys Arg Pro Pro  Val Arg Ser
    1070                1075                1080


Gly Lys  Arg Thr Ala Asp Gly  Ser Glu Phe Glu Ser  Pro Lys Lys
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Lys Arg  Lys Val Glu
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<220>
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<220>
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<400>  270
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu                            100
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<212> RNA
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<220>
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<400> 271
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu   100

<210> 272
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<212> RNA
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<220>
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<222> (69)..(100)

<223> 2'-O-Me nucleotide

<220>
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<222> (97)..(100)
<223> PS linkage

<400> 272
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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> 273
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<220>
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<400> 273
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ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

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gtcccgcagt cggcgtccag cggctctgct tgttcgtgtg tgtgtcgttg caggccttat 480

tcggatccat ggtgagcaag ggcgaggagc tgttcaccgg ggtggtgccc atcctggtcg 540

agctggacgg cgacgtaaac ggccacaagt tcagcgtgtc cggcgagggc gagggcgatg 600

ccacctacgg caagctgacc ctgaagttca tctgcaccac cggcaagctg cccgtgccct 660

ggcccaccct cgtgaccacc ctgacctacg gcgtgcagtg cttcagccgc taccccgacc 720

acatgaagca gcacgacttc ttcaagtccg ccatgcccga aggctacgtc caggagcgca 780

ccatcttctt caaggacgac ggcaactaca agacccgcgc cgaggtgaag ttcgagggcg 840

acaccctggt gaaccgcatc gagctgaagg gcatcgactt caaggaggac ggcaacatcc 900

tggggcacaa gctggagtac aactacaaca gccacaacgt ctatatcatg gccgacaagc 960

agaagaacgg catcaaggtg aacttcaaga tccgccacaa catcgaggac ggcagcgtgc 1020

agctcgccga ccactaccag cagaacaccc ccatcggcga cggccccgtg ctgctgcccg 1080

acaaccacta cctgagcacc cagtccgccc tgagcaaaga ccccaacgag aagcgcgatc 1140

acatggtcct gctggagttc gtgaccgccg ccgggatcac tctcggcatg gacgagctgt 1200

acaagtaata ggaattatgc agtctagcca tcacatttaa aagcatctca gcctaccatg 1260

agaataagag aaagaaaatg aagatcaata gcttattcat ctcttttttct ttttcgttgg 1320

tgtaaagcca acaccctgtc taaaaaacat aaatttcttt aatcattttg cctcttttct 1380

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aaaaaaaaaa aaaaaaaatc tagacttaag cttgatgagc tctagcttgg cgtaatcatg 1560

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ccctgacgag catcacaaaa atcgacgctc aagtcagagg tggcgaaacc cgacaggact 2040

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cccggtaaga cacgacttat cgccactggc agcagccact ggtaacagga ttagcagagc 2340

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aagaacagta tttggtatct gcgctctgct gaagccagtt accttcggaa aaagagttgg 2460

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gttggccgca gtgttatcac tcatggttat ggcagcactg cataattctc ttactgtcat 3240

gccatccgta agatgctttt ctgtgactgg tgagtactca accaagtcat tctgagaata 3300

gtgtatgcgg cgaccgagtt gctcttgccc ggcgtcaata cgggataata ccgcgccaca 3360

tagcagaact ttaaaagtgc tcatcattgg aaaacgttct tcggggcgaa aactctcaag 3420

gatcttaccg ctgttgagat ccagttcgat gtaacccact cgtgcaccca actgatcttc 3480

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ttattgaagc atttatcagg gttattgtct catgagcgga tacatatttg aatgtattta 3660

gaaaaataaa caaatagggg ttccgcgcac atttccccga aaagtgccac ctgacgtcta 3720

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tcg 3783

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<212> DNA
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<210> 276
<211> 42
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<400> 276
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<210> 277
<211> 42
<212> DNA
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<220>
<223> Synthetic: CMV-3 5 UTR

<400> 277
tgcattggaa cgcggattcc ccgtgccaag agtgactcac cg 42

<210> 278
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
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<400> 278

Pro Lys Lys Lys Arg Lys Val
1 5

<210> 279

<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 279

Leu Ala Ala Lys Arg Ser Arg Thr Thr
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<210> 280
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 280

Gln Ala Ala Lys Arg Ser Arg Thr Thr
1   5

<210> 281
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
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<400> 281

Pro Ala Pro Ala Lys Arg Glu Arg Thr Thr
1   5   10

<210> 282
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary NLS 4

<400> 282

Gln Ala Ala Lys Arg Pro Arg Thr Thr
1   5

<210> 283
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary NLS 5

<400> 283

Arg Ala Ala Lys Arg Pro Arg Thr Thr
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<210> 284
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
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<400> 284

Ala Ala Ala Lys Arg Ser Trp Ser Met Ala Ala
1   5   10

<210> 285
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary NLS 7

<400> 285

Ala Ala Ala Lys Arg Val Trp Ser Met Ala Phe
1   5   10

<210> 286
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary NLS 8

<400> 286

Ala Ala Ala Lys Arg Ser Trp Ser Met Ala Phe
1   5   10

<210> 287
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary NLS 9

<400> 287

Ala Ala Ala Lys Arg Lys Tyr Phe Ala Ala
1   5   10

<210> 288
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary NLS 10

<400> 288

Arg Ala Ala Lys Arg Lys Ala Phe Ala Ala
1   5   10

<210> 289
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
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<400> 289

Arg Ala Ala Lys Arg Lys Tyr Phe Ala Val
1 5 10

<210> 290
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
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<400> 290

Pro Lys Lys Lys Arg Arg Val
1 5

<210> 291
<211> 16
<212> PRT
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<220>
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<400> 291

Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys
1 5 10 15

<210> 292
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<212> DNA
<213> Artificial Sequence

<220>
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<400> 292
ccgaagaaga agagaaaggt c 21

<210> 293
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
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<400> 293
ctggcagcaa agagaagcag aacaaca 27

<210> 294
<211> 27

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for NLS2

<400> 294
caggcagcaa agagaagcag aacaaca 27

<210> 295
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for NLS3

<400> 295
ccggcaccgg caaagagaga aagaacaaca 30

<210> 296
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for NLS4

<400> 296
caggcagcaa agagaccgag aacaaca 27

<210> 297
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for NLS5

<400> 297
agagcagcaa agagaccgag aacaaca 27

<210> 298
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for NLS6

<400> 298
gcagcagcaa agagaagctg gagcatggca gca 33

<210> 299
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for NLS7

<400> 299
gcagcagcaa agagagtctg gagcatggca ttc 33

<210> 300
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for NLS8

<400> 300
gcagcagcaa agagaagctg gagcatggca ttc 33

<210> 301
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for NLS9

<400> 301
gcagcagcaa agagaaagta cttcgcagca 30

<210> 302
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<220>
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<400> 302
agagcagcaa agagaaaggc attcgcagca 30

<210> 303
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<212> DNA
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<220>
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<400> 303
agagcagcaa agagaaagta cttcgcagtc 30

<210> 304
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Exemplary coding sequence for alternate SV40 NLS

<400> 304
ccgaagaaga agagaagagt c 21

<210> 305
<211> 13
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: exemplary Kozak sequence

<400> 305
gccgccrcca ugg 13

<210> 306

<400> 306
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<210> 307
<211> 4140
<212> DNA
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<220>
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<400> 307
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atcaccgacg aatacaaggt cccatctaag aagttcaagg tcttgggtaa caccgacaga 120

cactctatca agaagaactt gatcggtgcc ttgttgttcg actctggtga aaccgccgaa 180

gccaccagat tgaagagaac cgccagaaga agatacacca gaagaaagaa cagaatctgc 240

tacttgcaag aaatcttctc taacgaaatg gccaaggtcg acgactcttt cttccacaga 300

ttggaagaat ctttcttggt cgaagaagac aagaagcacg aaagacaccc aatcttcggt 360

aacatcgtcg acgaagtcgc ctaccacgaa aagtacccaa ccatctacca cttgagaaag 420

aagttggtcg actctaccga caaggccgac ttgagattga tctacttggc cttggcccac 480

atgatcaagt tcagaggtca cttcttgatc gaaggtgact tgaacccaga caactctgac 540

gtcgacaagt tgttcatcca attggtccaa acctacaacc aattgttcga agaaaaccca 600

atcaacgcct ctggtgtcga cgccaaggcc atcttgtctg ccagattgtc taagagcaga 660

agattggaaa acttgatcgc ccaattgcca ggtgaaaaga agaacggttt gttcggtaac 720

ttgatcgcct tgtctttggg tttgacccca aacttcaagt ctaacttcga cttggccgaa 780

gacgccaagt tgcaattgtc taaggacacc tacgacgacg acttggacaa cttgttggcc 840

caaatcggtg accaatacgc cgacttgttc ttggccgcca agaacttgtc tgacgccatc 900

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atgatcaaga gatacgacga acaccaccaa gacttgacct tgttgaaggc cttggtcaga 1020

caacaattgc cagaaaagta caaggaaatc ttcttcgacc aatctaagaa cggttacgcc 1080

ggttacatcg acggtggtgc ctctcaagaa gaattctaca agttcatcaa gccaatcttg 1140

gaaaagatgg acggtaccga agaattgttg gtcaagttga acagagaaga cttgttgaga 1200

aagcaaagaa ccttcgacaa cggttctatc ccacaccaaa tccacttggg tgaattgcac 1260

gccatcttga gaagacaaga agacttctac ccattcttga aggacaacag agaaaagatc 1320

gaaaagatct tgaccttcag aatcccatac tacgtcggtc cattggccag aggtaacagc 1380

agattcgcct ggatgaccag aaagtctgaa gaaaccatca ccccatggaa cttcgaagaa 1440

gtcgtcgaca agggtgcctc tgcccaatct ttcatcgaaa gaatgaccaa cttcgacaag 1500

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aacttgccaa acgaaaaggt cttgccaaag cactctttgt tgtacgaata cttcaccgtc     1560

tacaacgaat tgaccaaggt caagtacgtc accgaaggta tgagaaagcc agccttcttg     1620

tctggtgaac aaaagaaggc catcgtcgac ttgttgttca agaccaacag aaaggtcacc     1680

gtcaagcaat tgaaggaaga ctacttcaag aagatcgaat gcttcgactc tgtcgaaatc     1740

tctggtgtcg aagacagatt caacgcctct ttgggtacct accacgactt gttgaagatc     1800

atcaaggaca aggacttctt ggacaacgaa gaaaacgaag acatcttgga agacatcgtc     1860

ttgaccttga ccttgttcga agacagagaa atgatcgaag aaagattgaa gacctacgcc     1920

cacttgttcg acgacaaggt catgaagcaa ttgaagagaa gaagatacac cggttggggt     1980

agattgagca gaaagttgat caacggtatc agagacaagc aatctggtaa gaccatcttg     2040

gacttcttga agtctgacgg tttcgccaac agaaacttca tgcaattgat ccacgacgac     2100

tctttgacct tcaaggaaga catccaaaag gcccaagtct ctggtcaagg tgactctttg     2160

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gtcaaggtcg tcgacgaatt ggtcaaggtc atgggtagac acaagccaga aaacatcgtc     2280

atcgaaatgg ccagagaaaa ccaaaccacc caaaagggtc aaaagaacag cagagaaaga     2340

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accaaggccg aaagaggtgg tttgtctgaa ttggacaagg ccggtttcat caagagacaa     2760

ttggtcgaaa ccagacaaat caccaagcac gtcgcccaaa tcttggacag cagaatgaac     2820

accaagtacg acgaaaacga caagttgatc agagaagtca aggtcatcac cttgaagtct     2880

aagttggtct ctgacttcag aaaggacttc caattctaca aggtcagaga aatcaacaac     2940

taccaccacg cccacgacgc ctacttgaac gccgtcgtcg gtaccgcctt gatcaagaag     3000

tacccaaagt tggaatctga attcgtctac ggtgactaca aggtctacga cgtcagaaag     3060

atgatcgcca agtctgaaca agaaatcggt aaggccaccg ccaagtactt cttctactct     3120

aacatcatga acttcttcaa gaccgaaatc accttggcca acggtgaaat cagaaagaga     3180

ccattgatcg aaaccaacgg tgaaaccggt gaaatcgtct gggacaaggg tagagacttc     3240

gccaccgtca gaaaggtctt gtctatgcca caagtcaaca tcgtcaagaa gaccgaagtc     3300

caaaccggtg gtttctctaa ggaatctatc ttgccaaaga gaaactctga caagttgatc     3360

gccagaaaga aggactggga cccaaagaag tacggtggtt tcgactctcc aaccgtcgcc     3420

tactctgtct tggtcgtcgc caaggtcgaa aagggtaagt ctaagaagtt gaagtctgtc     3480

aaggaattgt tgggtatcac catcatggaa agatcttctt tcgaaaagaa cccaatcgac     3540

ttcttggaag ccaagggtta caaggaagtc aagaaggact tgatcatcaa gttgccaaag     3600
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tactctttgt tcgaattgga aaacggtaga aagagaatgt tggcctctgc cggtgaattg 3660

caaaagggta acgaattggc cttgccatct aagtacgtca acttcttgta cttggcctct 3720

cactacgaaa agttgaaggg ttctccagaa gacaacgaac aaaagcaatt gttcgtcgaa 3780

caacacaagc actacttgga cgaaatcatc gaacaaatct ctgaattctc taagagagtc 3840

atcttggccg acgccaactt ggacaaggtc ttgtctgcct acaacaagca cagagacaag 3900

ccaatcagag aacaagccga aaacatcatc cacttgttca ccttgaccaa cttgggtgcc 3960

ccagccgcct tcaagtactt cgacaccacc atcgacagaa agagatacac ctctaccaag 4020

gaagtcttgg acgccacctt gatccaccaa tctatcaccg gtttgtacga aaccagaatc 4080

gacttgtctc aattgggtgg tgacggtggt ggttctccaa agaagaagag aaaggtctaa 4140

<210> 308
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using U rich codons of Table 4, with start and stop codons

<400> 308
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attactgatg aatataaagt tccttctaaa aaatttaaag ttttaggtaa tactgatcgt 120

cattctatta aaaaaaattt aattggtgct ttattatttg attctggtga aactgctgaa 180

gctactcgtt taaaacgtac tgctcgtcgt cgttatactc gtcgtaaaaa tcgtatttgt 240

tatttacaag aaatttttc taatgaaatg gctaaagttg atgattcttt ttttcatcgt 300

ttagaagaat ctttttttagt tgaagaagat aaaaaacatg aacgtcatcc tatttttggt 360

aatattgttg atgaagttgc ttatcatgaa aaatatccta ctatttatca tttacgtaaa 420

aaattagttg attctactga taaagctgat ttacgtttaa tttatttagc tttagctcat 480

atgattaaat ttcgtggtca ttttttaatt gaaggtgatt taaatcctga taattctgat 540

gttgataaat tatttattca attagttcaa acttataatc aattatttga agaaaatcct 600

attaatgctt ctggtgttga tgctaaagct attttatctg ctcgtttatc taaatctcgt 660

cgtttagaaa atttaattgc tcaattacct ggtgaaaaaa aaaatggttt atttggtaat 720

ttaattgctt tatctttagg tttaactcct aattttaaat ctaattttga tttagctgaa 780

gatgctaaat tacaattatc taaagatact tatgatgatg atttagataa tttattagct 840

caaattggtg atcaatatgc tgatttattt ttagctgcta aaaatttatc tgatgctatt 900

ttattatctg atattttacg tgttaatact gaaattacta aagctccttt atctgcttct 960

atgattaaac gttatgatga acatcatcaa gatttaactt tattaaaagc tttagttcgt 1020

caacaattac ctgaaaaata taaagaaatt ttttttgatc aatctaaaaa tggttatgct 1080

ggttatattg atggtggtgc ttctcaagaa gaattttata aatttattaa acctatttta 1140

gaaaaaatgg atggtactga agaattatta gttaaattaa atcgtgaaga tttattacgt 1200

aaacaacgta cttttgataa tggttctatt cctcatcaaa ttcatttagg tgaattacat 1260

gctattttac gtcgtcaaga agatttttat ccttttttaa aagataatcg tgaaaaaatt 1320

gaaaaaattt taacttttcg tattccttat tatgttggtc ctttagctcg tggtaattct 1380

cgttttgctt ggatgactcg taaatctgaa gaaactatta ctccttggaa ttttgaagaa 1440

gttgttgata aaggtgcttc tgctcaatct tttattgaac gtatgactaa ttttgataaa 1500

aatttaccta atgaaaaagt tttacctaaa cattctttat tatatgaata ttttactgtt 1560

tataatgaat taactaaagt taaatatgtt actgaaggta tgcgtaaacc tgctttttta 1620

tctggtgaac aaaaaaaagc tattgttgat ttattattta aaactaatcg taaagttact 1680

gttaaacaat taaaagaaga ttattttaaa aaaattgaat gttttgattc tgttgaaatt 1740

tctggtgttg aagatcgttt taatgcttct ttaggtactt atcatgattt attaaaaatt 1800

attaaagata aagatttttt agataatgaa gaaaatgaag atattttaga agatattgtt 1860

ttaactttaa ctttatttga agatcgtgaa atgattgaag aacgtttaaa aacttatgct 1920

catttatttg atgataaagt tatgaaacaa ttaaaacgtc gtcgttatac tggttggggt 1980

cgtttatctc gtaaattaat taatggtatt cgtgataaac aatctggtaa aactatttta 2040

gatttttaa aatctgatgg ttttgctaat cgtaatttta tgcaattaat tcatgatgat 2100

tctttaactt ttaaagaaga tattcaaaaa gctcaagttt ctggtcaagg tgattcttta 2160

catgaacata ttgctaattt agctggttct cctgctatta aaaaaggtat tttacaaact 2220

gttaaagttg ttgatgaatt agttaaagtt atgggtcgtc ataaacctga aaatattgtt 2280

attgaaatgg ctcgtgaaaa tcaaactact caaaaaggtc aaaaaaattc tcgtgaacgt 2340

atgaaacgta ttgaagaagg tattaaagaa ttaggttctc aaattttaaa agaacatcct 2400

gttgaaaata ctcaattaca aaatgaaaaa ttatatttat attatttaca aaatggtcgt 2460

gatatgtatg ttgatcaaga attagatatt aatcgtttat ctgattatga tgttgatcat 2520

attgttcctc aatctttttt aaaagatgat tctattgata ataaagtttt aactcgttct 2580

gataaaaatc gtggtaaatc tgataatgtt ccttctgaag aagttgttaa aaaaatgaaa 2640

aattattggc gtcaattatt aaatgctaaa ttaattactc aacgtaaatt tgataattta 2700

actaaagctg aacgtggtgg tttatctgaa ttagataaag ctggttttat taaacgtcaa 2760

ttagttgaaa ctcgtcaaat tactaaacat gttgctcaaa ttttagattc tcgtatgaat 2820

actaaatatg atgaaaatga taaattaatt cgtgaagtta aagttattac tttaaaatct 2880

aaattagttt ctgattttcg taaagatttt caattttata aagttcgtga aattaataat 2940

tatcatcatg ctcatgatgc ttatttaaat gctgttgttg gtactgcttt aattaaaaaa 3000

tatcctaaat tagaatctga atttgtttat ggtgattata aagtttatga tgttcgtaaa 3060

atgattgcta aatctgaaca agaaattggt aaagctactg ctaaatattt tttttattct 3120

aatattatga attttttaa aactgaaatt actttagcta atggtgaaat tcgtaaacgt 3180

cctttaattg aaactaatgg tgaaactggt gaaattgttt gggataaagg tcgtgatttt 3240

gctactgttc gtaaagtttt atctatgcct caagttaata ttgttaaaaa aactgaagtt 3300

caaactggtg gtttttctaa agaatctatt ttacctaaac gtaattctga taaattaatt 3360

gctcgtaaaa aagattggga tcctaaaaaa tatggtggtt ttgattctcc tactgttgct 3420

tattctgttt tagttgttgc taaagttgaa aaaggtaaat ctaaaaaatt aaaatctgtt 3480

aaagaattat taggtattac tattatggaa cgttcttctt ttgaaaaaaa tcctattgat 3540

tttttagaag ctaaaggtta taaagaagtt aaaaaagatt taattattaa attacctaaa 3600

tattctttat ttgaattaga aaatggtcgt aaacgtatgt tagcttctgc tggtgaatta 3660

caaaaaggta atgaattagc tttaccttct aaatatgtta atttttttata tttagcttct 3720

cattatgaaa aattaaaagg ttctcctgaa gataatgaac aaaaacaatt atttgttgaa 3780

caacataaac attatttaga tgaaattatt gaacaaattt ctgaattttc taaacgtgtt 3840

attttagctg atgctaattt agataaagtt ttatctgctt ataataaaca tcgtgataaa 3900

cctattcgtg aacaagctga aaatattatt catttattta ctttaactaa tttaggtgct 3960

cctgctgctt ttaaatattt tgatactact attgatcgta aacgttatac ttctactaaa 4020

gaagttttag atgctacttt aattcatcaa tctattactg gtttatatga aactcgtatt 4080

gatttatctc aattaggtgg tgatggtggt ggttctccta aaaaaaaacg taaagtttga 4140

<210> 309
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low G codons of Table 4, with start and stop codons

<400> 309
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atcaccgacg aatacaaagt cccctccaaa aaattcaaag tcctcggcaa caccgacaga 120

cactccatca aaaaaaacct catcggcgcc ctcctcttcg actccggcga aaccgccgaa 180

gccaccagac tcaaaagaac cgccagaaga agatacacca gaagaaaaaa cagaatctgc 240

tacctccaag aaatcttctc caacgaaatg gccaaagtcg acgactcctt cttccacaga 300

ctcgaagaat ccttcctcgt cgaagaagac aaaaaacacg aaagacaccc catcttcggc 360

aacatcgtcg acgaagtcgc ctaccacgaa aaatacccca ccatctacca cctcagaaaa 420

aaactcgtcg actccaccga caaagccgac ctcagactca tctacctcgc cctcgcccac 480

atgatcaaat tcagaggcca cttcctcatc gaaggcgacc tcaaccccga caactccgac 540

gtcgacaaac tcttcatcca actcgtccaa acctacaacc aactcttcga agaaaacccc 600

atcaacgcct ccggcgtcga cgccaaagcc atcctctccg ccagactctc caaatccaga 660

agactcgaaa acctcatcgc ccaactcccc ggcgaaaaaa aaaacggcct cttcggcaac 720

ctcatcgccc tctccctcgg cctcaccccc aacttcaaat ccaacttcga cctcgccgaa 780

gacgccaaac tccaactctc caaagacacc tacgacgacg acctcgacaa cctcctcgcc 840

caaatcggcg accaatacgc cgacctcttc ctcgccgcca aaaacctctc cgacgccatc 900

ctcctctccg acatcctcag agtcaacacc gaaatcacca aagcccccct ctccgcctcc 960

atgatcaaaa gatacgacga acaccaccaa gacctcaccc tcctcaaagc cctcgtcaga 1020

caacaactcc ccgaaaaata caaagaaatc ttcttcgacc aatccaaaaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaagaa gaattctaca aattcatcaa acccatcctc 1140

gaaaaaatgg acggcaccga agaactcctc gtcaaactca acagagaaga cctcctcaga 1200

aaacaaagaa ccttcgacaa cggctccatc ccccaccaaa tccacctcgg cgaactccac 1260

gccatcctca gaagacaaga agacttctac cccttcctca aagacaacag agaaaaaatc 1320

gaaaaaatcc tcaccttcag aatcccctac tacgtcggcc ccctcgccag aggcaactcc 1380

agattcgcct ggatgaccag aaaatccgaa gaaaccatca ccccctggaa cttcgaagaa 1440

gtcgtcgaca aaggcgcctc cgcccaatcc ttcatcgaaa gaatgaccaa cttcgacaaa 1500

aacctcccca acgaaaaagt cctccccaaa cactccctcc tctacgaata cttcaccgtc 1560

tacaacgaac tcaccaaagt caaatacgtc accgaaggca tgagaaaacc cgccttcctc 1620

tccggcgaac aaaaaaaagc catcgtcgac ctcctcttca aaaccaacag aaaagtcacc 1680

gtcaaacaac tcaaagaaga ctacttcaaa aaaatcgaat gcttcgactc cgtcgaaatc 1740

tccggcgtcg aagacagatt caacgcctcc ctcggcacct accacgacct cctcaaaatc 1800

atcaaagaca aagacttcct cgacaacgaa gaaaacgaag acatcctcga agacatcgtc 1860

ctcaccctca ccctcttcga agacagagaa atgatcgaag aaagactcaa aacctacgcc 1920

cacctcttcg acgacaaagt catgaaacaa ctcaaaagaa gaagatacac cggctggggc 1980

agactctcca gaaaactcat caacggcatc agagacaaac aatccggcaa aaccatcctc 2040

gacttcctca aatccgacgg cttcgccaac agaaacttca tgcaactcat ccacgacgac 2100

tccctcacct tcaaagaaga catccaaaaa gcccaagtct ccggccaagg cgactccctc 2160

cacgaacaca tcgccaacct cgccggctcc cccgccatca aaaaaggcat cctccaaacc 2220

gtcaaagtcg tcgacgaact cgtcaaagtc atgggcagac acaaacccga aaacatcgtc 2280

atcgaaatgg ccagagaaaa ccaaaccacc caaaaaggcc aaaaaaactc cagagaaaga 2340

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gtcgaaaaca cccaactcca aaacgaaaaa ctctacctct actacctcca aaacggcaga 2460

gacatgtacg tcgaccaaga actcgacatc aacagactct ccgactacga cgtcgaccac 2520

atcgtccccc aatccttcct caaagacgac tccatcgaca acaaagtcct caccagatcc 2580

gacaaaaaca gaggcaaatc cgacaacgtc ccctccgaag aagtcgtcaa aaaaatgaaa 2640

aactactgga gacaactcct caacgccaaa ctcatcaccc aaagaaaatt cgacaacctc 2700

accaaagccg aaagaggcgg cctctccgaa ctcgacaaag ccggcttcat caaaagacaa 2760

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aaactcgtct ccgacttcag aaaagacttc caattctaca aagtcagaga aatcaacaac 2940

taccaccacg cccacgacgc ctacctcaac gccgtcgtcg gcaccgccct catcaaaaaa 3000

taccccaaac tcgaatccga attcgtctac ggcgactaca aagtctacga cgtcagaaaa 3060

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cccctcatcg aaaccaacgg cgaaaccggc gaaatcgtct gggacaaagg cagagacttc 3240

gccaccgtca gaaaagtcct ctccatgccc caagtcaaca tcgtcaaaaa aaccgaagtc 3300

caaaccggcg gcttctccaa agaatccatc ctccccaaaa gaaactccga caaactcatc 3360

gccagaaaaa aagactggga ccccaaaaaa tacggcggct tcgactcccc caccgtcgcc 3420

tactccgtcc tcgtcgtcgc caaagtcgaa aaaggcaaat ccaaaaaact caaatccgtc 3480

aaagaactcc tcggcatcac catcatggaa agatcctcct tcgaaaaaaa ccccatcgac 3540

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atcctcgccg acgccaacct cgacaaagtc ctctccgcct acaacaaaca cagagacaaa 3900

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cccgccgcct tcaaatactt cgacaccacc atcgacagaa aaagatacac ctccaccaaa 4020

gaagtcctcg acgccaccct catccaccaa tccatcaccg gcctctacga aaccagaatc 4080

gacctctccc aactcggcgg cgacggcggc ggctccccca aaaaaaaaag aaaagtctga 4140

<210> 310
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low C codons of Table 4, with start and stop codons

<400> 310
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attacagatg agtataaggt gcctagtaag aagtttaagg tgttgggaaa tacagataga 120

catagtatta agaagaattt gattggagct ttgttgtttg atagtggaga gacagctgag 180

gctacaagat tgaagagaac agctagaaga agatatacaa gaagaaagaa tagaatttgt 240

tatttgcagg agatttttag taatgagatg gctaaggtgg atgatagttt ttttcataga 300

ttggaggaga gttttttggt ggaggaggat aagaagcatg agagacatcc tatttttgga 360

aatattgtgg atgaggtggc ttatcatgag aagtatccta caatttatca tttgagaaag 420

aagttggtgg atagtacaga taaggctgat ttgagattga tttatttggc tttggctcat 480

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gtggataagt tgtttattca gttggtgcag acatataatc agttgtttga ggagaatcct 600

attaatgcta gtggagtgga tgctaaggct attttgagtg ctagattgag taagagtaga 660

agattggaga atttgattgc tcagttgcct ggagagaaga agaatggatt gtttggaaat 720

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gatgctaagt tgcagttgag taaggataca tatgatgatg atttggataa tttgttggct 840

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gagaagatgg atggaacaga ggagttgttg gtgaagttga atagagagga tttgttgaga 1200

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gagaagattt tgacatttag aattccttat tatgtgggac ctttggctag aggaaatagt 1380

agatttgctt ggatgacaag aaagagtgag gagacaatta caccttggaa ttttgaggag 1440

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aatttgccta atgagaaggt gttgcctaag catagtttgt tgtatgagta ttttacagtg 1560

tataatgagt tgacaaaggt gaagtatgtg acagagggaa tgagaaagcc tgctttttg 1620

agtggagagc agaagaaggc tattgtggat ttgttgttta agacaaatag aaaggtgaca 1680

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gtggagaata cacagttgca gaatgagaag ttgtatttgt attatttgca gaatggaaga 2460

gatatgtatg tggatcagga gttggatatt aatagattga gtgattatga tgtggatcat 2520

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tatcctaagt tggagagtga gtttgtgtat ggagattata aggtgtatga tgtgagaaag 3060

atgattgcta agagtgagca ggagattgga aaggctacag ctaagtattt tttttatagt 3120

aatattatga attttttttaa gacagagatt acattggcta atggagagat tagaaagaga 3180

cctttgattg agacaaatgg agagacagga gagattgtgt gggataaggg aagagatttt 3240

gctacagtga gaaaggtgtt gagtatgcct caggtgaata ttgtgaagaa gacagaggtg 3300

cagacaggag gatttagtaa ggagagtatt ttgcctaaga gaaatagtga taagttgatt 3360

gctagaaaga aggattggga tcctaagaag tatggaggat ttgatagtcc tacagtggct 3420

tatagtgtgt tggtggtggc taaggtggag aagggaaaga gtaagaagtt gaagagtgtg 3480

aaggagttgt tgggaattac aattatggag agaagtagtt ttgagaagaa tcctattgat 3540

tttttggagg ctaagggata taaggaggtg aagaaggatt tgattattaa gttgcctaag 3600

tatagtttgt ttgagttgga gaatggaaga aagagaatgt tggctagtgc tggagagttg 3660

cagaagggaa atgagttggc tttgcctagt aagtatgtga atttttttgta tttggctagt 3720

cattatgaga agttgaaggg aagtcctgag gataatgagc agaagcagtt gtttgtggag 3780

cagcataagc attatttgga tgagattatt gagcagatta gtgagtttag taagagagtg 3840

attttggctg atgctaattt ggataaggtg ttgagtgctt ataataagca tagagataag 3900

cctattagag agcaggctga gaatattatt catttgttta cattgacaaa tttgggagct 3960

cctgctgctt ttaagtattt tgatacaaca attgatagaa agagatatac aagtacaaag 4020

gaggtgttgg atgctacatt gattcatcag agtattacag gattgtatga gacaagaatt 4080

gatttgagtc agttgggagg agatggagga ggaagtccta agaagaagag aaaggtgtga 4140

<210> 311
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A codons of Table 4, with start and stop codons

<400> 311
atggacaaga agtactccat cggcctggac atcggcacca actccgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

cactccatca agaagaacct gatcggcgcc ctgctgttcg actccggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttctc caacgagatg gccaaggtgg acgactcctt cttccaccgg 300

ctggaggagt ccttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg actccaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caactccgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcct ccggcgtgga cgccaaggcc atcctgtccg cccggctgtc caagtcccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgtccctggg cctgaccccc aacttcaagt ccaacttcga cctggccgag 780

gacgccaagc tgcagctgtc caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgtc cgacgccatc 900

ctgctgtccg acatcctgcg ggtgaacacc gagatcacca aggcccccct gtccgcctcc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agtccaagaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggctccatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaactcc 1380

cggttcgcct ggatgacccg gaagtccgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgcctc cgcccagtcc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cactccctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

tccggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgactc cgtggagatc 1740

tccggcgtgg aggaccggtt caacgcctcc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgtccc ggaagctgat caacggcatc cgggacaagc agtccggcaa gaccatcctg 2040

gacttcctga agtccgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

tccctgacct tcaaggagga catccagaag gcccaggtgt ccggccaggg cgactccctg 2160

cacgagcaca tcgccaacct ggccggctcc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaactc ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggctccc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctgt ccgactacga cgtggaccac 2520

atcgtgcccc agtccttcct gaaggacgac tccatcgaca acaaggtgct gacccggtcc 2580

gacaagaacc ggggcaagtc cgacaacgtg ccctccgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgtccgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggactc ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagtcc 2880

aagctggtgt ccgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagtccga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agtccgagca ggagatcggc aaggccaccg ccaagtactt cttctactcc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gtccatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttctccaa ggagtccatc ctgcccaagc ggaactccga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgactcccc caccgtggcc 3420

tactccgtgc tggtggtggc caaggtggag aagggcaagt ccaagaagct gaagtccgtg 3480

aaggagctgc tgggcatcac catcatggag cggtcctcct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tactccctgt tcgagctgga gaacggccgg aagcggatgc tggcctccgc cggcgagctg 3660

cagaagggca acgagctggc cctgccctcc aagtacgtga acttcctgta cctggcctcc 3720

cactacgaga agctgaaggg ctcccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatct ccgagttctc caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgtccgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac ctccaccaag 4020

gaggtgctgg acgccaccct gatccaccag tccatcaccg gcctgtacga gacccggatc 4080

gacctgtccc agctgggcgg cgacggcggc ggctccccca agaagaagcg gaaggtgtga 4140

<210> 312
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A/U codons of Table 4, with start and stop codons

<400> 312
atggacaaga agtacagcat cggcctggac atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg 300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag 780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc 900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc 1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggcagcatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc 1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgccag cgcccagagc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cacagcctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc 1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

```
cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc     1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg     2040

gacttcctga agagcgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac     2100

agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg     2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc     2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg     2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg     2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc     2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg     2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggaccac     2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc     2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag     2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg     2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag     2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac     2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc     2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac     2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag     3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag     3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc     3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg     3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc     3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg     3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc     3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc     3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg     3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac     3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag     3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg     3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc     3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag     3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg     3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag     3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc     3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag     4020
```

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgacggcggc ggcagcccca agaagaagcg gaaggtgtga 4140

<210> 313
<211> 4179
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A codons of Table 4, with two
C-terminal NLS sequences and start and stop codons

<400> 313
atggacaaga agtactccat cggcctggac atcggcacca actccgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

cactccatca agaagaacct gatcggcgcc ctgctgttcg actccggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttctc caacgagatg gccaaggtgg acgactcctt cttccaccgg 300

ctggaggagt ccttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg actccaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caactccgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcct ccggcgtgga cgccaaggcc atcctgtccg cccggctgtc caagtcccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgtccctggg cctgaccccc aacttcaagt ccaacttcga cctggccgag 780

gacgccaagc tgcagctgtc caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgtc cgacgccatc 900

ctgctgtccg acatcctgcg ggtgaacacc gagatcacca aggcccccct gtccgcctcc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agtccaagaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggctccatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaactcc 1380

cggttcgcct ggatgacccg gaagtccgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgcctc cgcccagtcc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cactccctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

tccggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgactc cgtggagatc 1740

tccggcgtgg aggaccggtt caacgcctcc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgtccc ggaagctgat caacggcatc cgggacaagc agtccggcaa gaccatcctg 2040

gacttcctga agtccgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

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cacgagcaca tcgccaacct ggccggctcc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaactc ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggctccc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctgt ccgactacga cgtggaccac 2520

atcgtgcccc agtccttcct gaaggacgac tccatcgaca acaaggtgct gacccggtcc 2580

gacaagaacc ggggcaagtc cgacaacgtg ccctccgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgtccgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggactc ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagtcc 2880

aagctggtgt ccgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagtccga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agtccgagca ggagatcggc aaggccaccg ccaagtactt cttctactcc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gtccatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttctccaa ggagtccatc ctgcccaagc ggaactccga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgactcccc caccgtggcc 3420

tactccgtgc tggtggtggc caaggtggag aagggcaagt ccaagaagct gaagtccgtg 3480

aaggagctgc tgggcatcac catcatggag cggtcctcct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tactccctgt tcgagctgga gaacggccgg aagcggatgc tggcctccgc cggcgagctg 3660

cagaagggca acgagctggc cctgccctcc aagtacgtga acttcctgta cctggcctcc 3720

cactacgaga agctgaaggg ctcccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatct ccgagttctc caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgtccgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac ctccaccaag 4020

gaggtgctgg acgccaccct gatccaccag tccatcaccg gcctgtacga gacccggatc 4080

gacctgtccc agctgggcgg cgacggctcc ggctccccca agaagaagcg gaaggtggac 4140

ggctccccca agaagaagcg gaaggtggac tccggctga 4179

<210> 314
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A codons of Table 4, with start and stop codons

<400> 314
atggacaaga agtactccat cggcctggcc atcggcacca actccgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

cactccatca agaagaacct gatcggcgcc ctgctgttcg actccggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttctc caacgagatg gccaaggtgg acgactcctt cttccaccgg 300

ctggaggagt ccttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg actccaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caactccgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcct ccggcgtgga cgccaaggcc atcctgtccg cccggctgtc caagtcccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgtccctggg cctgaccccc aacttcaagt ccaacttcga cctggccgag 780

gacgccaagc tgcagctgtc caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgtc cgacgccatc 900

ctgctgtccg acatcctgcg ggtgaacacc gagatcacca aggcccccct gtccgcctcc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agtccaagaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggctccatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaactcc 1380

cggttcgcct ggatgacccg gaagtccgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgcctc cgcccagtcc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cactccctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

tccggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgactc cgtggagatc 1740

tccggcgtgg aggaccggtt caacgcctcc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgtccc ggaagctgat caacggcatc cgggacaagc agtccggcaa gaccatcctg 2040

gacttcctga agtccgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

tccctgacct tcaaggagga catccagaag gcccaggtgt ccggccaggg cgactccctg 2160

cacgagcaca tcgccaacct ggccggctcc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaactc ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggctccc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctgt ccgactacga cgtggaccac 2520

atcgtgcccc agtccttcct gaaggacgac tccatcgaca acaaggtgct gacccggtcc 2580

gacaagaacc ggggcaagtc cgacaacgtg ccctccgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgtccgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggactc ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagtcc 2880

aagctggtgt ccgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagtccga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agtccgagca ggagatcggc aaggccaccg ccaagtactt cttctactcc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gtccatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttctccaa ggagtccatc ctgcccaagc ggaactccga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgactcccc caccgtggcc 3420

tactccgtgc tggtggtggc caaggtggag aagggcaagt ccaagaagct gaagtccgtg 3480

aaggagctgc tgggcatcac catcatggag cggtcctcct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tactccctgt tcgagctgga gaacggccgg aagcggatgc tggcctccgc cggcgagctg 3660

cagaagggca acgagctggc cctgccctcc aagtacgtga acttcctgta cctggcctcc 3720

cactacgaga agctgaaggg ctcccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatct ccgagttctc caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgtccgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac ctccaccaag 4020

gaggtgctgg acgccaccct gatccaccag tccatcaccg gcctgtacga gacccggatc 4080

gacctgtccc agctgggcgg cgacggcggc ggctccccca agaagaagcg gaaggtgtga 4140

<210> 315
<211> 4107
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A codons of Table 4, with start and stop codons and no NLS

<400> 315
atggacaaga agtactccat cggcctggcc atcggcacca actccgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

cactccatca agaagaacct gatcggcgcc ctgctgttcg actccggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttctc caacgagatg gccaaggtgg acgactcctt cttccaccgg 300

ctggaggagt ccttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg actccaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caactccgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcct ccggcgtgga cgccaaggcc atcctgtccg cccggctgtc caagtcccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgtccctggg cctgaccccc aacttcaagt ccaacttcga cctggccgag 780

gacgccaagc tgcagctgtc caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgtc cgacgccatc 900

ctgctgtccg acatcctgcg ggtgaacacc gagatcacca aggcccccct gtccgcctcc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agtccaagaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggctccatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaactcc 1380

cggttcgcct ggatgacccg gaagtccgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgcctc cgcccagtcc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cactccctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

tccggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgactc cgtggagatc 1740

tccggcgtgg aggaccggtt caacgcctcc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgtccc ggaagctgat caacggcatc cgggacaagc agtccggcaa gaccatcctg 2040

gacttcctga agtccgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

tccctgacct tcaaggagga catccagaag gcccaggtgt ccggccaggg cgactccctg 2160

cacgagcaca tcgccaacct ggccggctcc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaactc ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggctccc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctgt ccgactacga cgtggaccac 2520

atcgtgcccc agtccttcct gaaggacgac tccatcgaca acaaggtgct gacccggtcc 2580

gacaagaacc ggggcaagtc cgacaacgtg ccctccgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgtccgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggactc ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagtcc 2880

aagctggtgt ccgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagtccga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agtccgagca ggagatcggc aaggccaccg ccaagtactt cttctactcc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gtccatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttctccaa ggagtccatc ctgcccaagc ggaactccga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgactcccc caccgtggcc 3420

tactccgtgc tggtggtggc caaggtggag aagggcaagt ccaagaagct gaagtccgtg 3480

aaggagctgc tgggcatcac catcatggag cggtcctcct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tactccctgt tcgagctgga gaacggccgg aagcggatgc tggcctccgc cggcgagctg 3660

cagaagggca acgagctggc cctgccctcc aagtacgtga acttcctgta cctggcctcc 3720

cactacgaga agctgaaggg ctcccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatct ccgagttctc caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgtccgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac ctccaccaag 4020

gaggtgctgg acgccaccct gatccaccag tccatcaccg gcctgtacga gacccggatc 4080

gacctgtccc agctgggcgg cgactga 4107

<210> 316
<211> 4179
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A codons of Table 4, with two C-terminal NLS sequences and start and stop codons

<400> 316
atggacaaga agtactccat cggcctggcc atcggcacca actccgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

cactccatca agaagaacct gatcggcgcc ctgctgttcg actccggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttctc caacgagatg gccaaggtgg acgactcctt cttccaccgg 300

ctggaggagt ccttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg actccaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caactccgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcct ccggcgtgga cgccaaggcc atcctgtccg cccggctgtc caagtcccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgtccctggg cctgaccccc aacttcaagt ccaacttcga cctggccgag 780

gacgccaagc tgcagctgtc caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgtc cgacgccatc 900

ctgctgtccg acatcctgcg ggtgaacacc gagatcacca aggcccccct gtccgcctcc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agtccaagaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggctccatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaactcc 1380

cggttcgcct ggatgacccg gaagtccgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgcctc cgcccagtcc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cactccctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

tccggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgactc cgtggagatc 1740

tccggcgtgg aggaccggtt caacgcctcc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgtccc ggaagctgat caacggcatc cgggacaagc agtccggcaa gaccatcctg 2040

gacttcctga agtccgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

tccctgacct tcaaggagga catccagaag gcccaggtgt ccggccaggg cgactccctg 2160

cacgagcaca tcgccaacct ggccggctcc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaactc ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggctccc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctgt ccgactacga cgtggaccac 2520

atcgtgcccc agtccttcct gaaggacgac tccatcgaca acaaggtgct gacccggtcc 2580

gacaagaacc ggggcaagtc cgacaacgtg ccctccgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgtccgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggactc ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagtcc 2880

aagctggtgt ccgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagtccga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agtccgagca ggagatcggc aaggccaccg ccaagtactt cttctactcc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gtccatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttctccaa ggagtccatc ctgcccaagc ggaactccga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgactcccc caccgtggcc 3420

tactccgtgc tggtggtggc caaggtggag aagggcaagt ccaagaagct gaagtccgtg 3480

aaggagctgc tgggcatcac catcatggag cggtcctcct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tactccctgt tcgagctgga gaacggccgg aagcggatgc tggcctccgc cggcgagctg 3660

cagaagggca acgagctggc cctgccctcc aagtacgtga acttcctgta cctggcctcc 3720

cactacgaga agctgaaggg ctcccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatct ccgagttctc caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgtccgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac ctccaccaag 4020

gaggtgctgg acgccaccct gatccaccag tccatcaccg gcctgtacga gacccggatc 4080

gacctgtccc agctgggcgg cgacggctcc ggctccccca agaagaagcg gaaggtggac 4140

ggctccccca agaagaagcg gaaggtggac tccggctga 4179

<210> 317
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A codons of Table 4, with start and stop codons

<400> 317
atggacaaga agtactccat cggcctggcc atcggcacca actccgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

cactccatca agaagaacct gatcggcgcc ctgctgttcg actccggcga gaccgccgag 180

```
gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc      240
tacctgcagg agatcttctc caacgagatg gccaaggtgg acgactcctt cttccaccgg      300
ctggaggagt ccttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc      360
aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag      420
aagctggtgg actccaccga caaggccgac ctgcggctga tctacctggc cctggcccac      480
atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caactccgac      540
gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc      600
atcaacgcct ccggcgtgga cgccaaggcc atcctgtccg cccggctgtc caagtcccgg      660
cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac      720
ctgatcgccc tgtccctggg cctgaccccc aacttcaagt ccaacttcga cctggccgag      780
gacgccaagc tgcagctgtc caaggacacc tacgacgacg acctggacaa cctgctggcc      840
cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgtc cgacgccatc      900
ctgctgtccg acatcctgcg ggtgaacacc gagatcacca aggcccccct gtccgcctcc      960
atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg     1020
cagcagctgc ccgagaagta caaggagatc ttcttcgacc agtccaagaa cggctacgcc     1080
ggctacatcg acggcggcgc ctcccaggag gagttctaca agttcatcaa gcccatcctg     1140
gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg     1200
aagcagcgga ccttcgacaa cggctccatc ccccaccaga tccacctggg cgagctgcac     1260
gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc     1320
gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaactcc     1380
cggttcgcct ggatgacccg gaagtccgag gagaccatca ccccctggaa cttcgaggag     1440
gtggtggaca agggcgcctc cgcccagtcc ttcatcgagc ggatgaccaa cttcgacaag     1500
aacctgccca acgagaaggt gctgcccaag cactccctgc tgtacgagta cttcaccgtg     1560
tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg     1620
tccggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc     1680
gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgactc cgtggagatc     1740
tccggcgtgg aggaccggtt caacgcctcc ctgggcacct accacgacct gctgaagatc     1800
atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg     1860
ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc     1920
cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc     1980
cggctgtccc ggaagctgat caacggcatc cgggacaagc agtccggcaa gaccatcctg     2040
gacttcctga agtccgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac     2100
tccctgacct tcaaggagga catccagaag gcccaggtgt ccggccaggg cgactccctg     2160
cacgagcaca tcgccaacct ggccggctcc cccgccatca agaagggcat cctgcagacc     2220
```

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaactc ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggctccc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctgt ccgactacga cgtggacgcc 2520

atcgtgcccc agtccttcct gaaggacgac tccatcgaca acaaggtgct gacccggtcc 2580

gacaagaacc ggggcaagtc cgacaacgtg ccctccgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgtccgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggactc ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagtcc 2880

aagctggtgt ccgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagtccga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agtccgagca ggagatcggc aaggccaccg ccaagtactt cttctactcc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gtccatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttctccaa ggagtccatc ctgcccaagc ggaactccga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgactcccc caccgtggcc 3420

tactccgtgc tggtggtggc caaggtggag aagggcaagt ccaagaagct gaagtccgtg 3480

aaggagctgc tgggcatcac catcatggag cggtcctcct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tactccctgt tcgagctgga gaacggccgg aagcggatgc tggcctccgc cggcgagctg 3660

cagaagggca acgagctggc cctgccctcc aagtacgtga acttcctgta cctggcctcc 3720

cactacgaga agctgaaggg ctcccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatct ccgagttctc caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgtccgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac ctccaccaag 4020

gaggtgctgg acgccaccct gatccaccag tccatcaccg gcctgtacga gacccggatc 4080

gacctgtccc agctgggcgg cgacggcggc ggctccccca agaagaagcg gaaggtgtga 4140

<210> 318
<211> 4107
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A codons of Table 4, with start and stop codons and no NLS

<400> 318
atggacaaga agtactccat cggcctggcc atcggcacca actccgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

cactccatca agaagaacct gatcggcgcc ctgctgttcg actccggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttctc caacgagatg gccaaggtgg acgactcctt cttccaccgg 300

ctggaggagt ccttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg actccaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caactccgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcct ccggcgtgga cgccaaggcc atcctgtccg cccggctgtc caagtcccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgtccctggg cctgaccccc aacttcaagt ccaacttcga cctggccgag 780

gacgccaagc tgcagctgtc caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgtc cgacgccatc 900

ctgctgtccg acatcctgcg ggtgaacacc gagatcacca aggcccccct gtccgcctcc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agtccaagaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggctccatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaactcc 1380

cggttcgcct ggatgacccg gaagtccgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgcctc cgcccagtcc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cactccctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

tccggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgactc cgtggagatc 1740

tccggcgtgg aggaccggtt caacgcctcc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

```
cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc     1980

cggctgtccc ggaagctgat caacggcatc cgggacaagc agtccggcaa gaccatcctg     2040

gacttcctga agtccgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac     2100

tccctgacct tcaaggagga catccagaag gcccaggtgt ccggccaggg cgactccctg     2160

cacgagcaca tcgccaacct ggccggctcc cccgccatca agaagggcat cctgcagacc     2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg     2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaactc ccgggagcgg     2340

atgaagcgga tcgaggaggg catcaaggag ctgggctccc agatcctgaa ggagcacccc     2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg     2460

gacatgtacg tggaccagga gctggacatc aaccggctgt ccgactacga cgtggacgcc     2520

atcgtgcccc agtccttcct gaaggacgac tccatcgaca acaaggtgct gacccggtcc     2580

gacaagaacc ggggcaagtc cgacaacgtg ccctccgagg aggtggtgaa gaagatgaag     2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg     2700

accaaggccg agcggggcgg cctgtccgag ctggacaagg ccggcttcat caagcggcag     2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggactc ccggatgaac     2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagtcc     2880

aagctggtgt ccgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac     2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag     3000

taccccaagc tggagtccga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag     3060

atgatcgcca agtccgagca ggagatcggc aaggccaccg ccaagtactt cttctactcc     3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg     3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc     3240

gccaccgtgc ggaaggtgct gtccatgccc caggtgaaca tcgtgaagaa gaccgaggtg     3300

cagaccggcg gcttctccaa ggagtccatc ctgcccaagc ggaactccga caagctgatc     3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgactcccc caccgtggcc     3420

tactccgtgc tggtggtggc caaggtggag aagggcaagt ccaagaagct gaagtccgtg     3480

aaggagctgc tgggcatcac catcatggag cggtcctcct tcgagaagaa ccccatcgac     3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag     3600

tactccctgt tcgagctgga gaacggccgg aagcggatgc tggcctccgc cggcgagctg     3660

cagaagggca acgagctggc cctgccctcc aagtacgtga acttcctgta cctggcctcc     3720

cactacgaga agctgaaggg ctccccccgag gacaacgagc agaagcagct gttcgtggag     3780

cagcacaagc actacctgga cgagatcatc gagcagatct ccgagttctc caagcgggtg     3840

atcctggccg acgccaacct ggacaaggtg ctgtccgcct acaacaagca ccgggacaag     3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc     3960
```

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac ctccaccaag 4020

gaggtgctgg acgccaccct gatccaccag tccatcaccg gcctgtacga gacccggatc 4080

gacctgtccc agctgggcgg cgactga 4107

<210> 319
<211> 4179
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A codons of Table 4, with two C-terminal NLS sequences and start and stop codons

<400> 319
atggacaaga agtactccat cggcctggcc atcggcacca actccgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gccctccaag aagttcaagg tgctgggcaa caccgaccgg 120

cactccatca agaagaacct gatcggcgcc ctgctgttcg actccggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttctc caacgagatg gccaaggtgg acgactcctt cttccaccgg 300

ctggaggagt ccttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg actccaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caactccgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcct ccggcgtgga cgccaaggcc atcctgtccg cccggctgtc caagtcccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgtccctggg cctgaccccc aacttcaagt ccaacttcga cctggccgag 780

gacgccaagc tgcagctgtc caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgtc cgacgccatc 900

ctgctgtccg acatcctgcg ggtgaacacc gagatcacca aggcccccct gtccgcctcc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agtccaagaa cggctacgcc 1080

ggctacatcg acggcggcgc ctcccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggctccatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaactcc 1380

cggttcgcct ggatgacccg gaagtccgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgcctc cgcccagtcc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cactccctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

tccggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgactc cgtggagatc 1740

tccggcgtgg aggaccggtt caacgcctcc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgtccc ggaagctgat caacggcatc cgggacaagc agtccggcaa gaccatcctg 2040

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tccctgacct tcaaggagga catccagaag gcccaggtgt ccggccaggg cgactccctg 2160

cacgagcaca tcgccaacct ggccggctcc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaactc ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggctccc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctgt ccgactacga cgtggacgcc 2520

atcgtgcccc agtccttcct gaaggacgac tccatcgaca acaaggtgct gacccggtcc 2580

gacaagaacc ggggcaagtc cgacaacgtg ccctccgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgtccgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggactc ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagtcc 2880

aagctggtgt ccgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagtccga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agtccgagca ggagatcggc aaggccaccg ccaagtactt cttctactcc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gtccatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttctccaa ggagtccatc ctgcccaagc ggaactccga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgactcccc caccgtggcc 3420

tactccgtgc tggtggtggc caaggtggag aagggcaagt ccaagaagct gaagtccgtg 3480

aaggagctgc tgggcatcac catcatggag cggtcctcct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tactccctgt tcgagctgga gaacggccgg aagcggatgc tggcctccgc cggcgagctg 3660

cagaagggca acgagctggc cctgccctcc aagtacgtga acttcctgta cctggcctcc 3720

cactacgaga agctgaaggg ctcccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatct ccgagttctc caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgtccgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac ctccaccaag 4020

gaggtgctgg acgccaccct gatccaccag tccatcaccg gcctgtacga gacccggatc 4080

gacctgtccc agctgggcgg cgacggctcc ggctccccca agaagaagcg gaaggtggac 4140

ggctccccca agaagaagcg gaaggtggac tccggctga 4179

<210> 320
<211> 4179
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences and start and stop codons

<400> 320
atggacaaga agtacagcat cggcctggac atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg 300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag 780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc 900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc 1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggcagcatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc 1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgccag cgcccagagc ttcatcgagc ggatgaccaa cttcgacaag 1500

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tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc 1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg 2040

gacttcctga agagcgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg 2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggaccac 2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc 2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc 2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc 3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg 3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg 3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc 3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag 4020

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgacggcagc ggcagcccca agaagaagcg gaaggtggac 4140

ggcagcccca agaagaagcg gaaggtggac agcggctga 4179

<210> 321
<211> 4107
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A/U codons of Table 4, with start and stop codons and no NLS

<400> 321
atggacaaga agtacagcat cggcctggac atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg 300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag 780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc 900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc 1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggcagcatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc 1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgccag cgcccagagc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cacagcctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc 1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg 2040

gacttcctga agagcgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg 2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggaccac 2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc 2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc 2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc 3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg 3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg 3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc 3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag 4020

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgactga 4107

<210> 322
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A/U codons of Table 4, with start and stop codons

<400> 322
atggacaaga agtacagcat cggcctggcc atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg 300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag 780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc 900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc 1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggcagcatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc 1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgccag cgcccagagc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cacagcctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc 1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg 2040

gacttcctga agagcgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg 2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggaccac 2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc 2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc 2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc 3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg 3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg 3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc 3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag 4020

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgacggcggc ggcagcccca agaagaagcg gaaggtgtga 4140

<210> 323
<211> 4179
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A/U codons of Table 4, with
two C-terminal NLS sequences and start and stop codons

<400> 323
atggacaaga agtacagcat cggcctggcc atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

```
cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag      180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc      240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg      300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc      360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag      420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac      480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac      540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc      600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg      660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac      720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag      780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc      840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc      900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc      960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg     1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc     1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg     1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg     1200

aagcagcgga ccttcgacaa cggcagcatc ccccaccaga tccacctggg cgagctgcac     1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc     1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc     1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag     1440

gtggtggaca agggcgccag cgcccagagc ttcatcgagc ggatgaccaa cttcgacaag     1500

aacctgccca acgagaaggt gctgcccaag cacagcctgc tgtacgagta cttcaccgtg     1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg     1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc     1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc     1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc     1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg     1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc     1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc     1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg     2040

gacttcctga agagcgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac     2100

agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg     2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc     2220
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gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggaccac 2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc 2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc 2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc 3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg 3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg 3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc 3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag 4020

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgacggcagc ggcagcccca agaagaagcg gaaggtggac 4140

ggcagcccca agaagaagcg gaaggtggac agcggctga 4179

<210> 324

<211> 4107
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A/U codons of Table 4, with start and stop codons and no NLS

<400> 324
atggacaaga agtacagcat cggcctggcc atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg 300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag 780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc 900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc 1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggcagcatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc 1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgccag cgcccagagc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cacagcctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc 1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg 2040

gacttcctga agagcgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg 2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggaccac 2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc 2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc 2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc 3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg 3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg 3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc 3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag 4020

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgactga 4107

<210> 325
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A/U codons of Table 4, with start and stop codons

<400> 325
atggacaaga agtacagcat cggcctggcc atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg 300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag 780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc 900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc 1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggcagcatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc 1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgccag cgcccagagc ttcatcgagc ggatgaccaa cttcgacaag 1500

```
aacctgccca acgagaaggt gctgcccaag cacagcctgc tgtacgagta cttcaccgtg     1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg     1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc     1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc     1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc     1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg     1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc     1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc     1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg     2040

gacttcctga agagcgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac     2100

agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg     2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc     2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg     2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg     2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc     2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg     2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggacgcc     2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc     2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag     2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg     2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag     2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac     2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc     2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac     2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag     3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag     3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc     3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg     3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc     3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg     3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc     3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc     3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg     3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac     3540
```

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg 3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc 3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag 4020

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgacggcggc ggcagcccca agaagaagcg gaaggtgtga 4140

<210> 326
<211> 4179
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences and start and stop codons

<400> 326
atggacaaga agtacagcat cggcctggcc atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg 300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag 780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc 900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc 1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

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gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc 1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag 1440

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tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc 1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg 2040

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agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg 2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggacgcc 2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc 2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc 2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc 3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg 3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg 3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc 3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag 4020

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgacggcagc ggcagcccca agaagaagcg gaaggtggac 4140

ggcagcccca agaagaagcg gaaggtggac agcggctga 4179

<210> 327
<211> 4107
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A/U codons of Table 4, with start and stop codons and no NLS

<400> 327
atggacaaga agtacagcat cggcctggcc atcggcacca acagcgtggg ctgggccgtg 60

atcaccgacg agtacaaggt gcccagcaag aagttcaagg tgctgggcaa caccgaccgg 120

cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccacccggc tgaagcggac cgcccggcgg cggtacaccc ggcggaagaa ccggatctgc 240

tacctgcagg agatcttcag caacgagatg gccaaggtgg acgacagctt cttccaccgg 300

ctggaggaga gcttcctggt ggaggaggac aagaagcacg agcggcaccc catcttcggc 360

aacatcgtgg acgaggtggc ctaccacgag aagtacccca ccatctacca cctgcggaag 420

aagctggtgg acagcaccga caaggccgac ctgcggctga tctacctggc cctggcccac 480

atgatcaagt tccggggcca cttcctgatc gagggcgacc tgaaccccga caacagcgac 540

gtggacaagc tgttcatcca gctggtgcag acctacaacc agctgttcga ggagaacccc 600

atcaacgcca gcggcgtgga cgccaaggcc atcctgagcg cccggctgag caagagccgg 660

cggctggaga acctgatcgc ccagctgccc ggcgagaaga agaacggcct gttcggcaac 720

ctgatcgccc tgagcctggg cctgaccccc aacttcaaga gcaacttcga cctggccgag 780

gacgccaagc tgcagctgag caaggacacc tacgacgacg acctggacaa cctgctggcc 840

cagatcggcg accagtacgc cgacctgttc ctggccgcca agaacctgag cgacgccatc 900

ctgctgagcg acatcctgcg ggtgaacacc gagatcacca aggcccccct gagcgccagc 960

atgatcaagc ggtacgacga gcaccaccag gacctgaccc tgctgaaggc cctggtgcgg 1020

cagcagctgc ccgagaagta caaggagatc ttcttcgacc agagcaagaa cggctacgcc 1080

ggctacatcg acggcggcgc cagccaggag gagttctaca agttcatcaa gcccatcctg 1140

gagaagatgg acggcaccga ggagctgctg gtgaagctga accgggagga cctgctgcgg 1200

aagcagcgga ccttcgacaa cggcagcatc ccccaccaga tccacctggg cgagctgcac 1260

gccatcctgc ggcggcagga ggacttctac cccttcctga aggacaaccg ggagaagatc 1320

gagaagatcc tgaccttccg gatcccctac tacgtgggcc ccctggcccg gggcaacagc 1380

cggttcgcct ggatgacccg gaagagcgag gagaccatca ccccctggaa cttcgaggag 1440

gtggtggaca agggcgccag cgcccagagc ttcatcgagc ggatgaccaa cttcgacaag 1500

aacctgccca acgagaaggt gctgcccaag cacagcctgc tgtacgagta cttcaccgtg 1560

tacaacgagc tgaccaaggt gaagtacgtg accgagggca tgcggaagcc cgccttcctg 1620

agcggcgagc agaagaaggc catcgtggac ctgctgttca agaccaaccg gaaggtgacc 1680

gtgaagcagc tgaaggagga ctacttcaag aagatcgagt gcttcgacag cgtggagatc 1740

agcggcgtgg aggaccggtt caacgccagc ctgggcacct accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgag gagaacgagg acatcctgga ggacatcgtg 1860

ctgaccctga ccctgttcga ggaccgggag atgatcgagg agcggctgaa gacctacgcc 1920

cacctgttcg acgacaaggt gatgaagcag ctgaagcggc ggcggtacac cggctggggc 1980

cggctgagcc ggaagctgat caacggcatc cgggacaagc agagcggcaa gaccatcctg 2040

gacttcctga agagcgacgg cttcgccaac cggaacttca tgcagctgat ccacgacgac 2100

agcctgacct tcaaggagga catccagaag gcccaggtga gcggccaggg cgacagcctg 2160

cacgagcaca tcgccaacct ggccggcagc cccgccatca agaagggcat cctgcagacc 2220

gtgaaggtgg tggacgagct ggtgaaggtg atgggccggc acaagcccga gaacatcgtg 2280

atcgagatgg cccgggagaa ccagaccacc cagaagggcc agaagaacag ccgggagcgg 2340

atgaagcgga tcgaggaggg catcaaggag ctgggcagcc agatcctgaa ggagcacccc 2400

gtggagaaca cccagctgca gaacgagaag ctgtacctgt actacctgca gaacggccgg 2460

gacatgtacg tggaccagga gctggacatc aaccggctga gcgactacga cgtggacgcc 2520

atcgtgcccc agagcttcct gaaggacgac agcatcgaca acaaggtgct gacccggagc 2580

gacaagaacc ggggcaagag cgacaacgtg cccagcgagg aggtggtgaa gaagatgaag 2640

aactactggc ggcagctgct gaacgccaag ctgatcaccc agcggaagtt cgacaacctg 2700

accaaggccg agcggggcgg cctgagcgag ctggacaagg ccggcttcat caagcggcag 2760

ctggtggaga cccggcagat caccaagcac gtggcccaga tcctggacag ccggatgaac 2820

accaagtacg acgagaacga caagctgatc cgggaggtga aggtgatcac cctgaagagc 2880

aagctggtga gcgacttccg gaaggacttc cagttctaca aggtgcggga gatcaacaac 2940

taccaccacg cccacgacgc ctacctgaac gccgtggtgg gcaccgccct gatcaagaag 3000

taccccaagc tggagagcga gttcgtgtac ggcgactaca aggtgtacga cgtgcggaag 3060

atgatcgcca agagcgagca ggagatcggc aaggccaccg ccaagtactt cttctacagc 3120

aacatcatga acttcttcaa gaccgagatc accctggcca acggcgagat ccggaagcgg 3180

cccctgatcg agaccaacgg cgagaccggc gagatcgtgt gggacaaggg ccgggacttc 3240

gccaccgtgc ggaaggtgct gagcatgccc caggtgaaca tcgtgaagaa gaccgaggtg 3300

cagaccggcg gcttcagcaa ggagagcatc ctgcccaagc ggaacagcga caagctgatc 3360

gcccggaaga aggactggga ccccaagaag tacggcggct tcgacagccc caccgtggcc 3420

tacagcgtgc tggtggtggc caaggtggag aagggcaaga gcaagaagct gaagagcgtg 3480

aaggagctgc tgggcatcac catcatggag cggagcagct tcgagaagaa ccccatcgac 3540

ttcctggagg ccaagggcta caaggaggtg aagaaggacc tgatcatcaa gctgcccaag 3600

tacagcctgt tcgagctgga gaacggccgg aagcggatgc tggccagcgc cggcgagctg 3660

cagaagggca acgagctggc cctgcccagc aagtacgtga acttcctgta cctggccagc 3720

cactacgaga agctgaaggg cagccccgag gacaacgagc agaagcagct gttcgtggag 3780

cagcacaagc actacctgga cgagatcatc gagcagatca gcgagttcag caagcgggtg 3840

atcctggccg acgccaacct ggacaaggtg ctgagcgcct acaacaagca ccgggacaag 3900

cccatccggg agcaggccga gaacatcatc cacctgttca ccctgaccaa cctgggcgcc 3960

cccgccgcct tcaagtactt cgacaccacc atcgaccgga agcggtacac cagcaccaag 4020

gaggtgctgg acgccaccct gatccaccag agcatcaccg gcctgtacga gacccggatc 4080

gacctgagcc agctgggcgg cgactga 4107

<210> 328
<211> 3312
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Nme Cas9 ORF using low A codons of Table 4, with start and stop codons

<400> 328
atggccgcct tcaagcccaa ctccatcaac tacatcctgg gcctggacat cggcatcgcc 60

tccgtgggct gggccatggt ggagatcgac gaggaggaga accccatccg gctgatcgac 120

ctgggcgtgc gggtgttcga gcgggccgag gtgcccaaga ccggcgactc cctggccatg 180

gcccggcggc tggcccggtc cgtgcggcgg ctgacccggc ggcgggccca ccggctgctg 240

cggacccggc ggctgctgaa gcgggagggc gtgctgcagg ccgccaactt cgacgagaac 300

ggcctgatca agtccctgcc caacaccccc tggcagctgc gggccgccgc cctggaccgg 360

aagctgaccc ccctggagtg gtccgccgtg ctgctgcacc tgatcaagca ccggggctac 420

ctgtcccagc ggaagaacga gggcgagacc gccgacaagg agctgggcgc cctgctgaag 480

ggcgtggccg gcaacgccca cgccctgcag accggcgact tccggacccc cgccgagctg 540

gccctgaaca agttcgagaa ggagtccggc cacatccgga accagcggtc cgactactcc 600

cacaccttct cccggaagga cctgcaggcc gagctgatcc tgctgttcga gaagcagaag 660

gagttcggca acccccacgt gtccggcggc ctgaaggagg gcatcgagac cctgctgatg 720

acccagcggc ccgccctgtc cggcgacgcc gtgcagaaga tgctgggcca ctgcaccttc 780

gagcccgccg agcccaaggc cgccaagaac acctacaccg ccgagcggtt catctggctg 840

accaagctga acaacctgcg gatcctggag cagggctccg agcggcccct gaccgacacc 900

gagcgggcca ccctgatgga cgagccctac cggaagtcca agctgaccta cgcccaggcc 960

cggaagctgc tgggcctgga ggacaccgcc ttcttcaagg gcctgcggta cggcaaggac 1020

aacgccgagg cctccaccct gatggagatg aaggcctacc acgccatctc ccgggccctg 1080

gagaaggagg gcctgaagga caagaagtcc cccctgaacc tgtccccga gctgcaggac 1140

gagatcggca ccgccttctc cctgttcaag accgacgagg acatcaccgg ccggctgaag 1200

gaccggatcc agcccgagat cctggaggcc ctgctgaagc acatctcctt cgacaagttc 1260

gtgcagatct ccctgaaggc cctgcggcgg atcgtgcccc tgatggagca gggcaagcgg 1320

tacgacgagg cctgcgccga gatctacggc gaccactacg gcaagaagaa caccgaggag 1380

aagatctacc tgcccccat ccccgccgac gagatccgga accccgtggt gctgcgggcc 1440

ctgtcccagg cccggaaggt gatcaacggc gtggtgcggc ggtacggctc ccccgcccgg 1500

atccacatcg agaccgcccg ggaggtgggc aagtccttca aggaccggaa ggagatcgag 1560

aagcggcagg aggagaaccg gaaggaccgg gagaaggccg ccgccaagtt ccgggagtac 1620

ttccccaact tcgtgggcga gcccaagtcc aaggacatcc tgaagctgcg gctgtacgag 1680

cagcagcacg gcaagtgcct gtactccggc aaggagatca acctgggccg gctgaacgag 1740

aagggctacg tggagatcga ccacgccctg cccttctccc ggacctggga cgactccttc 1800

aacaacaagg tgctggtgct gggctccgag aaccagaaca agggcaacca gacccctac 1860

gagtacttca acggcaagga caactcccgg gagtggcagg agttcaaggc ccgggtggag 1920

acctcccggt tcccccggtc caagaagcag cggatcctgc tgcagaagtt cgacgaggac 1980

ggcttcaagg agcggaacct gaacgacacc cggtacgtga accggttcct gtgccagttc 2040

gtggccgacc ggatgcggct gaccggcaag ggcaagaagc gggtgttcgc ctccaacggc 2100

cagatcacca acctgctgcg gggcttctgg ggcctgcgga aggtgcgggc cgagaacgac 2160

cggcaccacg ccctggacgc cgtggtggtg gcctgctcca ccgtggccat gcagcagaag 2220

atcacccggt tcgtgcggta caaggagatg aacgccttcg acggcaagac catcgacaag 2280

gagaccggcg aggtgctgca ccagaagacc cacttccccc agccctggga gttcttcgcc 2340

caggaggtga tgatccgggt gttcggcaag cccgacggca agcccgagtt cgaggaggcc 2400

gacaccctgg agaagctgcg gaccctgctg gccgagaagc tgtcctcccg gcccgaggcc 2460

gtgcacgagt acgtgacccc cctgttcgtg tcccgggccc ccaaccggaa gatgtccggc 2520

cagggccaca tggagaccgt gaagtccgcc aagcggctgg acgagggcgt gtccgtgctg 2580

cgggtgcccc tgacccagct gaagctgaag gacctggaga agatggtgaa ccgggagcgg 2640

gagcccaagc tgtacgaggc cctgaaggcc cggctggagg cccacaagga cgaccccgcc 2700

aaggccttcg ccgagccctt ctacaagtac gacaaggccg gcaaccggac ccagcaggtg 2760

aaggccgtgc gggtggagca ggtgcagaag accggcgtgt gggtgcggaa ccacaacggc 2820

atcgccgaca acgccaccat ggtgcgggtg gacgtgttcg agaagggcga caagtactac 2880

ctggtgccca tctactcctg gcaggtggcc aagggcatcc tgcccgaccg ggccgtggtg 2940

cagggcaagg acgaggagga ctggcagctg atcgacgact ccttcaactt caagttctcc 3000

ctgcacccca acgacctggt ggaggtgatc accaagaagg cccggatgtt cggctacttc 3060

gcctcctgcc accggggcac cggcaacatc aacatccgga tccacgacct ggaccacaag 3120

atcggcaaga acggcatcct ggagggcatc ggcgtgaaga ccgccctgtc cttccagaag 3180

taccagatcg acgagctggg caaggagatc cggccctgcc ggctgaagaa gcggcccccc 3240

gtgcggtccg gcaagcggac cgccgacggc tccgagttcg agtccccaa gaagaagcgg 3300

aaggtggagt ga 3312

<210> 329
<211> 3312
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Nme Cas9 ORF using low A/U codons of Table 4, with start and stop codons

<400> 329
atggccgcct tcaagcccaa cagcatcaac tacatcctgg gcctggacat cggcatcgcc 60

agcgtgggct gggccatggt ggagatcgac gaggaggaga accccatccg gctgatcgac 120

ctgggcgtgc gggtgttcga gcgggccgag gtgcccaaga ccggcgacag cctggccatg 180

gcccggcggc tggcccggag cgtgcggcgg ctgacccggc ggcgggccca ccggctgctg 240

cggacccggc ggctgctgaa gcgggagggc gtgctgcagg ccgccaactt cgacgagaac 300

ggcctgatca agagcctgcc caacaccccc tggcagctgc gggccgccgc cctggaccgg 360

aagctgaccc ccctggagtg gagcgccgtg ctgctgcacc tgatcaagca ccggggctac 420

ctgagccagc ggaagaacga gggcgagacc gccgacaagg agctgggcgc cctgctgaag 480

ggcgtggccg gcaacgccca cgccctgcag accggcgact tccggacccc cgccgagctg 540

gccctgaaca agttcgagaa ggagagcggc cacatccgga accagcggag cgactacagc 600

cacaccttca gccggaagga cctgcaggcc gagctgatcc tgctgttcga gaagcagaag 660

gagttcggca acccccacgt gagcggcggc ctgaaggagg gcatcgagac cctgctgatg 720

acccagcggc ccgccctgag cggcgacgcc gtgcagaaga tgctgggcca ctgcaccttc 780

gagcccgccg agcccaaggc cgccaagaac acctacaccg ccgagcggtt catctggctg 840

accaagctga acaacctgcg gatcctggag cagggcagcg agcggcccct gaccgacacc 900

gagcgggcca ccctgatgga cgagccctac cggaagagca agctgaccta cgcccaggcc 960

cggaagctgc tgggcctgga ggacaccgcc ttcttcaagg gcctgcggta cggcaaggac 1020

aacgccgagg ccagcaccct gatggagatg aaggcctacc acgccatcag ccgggccctg 1080

gagaaggagg gcctgaagga caagaagagc cccctgaacc tgagccccga gctgcaggac 1140

gagatcggca ccgccttcag cctgttcaag accgacgagg acatcaccgg ccggctgaag 1200

gaccggatcc agcccgagat cctggaggcc ctgctgaagc acatcagctt cgacaagttc 1260

gtgcagatca gcctgaaggc cctgcggcgg atcgtgcccc tgatggagca gggcaagcgg 1320

tacgacgagg cctgcgccga gatctacggc gaccactacg gcaagaagaa caccgaggag 1380

aagatctacc tgcccccccat ccccgccgac gagatccgga accccgtggt gctgcgggcc 1440

ctgagccagg cccggaaggt gatcaacggc gtggtgcggc ggtacggcag ccccgcccgg 1500

atccacatcg agaccgcccg ggaggtgggc aagagcttca aggaccggaa ggagatcgag 1560

aagcggcagg aggagaaccg gaaggaccgg gagaaggccg ccgccaagtt ccgggagtac 1620

ttccccaact tcgtgggcga gcccaagagc aaggacatcc tgaagctgcg gctgtacgag 1680

cagcagcacg gcaagtgcct gtacagcggc aaggagatca acctgggccg gctgaacgag 1740

aagggctacg tggagatcga ccacgccctg cccttcagcc ggacctggga cgacagcttc 1800

aacaacaagg tgctggtgct gggcagcgag aaccagaaca agggcaacca gacccctac 1860

gagtacttca acggcaagga caacagccgg gagtggcagg agttcaaggc ccgggtggag 1920

accagccggt tcccccggag caagaagcag cggatcctgc tgcagaagtt cgacgaggac 1980

ggcttcaagg agcggaacct gaacgacacc cggtacgtga accggttcct gtgccagttc 2040

gtggccgacc ggatgcggct gaccggcaag ggcaagaagc gggtgttcgc cagcaacggc 2100

cagatcacca acctgctgcg gggcttctgg ggcctgcgga aggtgcgggc cgagaacgac 2160

cggcaccacg ccctggacgc cgtggtggtg gcctgcagca ccgtggccat gcagcagaag 2220

atcacccggt tcgtgcggta caaggagatg aacgccttcg acggcaagac catcgacaag 2280

gagaccggcg aggtgctgca ccagaagacc cacttccccc agccctggga gttcttcgcc 2340

caggaggtga tgatccgggt gttcggcaag cccgacggca agcccgagtt cgaggaggcc 2400

gacaccctgg agaagctgcg gaccctgctg gccgagaagc tgagcagccg gcccgaggcc 2460

gtgcacgagt acgtgacccc cctgttcgtg agccgggccc ccaaccggaa gatgagcggc 2520

cagggccaca tggagaccgt gaagagcgcc aagcggctgg acgagggcgt gagcgtgctg 2580

cgggtgcccc tgacccagct gaagctgaag gacctggaga agatggtgaa ccgggagcgg 2640

gagcccaagc tgtacgaggc cctgaaggcc cggctggagg cccacaagga cgaccccgcc 2700

aaggccttcg ccgagccctt ctacaagtac gacaaggccg gcaaccggac ccagcaggtg 2760

aaggccgtgc gggtggagca ggtgcagaag accggcgtgt gggtgcggaa ccacaacggc 2820

atcgccgaca acgccaccat ggtgcgggtg gacgtgttcg agaagggcga caagtactac 2880

ctggtgccca tctacagctg gcaggtggcc aagggcatcc tgcccgaccg ggccgtggtg 2940

cagggcaagg acgaggagga ctggcagctg atcgacgaca gcttcaactt caagttcagc 3000

ctgcacccca acgacctggt ggaggtgatc accaagaagg cccggatgtt cggctacttc 3060

gccagctgcc accggggcac cggcaacatc aacatccgga tccacgacct ggaccacaag 3120

atcggcaaga acggcatcct ggagggcatc ggcgtgaaga ccgccctgag cttccagaag 3180

taccagatcg acgagctggg caaggagatc cggccctgcc ggctgaagaa gcggcccccc 3240

gtgcggagcg gcaagcggac cgccgacggc agcgagttcg agagccccaa gaagaagcgg 3300

aaggtggagt ga 3312

<210> 330
<211> 4146
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS1, with start and stop codons

<400> 330
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

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agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

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agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

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agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

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gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

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acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

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aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

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tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

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cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcctgg cagcaaagag aagcagaaca 4140

acatag 4146

<210> 331
<211> 4146
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS2, with start and stop codons

<400> 331
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atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac aggatgggga 1980

agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

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agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagccagg cagcaaagag aagcagaaca 4140

acatag 4146

<210> 332
<211> 4149
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS3, with start and stop codons

<400> 332
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

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tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

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gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

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agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

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atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

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aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

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tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

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cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

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ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcccgg caccggcaaa gagagaaaga 4140

acaacatag 4149

<210> 333
<211> 4146
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS4, with start and stop codons

<400> 333
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atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

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agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

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cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

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gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

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tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

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ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac aggatgggga 1980

agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

gacttcctga agagcgacgg attcgcaaac agaaacttca tgcagctgat ccacgacgac 2100

agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

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gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

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gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

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aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagccagg cagcaaagag accgagaaca 4140

acatag 4146

<210> 334
<211> 4146

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS5, with start and stop codons

<400> 334
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

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agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

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gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcagag cagcaaagag accgagaaca 4140

acatag 4146

<210> 335
<211> 4152
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS6, with start and stop codons

<400> 335
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

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agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

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gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

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tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

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cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

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ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcgcag cagcaaagag aagctggagc 4140

atggcagcat ag 4152

<210> 336
<211> 4152
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS7, with start and stop codons

<400> 336
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atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

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cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

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gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

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ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

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gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

ctggtcgaaa caagacagat cacaaagcac gtcgcacaga tcctggacag cagaatgaac 2820

acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

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cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcgcag cagcaaagag agtctggagc 4140

atggcattct ag 4152

<210> 337
<211> 4152
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS8, with start and stop codons

<400> 337
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

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cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac aggatgggga 1980

agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

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agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcgcag cagcaaagag aagctggagc 4140

atggcattct ag 4152

<210> 338
<211> 4149
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS9, with start and stop codons

<400> 338
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

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agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

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agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

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tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

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ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

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tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

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gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcgcag cagcaaagag aaagtacttc 4140

gcagcatag 4149

<210> 339
<211> 4149
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic: Open reading frame for Cas9 with NLS10, with start and stop codons

<400> 339

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atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

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agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

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cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

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gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

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gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

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ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

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gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

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tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcagag cagcaaagag aaaggcattc 4140

gcagcatag 4149

<210> 340
<211> 4149
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS11, with start and stop codons

<400> 340
atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac aggatgggga 1980

agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

gacttcctga agagcgacgg attcgcaaac agaaacttca tgcagctgat ccacgacgac 2100

agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

cacgaacaca tcgcaaacct ggcaggaagc ccggcaatca agaagggaat cctgcagaca 2220

gtcaaggtcg tcgacgaact ggtcaaggtc atgggaagac acaagccgga aaacatcgtc 2280

atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

ctggtcgaaa caagacagat cacaaagcac gtcgcacaga tcctggacag cagaatgaac 2820

acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcagag cagcaaagag aaagtacttc 4140

gcagtctag 4149

<210> 341
<211> 4128
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using codons with generally high expression in humans (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 341
cctaagaaaa agcggaaggt cgacggggat aagaagtact caatcgggct ggatatcgga 60

actaattccg tgggttgggc agtgatcacg gatgaataca aagtgccgtc caagaagttc 120

aaggtcctgg ggaacaccga tagacacagc atcaagaaaa atctcatcgg agccctgctg 180

tttgactccg gcgaaaccgc agaagcgacc cggctcaaac gtaccgcgag gcgacgctac 240

acccggcgga agaatcgcat ctgctatctg caagagatct tttcgaacga aatggcaaag 300

gtcgacgaca gcttcttcca ccgcctggaa gaatctttcc tggtggagga ggacaagaag 360

catgaacggc atcctatctt tggaaacatc gtcgacgaag tggcgtacca cgaaaagtac 420

ccgaccatct accatctgcg gaagaagttg gttgactcaa ctgacaaggc cgacctcaga 480

ttgatctact tggccctcgc ccatatgatc aaattccgcg gacacttcct gatcgaaggc 540

gatctgaacc ctgataactc cgacgtggat aagcttttca ttcaactggt gcagacctac 600

aaccaactgt tcgaagaaaa cccaatcaat gctagcggcg tcgatgccaa ggccatcctg 660

tccgcccggc tgtcgaagtc gcggcgcctc gaaaacctga tcgcacagct gccgggagag 720

aaaaagaacg gacttttcgg caacttgatc gctctctcac tgggactcac tcccaatttc 780

aagtccaatt ttgacctggc cgaggacgcg aagctgcaac tctcaaagga cacctacgac 840

gacgacttgg acaatttgct ggcacaaatt ggcgatcagt acgcggatct gttccttgcc 900

gctaagaacc tttcggacgc aatcttgctg tccgatatcc tgcgcgtgaa caccgaaata 960

accaaagcgc cgcttagcgc ctcgatgatt aagcggtacg acgagcatca ccaggatctc 1020

acgctgctca aagcgctcgt gagacagcaa ctgcctgaaa agtacaagga gatcttcttc 1080

gaccagtcca agaatgggta cgcagggtac atcgatggag gcgctagcca ggaagagttc 1140

tataagttca tcaagccaat cctggaaaag atggacggaa ccgaagaact gctggtcaag 1200

ctgaacaggg aggatctgct ccggaaacag agaacctttg acaacggatc cattccccac 1260

cagatccatc tgggtgagct gcacgccatc ttgcggcgcc aggaggactt ttacccattc 1320

ctcaaggaca accgggaaaa gatcgagaaa attctgacgt tccgcatccc gtattacgtg 1380

ggcccactgg cgcgcggcaa ttcgcgcttc gcgtggatga ctagaaaatc agaggaaacc 1440

atcactcctt ggaatttcga ggaagttgtg gataagggag cttcggcaca aagcttcatc 1500

gaacgaatga ccaacttcga caagaatctc ccaaacgaga aggtgcttcc taagcacagc 1560

ctcctttacg aatacttcac tgtctacaac gaactgacta aagtgaaata cgttactgaa 1620

ggaatgagga agccggcctt tctgtccgga gaacagaaga aagcaattgt cgatctgctg 1680

ttcaagacca accgcaaggt gaccgtcaag cagcttaaag aggactactt caagaagatc 1740

gagtgtttcg actcagtgga aatcagcggg gtggaggaca gattcaacgc ttcgctggga 1800

acctatcatg atctcctgaa gatcatcaag gacaaggact tccttgacaa cgaggagaac 1860

gaggacatcc tggaagatat cgtcctgacc ttgacccttt tcgaggatcg cgagatgatc 1920

gaggagaggc ttaagaccta cgctcatctc ttcgacgata aggtcatgaa acaactcaag 1980

cgccgccggt acactggttg gggccgcctc tcccgcaagc tgatcaacgg tattcgcgat 2040

aaacagagcg gtaaaactat cctggatttc ctcaaatcgg atggcttcgc taatcgtaac 2100

ttcatgcaat tgatccacga cgacagcctg acctttaagg aggacatcca aaaagcacaa 2160

gtgtccggac agggagactc actccatgaa cacatcgcga atctggccgg ttcgccggcg 2220

attaagaagg gaattctgca aactgtgaag gtggtcgacg agctggtgaa ggtcatggga 2280

cggcacaaac cggagaatat cgtgattgaa atggcccgag aaaaccagac tacccagaag 2340

ggccagaaaa actcccgcga aaggatgaag cggatcgaag aaggaatcaa ggagctgggc 2400

agccagatcc tgaaagagca cccggtggaa aacacgcagc tgcagaacga gaagctctac 2460

ctgtactatt tgcaaaatgg acgggacatg tacgtggacc aagagctgga catcaatcgg 2520

ttgtctgatt acgacgtgga ccacatcgtt ccacagtcct ttctgaagga tgactcgatc 2580

gataacaagg tgttgactcg cagcgacaag aacagaggga agtcagataa tgtgccatcg 2640

gaggaggtcg tgaagaagat gaagaattac tggcggcagc tcctgaatgc gaagctgatt 2700

acccagagaa agtttgacaa tctcactaaa gccgagcgcg gcggactctc agagctggat 2760

aaggctggat tcatcaaacg gcagctggtc gagactcggc agattaccaa gcacgtggcg 2820

cagatcttgg actcccgcat gaacactaaa tacgacgaga acgataagct catccgggaa 2880

gtgaaggtga ttaccctgaa aagcaaactt gtgtcggact ttcggaagga ctttcagttt 2940

tacaaagtga gagaaatcaa caactaccat cacgcgcatg acgcatacct caacgctgtg 3000

gtcggtaccg ccctgatcaa aaagtaccct aaacttgaat cggagtttgt gtacggagac 3060

tacaaggtct acgacgtgag gaagatgata gccaagtccg aacaggaaat cgggaaagca 3120

actgcgaaat acttctttta ctcaaacatc atgaactttt tcaagactga aattacgctg 3180

gccaatggag aaatcaggaa gaggccactg atcgaaacta acggagaaac gggcgaaatc 3240

gtgtgggaca agggcaggga cttcgcaact gttcgcaaag tgctctctat gccgcaagtc 3300

aatattgtga agaaaaccga agtgcaaacc ggcggatttt caaaggaatc gatcctccca 3360

aagagaaata gcgacaagct cattgcacgc aagaaagact gggacccgaa gaagtacgga 3420

ggattcgatt cgccgactgt cgcatactcc gtcctcgtgg tggccaaggt ggagaaggga 3480

aagagcaaaa agctcaaatc cgtcaaagag ctgctgggga ttaccatcat ggaacgatcc 3540

tcgttcgaga agaacccgat tgatttcctc gaggcgaagg gttacaagga ggtgaagaag 3600

gatctgatca tcaaactccc caagtactca ctgttcgaac tggaaaatgg tcggaagcgc 3660

atgctggctt cggccggaga actccaaaaa ggaaatgagc tggccttgcc tagcaagtac 3720

gtcaacttcc tctatcttgc ttcgcactac gaaaaactca aagggtcacc ggaagataac 3780

gaacagaagc agcttttcgt ggagcagcac aagcattatc tggatgaaat catcgaacaa 3840

atctccgagt tttcaaagcg cgtgatcctc gccgacgcca acctcgacaa agtcctgtcg 3900

gcctacaata agcatagaga taagccgatc agagaacagg ccgagaacat tatccacttg 3960

ttcaccctga ctaacctggg agccccagcc gccttcaagt acttcgatac tactatcgat 4020

cgcaaaagat acacgtccac caaggaagtt ctggacgcga ccctgatcca ccaaagcatc 4080

actggactct acgaaactag gatcgatctg tcgcagctgg gtggcgat 4128

<210> 342
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using long half life codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 342
gacaagaagt actctatcgg tttggacatc ggtaccaact ctgtcggttg ggccgtcatc 60

accgacgaat acaaggtccc atctaagaag ttcaaggtct tgggtaacac cgacagacac 120

tctatcaaga agaacttgat cggtgccttg ttgttcgact ctggtgaaac cgccgaagcc 180

accagattga agagaaccgc cagaagaaga tacaccagaa gaaagaacag aatctgctac 240

ttgcaagaaa tcttctctaa cgaaatggcc aaggtcgacg actctttctt ccacagattg 300

gaagaatctt tcttggtcga agaagacaag aagcacgaaa gacacccaat cttcggtaac 360

atcgtcgacg aagtcgccta ccacgaaaag tacccaacca tctaccactt gagaaagaag 420

ttggtcgact ctaccgacaa ggccgacttg agattgatct acttggcctt ggcccacatg 480

atcaagttca gaggtcactt cttgatcgaa ggtgacttga acccagacaa ctctgacgtc 540

gacaagttgt tcatccaatt ggtccaaacc tacaaccaat tgttcgaaga aaacccaatc 600

aacgcctctg gtgtcgacgc caaggccatc ttgtctgcca gattgtctaa gagcagaaga 660

ttggaaaact tgatcgccca attgccaggt gaaaagaaga acggtttgtt cggtaacttg 720

atcgccttgt ctttgggttt gaccccaaac ttcaagtcta acttcgactt ggccgaagac 780

gccaagttgc aattgtctaa ggacacctac gacgacgact tggacaactt gttggcccaa 840

atcggtgacc aatacgccga cttgttcttg gccgccaaga acttgtctga cgccatcttg 900

ttgtctgaca tcttgagagt caacaccgaa atcaccaagg ccccattgtc tgcctctatg 960

atcaagagat acgacgaaca ccaccaagac ttgaccttgt tgaaggcctt ggtcagacaa 1020

caattgccag aaaagtacaa ggaaatcttc ttcgaccaat ctaagaacgg ttacgccggt 1080

tacatcgacg gtggtgcctc tcaagaagaa ttctacaagt tcatcaagcc aatcttggaa 1140

aagatggacg gtaccgaaga attgttggtc aagttgaaca gagaagactt gttgagaaag 1200

caaagaacct tcgacaacgg ttctatccca caccaaatcc acttgggtga attgcacgcc 1260

atcttgagaa gacaagaaga cttctaccca ttcttgaagg acaacagaga aaagatcgaa 1320

aagatcttga ccttcagaat cccatactac gtcggtccat tggccagagg taacagcaga 1380

ttcgcctgga tgaccagaaa gtctgaagaa accatcaccc catggaactt cgaagaagtc 1440

gtcgacaagg gtgcctctgc ccaatctttc atcgaaagaa tgaccaactt cgacaagaac 1500

ttgccaaacg aaaaggtctt gccaaagcac tctttgttgt acgaatactt caccgtctac 1560

aacgaattga ccaaggtcaa gtacgtcacc gaaggtatga gaaagccagc cttcttgtct 1620

ggtgaacaaa agaaggccat cgtcgacttg ttgttcaaga ccaacagaaa ggtcaccgtc 1680

aagcaattga aggaagacta cttcaagaag atcgaatgct tcgactctgt cgaaatctct 1740

ggtgtcgaag acagattcaa cgcctctttg ggtacctacc acgacttgtt gaagatcatc 1800

aaggacaagg acttcttgga caacgaagaa aacgaagaca tcttggaaga catcgtcttg 1860

accttgacct tgttcgaaga cagagaaatg atcgaagaaa gattgaagac ctacgcccac 1920

ttgttcgacg acaaggtcat gaagcaattg aagagaagaa gatacaccgg ttggggtaga 1980

ttgagcagaa agttgatcaa cggtatcaga gacaagcaat ctggtaagac catcttggac 2040

ttcttgaagt ctgacggttt cgccaacaga aacttcatgc aattgatcca cgacgactct 2100

ttgaccttca aggaagacat ccaaaaggcc caagtctctg gtcaaggtga ctctttgcac 2160

gaacacatcg ccaacttggc cggttctcca gccatcaaga agggtatctt gcaaaccgtc 2220

aaggtcgtcg acgaattggt caaggtcatg ggtagacaca agccagaaaa catcgtcatc 2280

gaaatggcca gagaaaacca aaccacccaa aagggtcaaa agaacagcag agaaagaatg 2340

aagagaatcg aagaaggtat caaggaattg ggttctcaaa tcttgaagga acacccagtc 2400

gaaaacaccc aattgcaaaa cgaaaagttg tacttgtact acttgcaaaa cggtagagac 2460

atgtacgtcg accaagaatt ggacatcaac agattgtctg actacgacgt cgaccacatc 2520

gtcccacaat ctttcttgaa ggacgactct atcgacaaca aggtcttgac cagatctgac 2580

aagaacagag gtaagtctga caacgtccca tctgaagaag tcgtcaagaa gatgaagaac 2640

tactggagac aattgttgaa cgccaagttg atcacccaaa gaaagttcga caacttgacc 2700

aaggccgaaa gaggtggttt gtctgaattg gacaaggccg gtttcatcaa gagacaattg 2760

gtcgaaacca gacaaatcac caagcacgtc gcccaaatct tggacagcag aatgaacacc 2820

aagtacgacg aaaacgacaa gttgatcaga gaagtcaagg tcatcacctt gaagtctaag 2880

ttggtctctg acttcagaaa ggacttccaa ttctacaagg tcagagaaat caacaactac 2940

caccacgccc acgacgccta cttgaacgcc gtcgtcggta ccgccttgat caagaagtac 3000

ccaaagttgg aatctgaatt cgtctacggt gactacaagg tctacgacgt cagaaagatg 3060

atcgccaagt ctgaacaaga aatcggtaag gccaccgcca agtacttctt ctactctaac 3120

atcatgaact tcttcaagac cgaaatcacc ttggccaacg gtgaaatcag aaagagacca 3180

ttgatcgaaa ccaacggtga aaccggtgaa atcgtctggg acaagggtag agacttcgcc 3240

accgtcagaa aggtcttgtc tatgccacaa gtcaacatcg tcaagaagac cgaagtccaa 3300

accggtggtt tctctaagga atctatcttg ccaaagagaa actctgacaa gttgatcgcc 3360

agaaagaagg actgggaccc aaagaagtac ggtggtttcg actctccaac cgtcgcctac 3420

tctgtcttgg tcgtcgccaa ggtcgaaaag ggtaagtcta agaagttgaa gtctgtcaag 3480

gaattgttgg gtatcaccat catggaaaga tcttctttcg aaaagaaccc aatcgacttc 3540

ttggaagcca agggttacaa ggaagtcaag aaggacttga tcatcaagtt gccaaagtac 3600

tctttgttcg aattggaaaa cggtagaaag agaatgttgg cctctgccgg tgaattgcaa 3660

aagggtaacg aattggcctt gccatctaag tacgtcaact tcttgtactt ggcctctcac 3720

tacgaaaagt tgaagggttc tccagaagac aacgaacaaa agcaattgtt cgtcgaacaa 3780

cacaagcact acttggacga aatcatcgaa caaatctctg aattctctaa gagagtcatc 3840

ttggccgacg ccaacttgga caaggtcttg tctgcctaca acaagcacag agacaagcca 3900

atcagagaac aagccgaaaa catcatccac ttgttcacct tgaccaactt gggtgcccca 3960

gccgccttca agtacttcga caccaccatc gacagaaaga gatacacctc taccaaggaa 4020

gtcttggacg ccaccttgat ccaccaatct atcaccggtt tgtacgaaac cagaatcgac 4080

ttgtctcaat tgggtggtga cggtggtggt tctccaaaga agaagagaaa ggtc 4134

<210> 343
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using U rich codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 343
gataaaaaat attctattgg tttagatatt ggtactaatt ctgttggttg ggctgttatt 60

actgatgaat ataaagttcc ttctaaaaaa tttaaagttt taggtaatac tgatcgtcat 120

tctattaaaa aaaatttaat tggtgcttta ttatttgatt ctggtgaaac tgctgaagct 180

actcgtttaa aacgtactgc tcgtcgtcgt tatactcgtc gtaaaaatcg tatttgttat 240

ttacaagaaa ttttttctaa tgaaatggct aaagttgatg attctttttt tcatcgttta 300

gaagaatctt ttttagttga agaagataaa aaacatgaac gtcatcctat ttttggtaat 360

attgttgatg aagttgctta tcatgaaaaa tatcctacta tttatcattt acgtaaaaaa 420

ttagttgatt ctactgataa agctgattta cgtttaattt atttagcttt agctcatatg 480

attaaatttc gtggtcattt tttaattgaa ggtgatttaa atcctgataa ttctgatgtt 540

gataaattat ttattcaatt agttcaaact tataatcaat tatttgaaga aaatcctatt 600

aatgcttctg gtgttgatgc taaagctatt ttatctgctc gtttatctaa atctcgtcgt 660

ttagaaaatt taattgctca attacctggt gaaaaaaaaa atggtttatt tggtaattta 720

attgctttat ctttaggttt aactcctaat tttaaatcta attttgattt agctgaagat 780

gctaaattac aattatctaa agatacttat gatgatgatt tagataattt attagctcaa 840

attggtgatc aatatgctga tttattttta gctgctaaaa atttatctga tgctatttta 900

ttatctgata ttttacgtgt taatactgaa attactaaag ctcctttatc tgcttctatg 960

attaaacgtt atgatgaaca tcatcaagat ttaactttat taaaagcttt agttcgtcaa 1020

caattacctg aaaaatataa agaaattttt tttgatcaat ctaaaaatgg ttatgctggt 1080

tatattgatg gtggtgcttc tcaagaagaa ttttataaat ttattaaacc tattttagaa 1140

aaaatggatg gtactgaaga attattagtt aaattaaatc gtgaagattt attacgtaaa 1200

caacgtactt ttgataatgg ttctattcct catcaaattc atttaggtga attacatgct 1260

attttacgtc gtcaagaaga tttttatcct tttttaaaag ataatcgtga aaaaattgaa 1320

aaaattttaa cttttcgtat tccttattat gttggtcctt tagctcgtgg taattctcgt 1380

tttgcttgga tgactcgtaa atctgaagaa actattactc cttggaattt tgaagaagtt 1440

gttgataaag gtgcttctgc tcaatctttt attgaacgta tgactaattt tgataaaaat 1500

ttacctaatg aaaaagtttt acctaaacat tctttattat atgaatattt tactgtttat 1560

aatgaattaa ctaaagttaa atatgttact gaaggtatgc gtaaacctgc ttttttatct 1620

ggtgaacaaa aaaaagctat tgttgattta ttatttaaaa ctaatcgtaa agttactgtt 1680

aaacaattaa aagaagatta ttttaaaaaa attgaatgtt ttgattctgt tgaaatttct 1740

ggtgttgaag atcgttttaa tgcttcttta ggtacttatc atgatttatt aaaaattatt 1800

aaagataaag attttttaga taatgaagaa aatgaagata ttttagaaga tattgtttta 1860

actttaactt tatttgaaga tcgtgaaatg attgaagaac gtttaaaaac ttatgctcat 1920

ttatttgatg ataaagttat gaaacaatta aaacgtcgtc gttatactgg ttggggtcgt 1980

ttatctcgta aattaattaa tggtattcgt gataaacaat ctggtaaaac tattttagat 2040

tttttaaaat ctgatggttt tgctaatcgt aattttatgc aattaattca tgatgattct 2100

ttaactttta aagaagatat tcaaaaagct caagtttctg gtcaaggtga ttctttacat 2160

gaacatattg ctaatttagc tggttctcct gctattaaaa aaggtatttt acaaactgtt 2220

aaagttgttg atgaattagt taaagttatg ggtcgtcata aacctgaaaa tattgttatt 2280

gaaatggctc gtgaaaatca aactactcaa aaaggtcaaa aaaattctcg tgaacgtatg 2340

aaacgtattg aagaaggtat taaagaatta ggttctcaaa ttttaaaaga acatcctgtt 2400

gaaaatactc aattacaaaa tgaaaaatta tatttatatt atttacaaaa tggtcgtgat 2460

atgtatgttg atcaagaatt agatattaat cgtttatctg attatgatgt tgatcatatt 2520

gttcctcaat ctttttaaa agatgattct attgataata aagttttaac tcgttctgat 2580

aaaaatcgtg gtaaatctga taatgttcct tctgaagaag ttgttaaaaa aatgaaaaat 2640

tattggcgtc aattattaaa tgctaaatta attactcaac gtaaatttga taatttaact 2700

aaagctgaac gtggtggttt atctgaatta gataaagctg gttttattaa acgtcaatta 2760

gttgaaactc gtcaaattac taaacatgtt gctcaaattt tagattctcg tatgaatact 2820

aaatatgatg aaaatgataa attaattcgt gaagttaaag ttattacttt aaaatctaaa 2880

ttagtttctg attttcgtaa agattttcaa ttttataaag ttcgtgaaat taataattat 2940

catcatgctc atgatgctta tttaaatgct gttgttggta ctgctttaat taaaaaatat 3000

cctaaattag aatctgaatt tgtttatggt gattataaag tttatgatgt tcgtaaaatg 3060

attgctaaat ctgaacaaga aattggtaaa gctactgcta aatatttttt ttattctaat 3120

attatgaatt tttttaaaac tgaaattact ttagctaatg gtgaaattcg taaacgtcct 3180

ttaattgaaa ctaatggtga aactggtgaa attgtttggg ataaaggtcg tgattttgct 3240

actgttcgta aagttttatc tatgcctcaa gttaatattg ttaaaaaaac tgaagttcaa 3300

actggtggtt tttctaaaga atctatttta cctaaacgta attctgataa attaattgct 3360

cgtaaaaaag attgggatcc taaaaaatat ggtggttttg attctcctac tgttgcttat 3420

tctgttttag ttgttgctaa agttgaaaaa ggtaaatcta aaaaattaaa atctgttaaa 3480

gaattattag gtattactat tatggaacgt tcttcttttg aaaaaaatcc tattgatttt 3540

ttagaagcta aaggttataa agaagttaaa aaagatttaa ttattaaatt acctaaatat 3600

tctttatttg aattagaaaa tggtcgtaaa cgtatgttag cttctgctgg tgaattacaa 3660

aaaggtaatg aattagcttt accttctaaa tatgttaatt ttttatattt agcttctcat 3720

tatgaaaaat taaaaggttc tcctgaagat aatgaacaaa aacaattatt tgttgaacaa 3780

cataaacatt atttagatga aattattgaa caaatttctg aattttctaa acgtgttatt 3840

ttagctgatg ctaatttaga taaagtttta tctgcttata ataaacatcg tgataaacct 3900

attcgtgaac aagctgaaaa tattattcat ttatttactt taactaattt aggtgctcct 3960

gctgctttta aatattttga tactactatt gatcgtaaac gttatacttc tactaaagaa 4020

gttttagatg ctactttaat tcatcaatct attactggtt tatatgaaac tcgtattgat 4080

ttatctcaat taggtggtga tggtggtggt tctcctaaaa aaaaacgtaa agtt 4134

<210> 344
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low G codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 344
gacaaaaaat actccatcgg cctcgacatc ggcaccaact ccgtcggctg ggccgtcatc 60

accgacgaat acaaagtccc ctccaaaaaa ttcaaagtcc tcggcaacac cgacagacac 120

tccatcaaaa aaaacctcat cggcgccctc ctcttcgact ccggcgaaac cgccgaagcc 180

accagactca aaagaaccgc cagaagaaga tacaccagaa gaaaaaacag aatctgctac 240

ctccaagaaa tcttctccaa cgaaatggcc aaagtcgacg actccttctt ccacagactc 300

gaagaatcct tcctcgtcga agaagacaaa aaacacgaaa gacaccccat cttcggcaac 360

atcgtcgacg aagtcgccta ccacgaaaaa taccccacca tctaccacct cagaaaaaaa 420

ctcgtcgact ccaccgacaa agccgacctc agactcatct acctcgccct cgcccacatg 480

atcaaattca gaggccactt cctcatcgaa ggcgacctca accccgacaa ctccgacgtc 540

gacaaactct tcatccaact cgtccaaacc tacaaccaac tcttcgaaga aaaccccatc 600

aacgcctccg gcgtcgacgc caaagccatc ctctccgcca gactctccaa atccagaaga 660

ctcgaaaacc tcatcgccca actccccggc gaaaaaaaaa acggcctctt cggcaacctc 720

atcgccctct ccctcggcct caccccccaac ttcaaatcca acttcgacct cgccgaagac 780

gccaaactcc aactctccaa agacacctac gacgacgacc tcgacaacct cctcgcccaa 840

atcggcgacc aatacgccga cctcttcctc gccgccaaaa acctctccga cgccatcctc 900

ctctccgaca tcctcagagt caacaccgaa atcaccaaag ccccccctctc cgcctccatg 960

atcaaaagat acgacgaaca ccaccaagac ctcaccctcc tcaaagccct cgtcagacaa 1020

caactccccg aaaaatacaa agaaatcttc ttcgaccaat ccaaaaacgg ctacgccggc 1080

tacatcgacg gcggcgcctc ccaagaagaa ttctacaaat tcatcaaacc catcctcgaa 1140

aaaatggacg gcaccgaaga actcctcgtc aaactcaaca gagaagacct cctcagaaaa 1200

caaagaacct tcgacaacgg ctccatcccc caccaaatcc acctcggcga actccacgcc 1260

atcctcagaa gacaagaaga cttctacccc ttcctcaaag acaacagaga aaaaatcgaa 1320

aaaatcctca ccttcagaat cccctactac gtcggccccc tcgccagagg caactccaga 1380

ttcgcctgga tgaccagaaa atccgaagaa accatcaccc cctggaactt cgaagaagtc 1440

gtcgacaaag gcgcctccgc ccaatccttc atcgaaagaa tgaccaactt cgacaaaaac 1500

ctccccaacg aaaaagtcct ccccaaacac tccctcctct acgaatactt caccgtctac 1560

aacgaactca ccaaagtcaa atacgtcacc gaaggcatga gaaaacccgc cttcctctcc 1620

ggcgaacaaa aaaaagccat cgtcgacctc ctcttcaaaa ccaacagaaa agtcaccgtc 1680

aaacaactca aagaagacta cttcaaaaaa atcgaatgct tcgactccgt cgaaatctcc 1740

ggcgtcgaag acagattcaa cgcctccctc ggcacctacc acgacctcct caaaatcatc 1800

aaagacaaag acttcctcga caacgaagaa aacgaagaca tcctcgaaga catcgtcctc 1860

accctcaccc tcttcgaaga cagagaaatg atcgaagaaa gactcaaaac ctacgcccac 1920

ctcttcgacg acaaagtcat gaaacaactc aaaagaagaa gatacaccgg ctggggcaga 1980

ctctccagaa aactcatcaa cggcatcaga gacaaacaat ccggcaaaac catcctcgac 2040

ttcctcaaat ccgacggctt cgccaacaga aacttcatgc aactcatcca cgacgactcc 2100

ctcaccttca aagaagacat ccaaaaagcc caagtctccg gccaaggcga ctccctccac 2160

gaacacatcg ccaacctcgc cggctccccc gccatcaaaa aaggcatcct ccaaaccgtc 2220

aaagtcgtcg acgaactcgt caaagtcatg ggcagacaca aacccgaaaa catcgtcatc 2280

gaaatggcca gagaaaacca aaccacccaa aaaggccaaa aaaactccag agaaagaatg 2340

aaaagaatcg aagaaggcat caaagaactc ggctcccaaa tcctcaaaga acaccccgtc 2400

gaaaacaccc aactccaaaa cgaaaaactc tacctctact acctccaaaa cggcagagac 2460

atgtacgtcg accaagaact cgacatcaac agactctccg actacgacgt cgaccacatc 2520

gtcccccaat ccttcctcaa agacgactcc atcgacaaca aagtcctcac cagatccgac 2580

aaaaacagag gcaaatccga caacgtcccc tccgaagaag tcgtcaaaaa aatgaaaaac 2640

tactggagac aactcctcaa cgccaaactc atcacccaaa gaaaattcga caacctcacc 2700

aaagccgaaa gaggcggcct ctccgaactc gacaaagccg gcttcatcaa aagacaactc 2760

gtcgaaacca gacaaatcac caaacacgtc gcccaaatcc tcgactccag aatgaacacc 2820

aaatacgacg aaaacgacaa actcatcaga gaagtcaaag tcatcaccct caaatccaaa 2880

ctcgtctccg acttcagaaa agacttccaa ttctacaaag tcagagaaat caacaactac 2940

caccacgccc acgacgccta cctcaacgcc gtcgtcggca ccgccctcat caaaaaatac 3000

cccaaactcg aatccgaatt cgtctacggc gactacaaag tctacgacgt cagaaaaatg 3060

atcgccaaat ccgaacaaga aatcggcaaa gccaccgcca aatacttctt ctactccaac 3120

atcatgaact tcttcaaaac cgaaatcacc ctcgccaacg gcgaaatcag aaaaagaccc 3180

ctcatcgaaa ccaacggcga aaccggcgaa atcgtctggg acaaaggcag agacttcgcc 3240

accgtcagaa aagtcctctc catgccccaa gtcaacatcg tcaaaaaaac cgaagtccaa 3300

accggcggct tctccaaaga atccatcctc cccaaaagaa actccgacaa actcatcgcc 3360

agaaaaaaag actgggaccc caaaaaatac ggcggcttcg actcccccac cgtcgcctac 3420

tccgtcctcg tcgtcgccaa agtcgaaaaa ggcaaatcca aaaaactcaa atccgtcaaa 3480

gaactcctcg gcatcaccat catggaaaga tcctccttcg aaaaaaaccc catcgacttc 3540

ctcgaagcca aaggctacaa agaagtcaaa aaagacctca tcatcaaact ccccaaatac 3600

tccctcttcg aactcgaaaa cggcagaaaa agaatgctcg cctccgccgg cgaactccaa 3660

aaaggcaacg aactcgccct cccctccaaa tacgtcaact tcctctacct cgcctcccac 3720

tacgaaaaac tcaaaggctc ccccgaagac aacgaacaaa aacaactctt cgtcgaacaa 3780

cacaaacact acctcgacga aatcatcgaa caaatctccg aattctccaa aagagtcatc 3840

ctcgccgacg ccaacctcga caaagtcctc tccgcctaca acaaacacag agacaaaccc 3900

atcagagaac aagccgaaaa catcatccac ctcttcaccc tcaccaacct cggcgccccc 3960

gccgccttca aatacttcga caccaccatc gacagaaaaa gatacacctc caccaaagaa 4020

gtcctcgacg ccaccctcat ccaccaatcc atcaccggcc tctacgaaac cagaatcgac 4080

ctctcccaac tcggcggcga cggcggcggc tcccccaaaa aaaaaagaaa agtc 4134

<210> 345

<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low C codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 345
gataagaagt atagtattgg attggatatt ggaacaaata gtgtgggatg ggctgtgatt 60

acagatgagt ataaggtgcc tagtaagaag tttaaggtgt tgggaaatac agatagacat 120

agtattaaga agaatttgat tggagctttg ttgtttgata gtggagagac agctgaggct 180

acaagattga agagaacagc tagaagaaga tatacaagaa gaaagaatag aatttgttat 240

ttgcaggaga tttttagtaa tgagatggct aaggtggatg atagtttttt tcatagattg 300

gaggagagtt ttttggtgga ggaggataag aagcatgaga gacatcctat ttttggaaat 360

attgtggatg aggtggctta tcatgagaag tatcctacaa tttatcattt gagaaagaag 420

ttggtggata gtacagataa ggctgatttg agattgattt atttggcttt ggctcatatg 480

attaagttta gaggacattt tttgattgag ggagatttga atcctgataa tagtgatgtg 540

gataagttgt ttattcagtt ggtgcagaca tataatcagt tgtttgagga gaatcctatt 600

aatgctagtg gagtggatgc taaggctatt ttgagtgcta gattgagtaa gagtagaaga 660

ttggagaatt tgattgctca gttgcctgga gagaagaaga atggattgtt tggaaatttg 720

attgctttga gtttgggatt gacacctaat tttaagagta attttgattt ggctgaggat 780

gctaagttgc agttgagtaa ggatacatat gatgatgatt tggataattt gttggctcag 840

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ttgagtgata ttttgagagt gaatacagag attacaaagg ctcctttgag tgctagtatg 960

attaagagat atgatgagca tcatcaggat ttgacattgt tgaaggcttt ggtgagacag 1020

cagttgcctg agaagtataa ggagattttt tttgatcaga gtaagaatgg atatgctgga 1080

tatattgatg gaggagctag tcaggaggag ttttataagt ttattaagcc tattttggag 1140

aagatggatg gaacagagga gttgttggtg aagttgaata gagaggattt gttgagaaag 1200

cagagaacat ttgataatgg aagtattcct catcagattc atttgggaga gttgcatgct 1260

attttgagaa gacaggagga tttttatcct tttttgaagg ataatagaga gaagattgag 1320

aagattttga catttagaat tccttattat gtgggacctt tggctagagg aaatagtaga 1380

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aatgagttga caaaggtgaa gtatgtgaca gagggaatga gaaagcctgc ttttttgagt 1620

ggagagcaga agaaggctat tgtggatttg ttgtttaaga caaatagaaa ggtgacagtg 1680

aagcagttga aggaggatta ttttaagaag attgagtgtt ttgatagtgt ggagattagt 1740

ggagtggagg atagatttaa tgctagtttg ggaacatatc atgatttgtt gaagattatt 1800

aaggataagg atttttgga taatgaggag aatgaggata ttttggagga tattgtgttg 1860

acattgacat tgtttgagga tagagagatg attgaggaga gattgaagac atatgctcat 1920

ttgtttgatg ataaggtgat gaagcagttg aagagaagaa gatatacagg atggggaaga 1980

ttgagtagaa agttgattaa tggaattaga gataagcaga gtggaaagac aattttggat 2040

tttttgaaga gtgatggatt tgctaataga aattttatgc agttgattca tgatgatagt 2100

ttgacattta aggaggatat tcagaaggct caggtgagtg gacagggaga tagtttgcat 2160

gagcatattg ctaatttggc tggaagtcct gctattaaga agggaatttt gcagacagtg 2220

aaggtggtgg atgagttggt gaaggtgatg ggaagacata agcctgagaa tattgtgatt 2280

gagatggcta gagagaatca gacaacacag aagggacaga agaatagtag agagagaatg 2340

aagagaattg aggagggaat taaggagttg ggaagtcaga ttttgaagga gcatcctgtg 2400

gagaatacac agttgcagaa tgagaagttg tatttgtatt atttgcagaa tggaagagat 2460

atgtatgtgg atcaggagtt ggatattaat agattgagtg attatgatgt ggatcatatt 2520

gtgcctcaga gttttttgaa ggatgatagt attgataata aggtgttgac aagaagtgat 2580

aagaatagag gaaagagtga taatgtgcct agtgaggagg tggtgaagaa gatgaagaat 2640

tattggagac agttgttgaa tgctaagttg attacacaga gaaagtttga taatttgaca 2700

aaggctgaga gaggaggatt gagtgagttg gataaggctg gatttattaa gagacagttg 2760

gtggagacaa gacagattac aaagcatgtg gctcagattt tggatagtag aatgaataca 2820

aagtatgatg agaatgataa gttgattaga gaggtgaagg tgattacatt gaagagtaag 2880

ttggtgagtg attttagaaa ggattttcag ttttataagg tgagagagat taataattat 2940

catcatgctc atgatgctta tttgaatgct gtggtgggaa cagctttgat taagaagtat 3000

cctaagttgg agagtgagtt tgtgtatgga gattataagg tgtatgatgt gagaaagatg 3060

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acagtgagaa aggtgttgag tatgcctcag gtgaatattg tgaagaagac agaggtgcag 3300

acaggaggat ttagtaagga gagtattttg cctaagagaa atagtgataa gttgattgct 3360

agaaagaagg attgggatcc taagaagtat ggaggatttg atagtcctac agtggcttat 3420

agtgtgttgg tggtggctaa ggtggagaag ggaaagagta agaagttgaa gagtgtgaag 3480

gagttgttgg gaattacaat tatggagaga agtagttttg agaagaatcc tattgatttt 3540

ttggaggcta agggatataa ggaggtgaag aaggatttga ttattaagtt gcctaagtat 3600

agtttgtttg agttggagaa tggaagaaag agaatgttgg ctagtgctgg agagttgcag 3660

aagggaaatg agttggcttt gcctagtaag tatgtgaatt ttttgtattt ggctagtcat 3720

tatgagaagt tgaagggaag tcctgaggat aatgagcaga agcagttgtt tgtggagcag 3780

cataagcatt atttggatga gattattgag cagattagtg agtttagtaa gagagtgatt 3840

ttggctgatg ctaatttgga taaggtgttg agtgcttata ataagcatag agataagcct 3900

attagagagc aggctgagaa tattattcat ttgtttacat tgacaaattt gggagctcct 3960

gctgctttta agtattttga tacaacaatt gatagaaaga gatatacaag tacaaaggag 4020

gtgttggatg ctacattgat tcatcagagt attacaggat tgtatgagac aagaattgat 4080

ttgagtcagt tgggaggaga tggaggagga agtcctaaga agaagagaaa ggtg 4134

<210> 346
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 346
gacaagaagt actccatcgg cctggacatc ggcaccaact ccgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc ctccaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

tccatcaaga agaacctgat cggcgccctg ctgttcgact ccggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttctccaa cgagatggcc aaggtggacg actccttctt ccaccggctg 300

gaggagtcct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggact ccaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa ctccgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgcctccg gcgtggacgc caaggccatc ctgtccgccc ggctgtccaa gtcccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctgt ccctgggcct gaccccaac ttcaagtcca acttcgacct ggccgaggac 780

gccaagctgc agctgtccaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgtccga cgccatcctg 900

ctgtccgaca tcctgcgggt gaacaccgag atcaccaagg ccccctgtc cgcctccatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccagt ccaagaacgg ctacgccggc 1080

tacatcgacg gcggcgcctc ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg ctccatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caactcccgg 1380

ttcgcctgga tgacccggaa gtccgaggag accatcaccc cctggaactt cgaggaggtg 1440

gtggacaagg gcgcctccgc ccagtccttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac tccctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgtcc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgactccgt ggagatctcc 1740

ggcgtggagg accggttcaa cgcctccctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgtcccgga agctgatcaa cggcatccgg gacaagcagt ccggcaagac catcctggac 2040

ttcctgaagt ccgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgactcc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgtccg gccagggcga ctccctgcac 2160

gagcacatcg ccaacctggc cggctccccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaactcccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggctcccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagtccaag 2880

ctggtgtccg acttccggaa ggacttccag ttctacaagg tgcgggagat caacaactac 2940

caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

cccaagctgg agtccgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg 3060

atcgccaagt ccgagcagga gatcggcaag gccaccgcca agtacttctt ctactccaac 3120

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ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgtc catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tctccaagga gtccatcctg cccaagcgga actccgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg actcccccac cgtggcctac 3420

tccgtgctgg tggtggccaa ggtggagaag ggcaagtcca agaagctgaa gtccgtgaag 3480

gagctgctgg gcatcaccat catggagcgg tcctccttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

tccctgttcg agctggagaa cggccggaag cggatgctgg cctccgccgg cgagctgcag 3660

aagggcaacg agctggccct gccctccaag tacgtgaact tcctgtacct ggcctcccac 3720

tacgagaagc tgaagggctc ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatctccg agttctccaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg tccgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacacctc caccaaggag 4020

gtgctggacg ccaccctgat ccaccagtcc atcaccggcc tgtacgagac ccggatcgac 4080

ctgtcccagc tgggcggcga cggcggcggc tcccccaaga agaagcggaa ggtg 4134

<210> 347
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A/U codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 347
gacaagaagt acagcatcgg cctggacatc ggcaccaaca gcgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc cagcaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttcagcaa cgagatggcc aaggtggacg acagcttctt ccaccggctg 300

gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa cagcgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgccagcg gcgtggacgc caaggccatc ctgagcgccc ggctgagcaa gagccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctga gcctgggcct gaccccaac ttcaagagca acttcgacct ggccgaggac 780

gccaagctgc agctgagcaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgagcga cgccatcctg 900

ctgagcgaca tcctgcgggt gaacaccgag atcaccaagg ccccctgag cgccagcatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccaga gcaagaacgg ctacgccggc 1080

tacatcgacg gcggcgccag ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg cagcatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caacagccgg 1380

ttcgcctgga tgacccggaa gagcgaggag accatcaccc cctggaactt cgaggaggtg 1440

gtggacaagg gcgccagcgc ccagagcttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac agcctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgagc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgacagcgt ggagatcagc 1740

ggcgtggagg accggttcaa cgccagcctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgagccgga agctgatcaa cggcatccgg gacaagcaga gcggcaagac catcctggac 2040

ttcctgaaga gcgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgacagc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgagcg gccagggcga cagcctgcac 2160

gagcacatcg ccaacctggc cggcagcccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaacagccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggcagccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

atgtacgtgg accaggagct ggacatcaac cggctgagcg actacgacgt ggaccacatc 2520

gtgccccaga gcttcctgaa ggacgacagc atcgacaaca aggtgctgac ccggagcgac 2580

aagaaccggg gcaagagcga caacgtgccc agcgaggagg tggtgaagaa gatgaagaac 2640

tactggcggc agctgctgaa cgccaagctg atcacccagc ggaagttcga caacctgacc 2700

aaggccgagc ggggcggcct gagcgagctg gacaaggccg gcttcatcaa gcggcagctg 2760

gtggagaccc ggcagatcac caagcacgtg gcccagatcc tggacagccg gatgaacacc 2820

aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagagcaag 2880

ctggtgagcg acttccggaa ggacttccag ttctacaagg tgcgggagat caacaactac 2940

caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

cccaagctgg agagcgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg 3060

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atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tcagcaagga gagcatcctg cccaagcgga acagcgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg acagccccac cgtggcctac 3420

agcgtgctgg tggtggccaa ggtggagaag ggcaagagca agaagctgaa gagcgtgaag 3480

gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacaccag caccaaggag 4020

gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080

ctgagccagc tgggcggcga cggcggcggc agccccaaga agaagcggaa ggtg 4134

<210> 348
<211> 4173
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A codons of Table 4, with two
C-terminal NLS sequences (no start or stop codons; suitable for
inclusion in fusion protein coding sequence)

<400> 348
gacaagaagt actccatcgg cctggacatc ggcaccaact ccgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc ctccaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

tccatcaaga agaacctgat cggcgccctg ctgttcgact ccggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttctccaa cgagatggcc aaggtggacg actccttctt ccaccggctg 300

gaggagtcct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggact ccaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa ctccgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgcctccg gcgtggacgc caaggccatc ctgtccgccc ggctgtccaa gtcccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctgt ccctgggcct gacccccaac ttcaagtcca acttcgacct ggccgaggac 780

gccaagctgc agctgtccaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgtccga cgccatcctg 900

ctgtccgaca tcctgcgggt gaacaccgag atcaccaagg ccccccctgtc cgcctccatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccagt ccaagaacgg ctacgccggc 1080

tacatcgacg gcggcgcctc ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg ctccatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caactcccgg 1380

ttcgcctgga tgacccggaa gtccgaggag accatcaccc cctggaactt cgaggaggtg 1440

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ctgcccaacg agaaggtgct gcccaagcac tccctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgtcc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgactccgt ggagatctcc 1740

ggcgtggagg accggttcaa cgcctccctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgtcccgga agctgatcaa cggcatccgg gacaagcagt ccggcaagac catcctggac 2040

ttcctgaagt ccgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgactcc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgtccg gccagggcga ctccctgcac 2160

gagcacatcg ccaacctggc cggctccccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaactcccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggctcccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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tactggcggc agctgctgaa cgccaagctg atcacccagc ggaagttcga caacctgacc 2700

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aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagtccaag 2880

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caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

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atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgtc catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tctccaagga gtccatcctg cccaagcgga actccgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg actcccccac cgtggcctac 3420

tccgtgctgg tggtggccaa ggtggagaag ggcaagtcca agaagctgaa gtccgtgaag 3480

gagctgctgg gcatcaccat catggagcgg tcctccttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

tccctgttcg agctggagaa cggccggaag cggatgctgg cctccgccgg cgagctgcag 3660

aagggcaacg agctggccct gccctccaag tacgtgaact tcctgtacct ggcctcccac 3720

tacgagaagc tgaagggctc ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatctccg agttctccaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg tccgcctaca acaagcaccg ggacaagccc 3900

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gtgctggacg ccaccctgat ccaccagtcc atcaccggcc tgtacgagac ccggatcgac 4080

ctgtcccagc tgggcggcga cggctccggc tcccccaaga agaagcggaa ggtggacggc 4140

tcccccaaga agaagcggaa ggtggactcc ggc 4173

<210> 349
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 349
gacaagaagt actccatcgg cctggccatc ggcaccaact ccgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc ctccaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

tccatcaaga agaacctgat cggcgccctg ctgttcgact ccggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttctccaa cgagatggcc aaggtggacg actccttctt ccaccggctg 300

```
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atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag      420
ctggtggact ccaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg      480
atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa ctccgacgtg      540
gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc      600
aacgcctccg gcgtggacgc caaggccatc ctgtccgccc ggctgtccaa gtcccggcgg      660
ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg      720
atcgccctgt ccctgggcct gacccccaac ttcaagtcca acttcgacct ggccgaggac      780
gccaagctgc agctgtccaa ggacacctac gacgacgacc tggacaacct gctggcccag      840
atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgtccga cgccatcctg      900
ctgtccgaca tcctgcgggt gaacaccgag atcaccaagg ccccccctgtc cgcctccatg      960
atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag     1020
cagctgcccg agaagtacaa ggagatcttc ttcgaccagt ccaagaacgg ctacgccggc     1080
tacatcgacg gcggcgcctc ccaggaggag ttctacaagt tcatcaagcc catcctggag     1140
aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag     1200
cagcggacct tcgacaacgg ctccatcccc caccagatcc acctgggcga gctgcacgcc     1260
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ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg     1680
aagcagctga aggaggacta cttcaagaag atcgagtgct tcgactccgt ggagatctcc     1740
ggcgtggagg accggttcaa cgcctccctg ggcacctacc acgacctgct gaagatcatc     1800
aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg     1860
accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac     1920
ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg     1980
ctgtcccgga agctgatcaa cggcatccgg gacaagcagt ccggcaagac catcctggac     2040
ttcctgaagt ccgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgactcc     2100
ctgaccttca aggaggacat ccagaaggcc caggtgtccg gccagggcga ctccctgcac     2160
gagcacatcg ccaacctggc cggctccccc gccatcaaga agggcatcct gcagaccgtg     2220
aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc     2280
gagatggccc gggagaacca gaccacccag aagggccaga agaactcccg ggagcggatg     2340
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aagcggatcg aggagggcat caaggagctg ggctcccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

atgtacgtgg accaggagct ggacatcaac cggctgtccg actacgacgt ggaccacatc 2520

gtgccccagt ccttcctgaa ggacgactcc atcgacaaca aggtgctgac ccggtccgac 2580

aagaaccggg gcaagtccga caacgtgccc tccgaggagg tggtgaagaa gatgaagaac 2640

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ctggtgtccg acttccggaa ggacttccag ttctacaagg tgcgggagat caacaactac 2940

caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

cccaagctgg agtccgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg 3060

atcgccaagt ccgagcagga gatcggcaag gccaccgcca agtacttctt ctactccaac 3120

atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgtc catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tctccaagga gtccatcctg cccaagcgga actccgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg actcccccac cgtggcctac 3420

tccgtgctgg tggtggccaa ggtggagaag ggcaagtcca agaagctgaa gtccgtgaag 3480

gagctgctgg gcatcaccat catggagcgg tcctccttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

tccctgttcg agctggagaa cggccggaag cggatgctgg cctccgccgg cgagctgcag 3660

aagggcaacg agctggccct gccctccaag tacgtgaact tcctgtacct ggcctcccac 3720

tacgagaagc tgaagggctc ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatctccg agttctccaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg tccgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacacctc caccaaggag 4020

gtgctggacg ccaccctgat ccaccagtcc atcaccggcc tgtacgagac ccggatcgac 4080

ctgtcccagc tgggcggcga cggcggcggc tcccccaaga agaagcggaa ggtg 4134

<210> 350
<211> 4101
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion

protein coding sequence)

<400> 350
gacaagaagt actccatcgg cctggccatc ggcaccaact ccgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc ctccaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

tccatcaaga agaacctgat cggcgccctg ctgttcgact ccggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttctccaa cgagatggcc aaggtggacg actccttctt ccaccggctg 300

gaggagtcct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggact ccaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa ctccgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgcctccg gcgtggacgc caaggccatc ctgtccgccc ggctgtccaa gtcccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctgt ccctgggcct gacccccaac ttcaagtcca acttcgacct ggccgaggac 780

gccaagctgc agctgtccaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgtccga cgccatcctg 900

ctgtccgaca tcctgcgggt gaacaccgag atcaccaagg ccccccctgtc cgcctccatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccagt ccaagaacgg ctacgccggc 1080

tacatcgacg gcggcgcctc ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg ctccatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caactcccgg 1380

ttcgcctgga tgacccggaa gtccgaggag accatcaccc cctggaactt cgaggaggtg 1440

gtggacaagg gcgcctccgc ccagtccttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac tccctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgtcc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgactccgt ggagatctcc 1740

ggcgtggagg accggttcaa cgcctccctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgtcccgga agctgatcaa cggcatccgg gacaagcagt ccggcaagac catcctggac 2040

ttcctgaagt ccgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgactcc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgtccg gccagggcga ctccctgcac 2160

gagcacatcg ccaacctggc cggctccccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaactcccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggctcccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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tactggcggc agctgctgaa cgccaagctg atcacccagc ggaagttcga caacctgacc 2700

aaggccgagc ggggcggcct gtccgagctg gacaaggccg gcttcatcaa gcggcagctg 2760

gtggagaccc ggcagatcac caagcacgtg gcccagatcc tggactcccg gatgaacacc 2820

aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagtccaag 2880

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caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

cccaagctgg agtccgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg 3060

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atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgtc catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tctccaagga gtccatcctg cccaagcgga actccgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg actcccccac cgtggcctac 3420

tccgtgctgg tggtggccaa ggtggagaag ggcaagtcca agaagctgaa gtccgtgaag 3480

gagctgctgg gcatcaccat catggagcgg tcctccttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

tccctgttcg agctggagaa cggccggaag cggatgctgg cctccgccgg cgagctgcag 3660

aagggcaacg agctggccct gccctccaag tacgtgaact tcctgtacct ggcctcccac 3720

tacgagaagc tgaagggctc ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatctccg agttctccaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg tccgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacacctc caccaaggag 4020

gtgctggacg ccaccctgat ccaccagtcc atcaccggcc tgtacgagac ccggatcgac 4080

ctgtcccagc tgggcggcga c 4101

<210> 351
<211> 4173
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 351
gacaagaagt actccatcgg cctggccatc ggcaccaact ccgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc ctccaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

tccatcaaga agaacctgat cggcgccctg ctgttcgact ccggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttctccaa cgagatggcc aaggtggacg actccttctt ccaccggctg 300

gaggagtcct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggact ccaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa ctccgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgcctccg gcgtggacgc caaggccatc ctgtccgccc ggctgtccaa gtcccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctgt ccctgggcct gaccccaac ttcaagtcca acttcgacct ggccgaggac 780

gccaagctgc agctgtccaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgtccga cgccatcctg 900

ctgtccgaca tcctgcgggt gaacaccgag atcaccaagg ccccctgtc cgcctccatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccagt ccaagaacgg ctacgccggc 1080

tacatcgacg gcggcgcctc ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg ctccatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caactcccgg 1380

ttcgcctgga tgacccggaa gtccgaggag accatcaccc cctggaactt cgaggaggtg 1440

gtggacaagg gcgcctccgc ccagtccttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac tccctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgtcc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgactccgt ggagatctcc 1740

ggcgtggagg accggttcaa cgcctccctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgtcccgga agctgatcaa cggcatccgg gacaagcagt ccggcaagac catcctggac 2040

ttcctgaagt ccgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgactcc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgtccg gccagggcga ctccctgcac 2160

gagcacatcg ccaacctggc cggctccccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaactcccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggctcccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgtc catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tctccaagga gtccatcctg cccaagcgga actccgacaa gctgatcgcc 3360

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tccgtgctgg tggtggccaa ggtggagaag ggcaagtcca agaagctgaa gtccgtgaag 3480

gagctgctgg gcatcaccat catggagcgg tcctccttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

tccctgttcg agctggagaa cggccggaag cggatgctgg cctccgccgg cgagctgcag 3660

aagggcaacg agctggccct gccctccaag tacgtgaact tcctgtacct ggcctcccac 3720

tacgagaagc tgaagggctc ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatctccg agttctccaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg tccgcctaca acaagcaccg ggacaagccc 3900

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gccgccttca agtacttcga caccaccatc gaccggaagc ggtacacctc caccaaggag 4020

gtgctggacg ccaccctgat ccaccagtcc atcaccggcc tgtacgagac ccggatcgac 4080

ctgtcccagc tgggcggcga cggctccggc tcccccaaga agaagcggaa ggtggacggc 4140

tcccccaaga agaagcggaa ggtggactcc ggc 4173

<210> 352
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 352
gacaagaagt actccatcgg cctggccatc ggcaccaact ccgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc ctccaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

tccatcaaga agaacctgat cggcgccctg ctgttcgact ccggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttctccaa cgagatggcc aaggtggacg actccttctt ccaccggctg 300

gaggagtcct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggact ccaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa ctccgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgcctccg gcgtggacgc caaggccatc ctgtccgccc ggctgtccaa gtcccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctgt ccctgggcct gaccccaac ttcaagtcca acttcgacct ggccgaggac 780

gccaagctgc agctgtccaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgtccga cgccatcctg 900

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cagctgcccg agaagtacaa ggagatcttc ttcgaccagt ccaagaacgg ctacgccggc 1080

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aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

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cagcggacct tcgacaacgg ctccatcccc caccagatcc acctgggcga gctgcacgcc     1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag     1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caactcccgg     1380

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ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg     1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgactccgt ggagatctcc     1740

ggcgtggagg accggttcaa cgcctccctg ggcacctacc acgacctgct gaagatcatc     1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg     1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac     1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg     1980

ctgtcccgga agctgatcaa cggcatccgg gacaagcagt ccggcaagac catcctggac     2040

ttcctgaagt ccgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgactcc     2100

ctgaccttca aggaggacat ccagaaggcc caggtgtccg gccagggcga ctccctgcac     2160

gagcacatcg ccaacctggc cggctccccc gccatcaaga agggcatcct gcagaccgtg     2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc     2280

gagatggccc gggagaacca gaccacccag aagggccaga agaactcccg ggagcggatg     2340

aagcggatcg aggagggcat caaggagctg ggctcccaga tcctgaagga gcaccccgtg     2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac     2460

atgtacgtgg accaggagct ggacatcaac cggctgtccg actacgacgt ggacgccatc     2520

gtgccccagt ccttcctgaa ggacgactcc atcgacaaca aggtgctgac ccggtccgac     2580

aagaaccggg gcaagtccga caacgtgccc tccgaggagg tggtgaagaa gatgaagaac     2640

tactggcggc agctgctgaa cgccaagctg atcacccagc ggaagttcga caacctgacc     2700

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ctggtgtccg acttccggaa ggacttccag ttctacaagg tgcgggagat caacaactac     2940

caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac     3000

cccaagctgg agtccgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg     3060

atcgccaagt ccgagcagga gatcggcaag gccaccgcca agtacttctt ctactccaac     3120

atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc     3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc     3240

accgtgcgga aggtgctgtc catgccccag gtgaacatcg tgaagaagac cgaggtgcag     3300
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accggcggct tctccaagga gtccatcctg cccaagcgga actccgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg actcccccac cgtggcctac 3420

tccgtgctgg tggtggccaa ggtggagaag ggcaagtcca agaagctgaa gtccgtgaag 3480

gagctgctgg gcatcaccat catggagcgg tcctccttcg agaagaaccc catcgacttc 3540

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tacgagaagc tgaagggctc ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatctccg agttctccaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg tccgcctaca acaagcaccg ggacaagccc 3900

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gtgctggacg ccaccctgat ccaccagtcc atcaccggcc tgtacgagac ccggatcgac 4080

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<210> 353
<211> 4101
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 353
gacaagaagt actccatcgg cctggccatc ggcaccaact ccgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc ctccaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

tccatcaaga agaacctgat cggcgccctg ctgttcgact ccggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttctccaa cgagatggcc aaggtggacg actccttctt ccaccggctg 300

gaggagtcct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggact ccaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa ctccgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgcctccg gcgtggacgc caaggccatc ctgtccgccc ggctgtccaa gtcccggcgg 660

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ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

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gtgctggacg ccaccctgat ccaccagtcc atcaccggcc tgtacgagac ccggatcgac 4080

ctgtcccagc tgggcggcga c 4101

<210> 354
<211> 4173
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 354
gacaagaagt actccatcgg cctggccatc ggcaccaact ccgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc ctccaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

tccatcaaga agaacctgat cggcgccctg ctgttcgact ccggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttctccaa cgagatggcc aaggtggacg actccttctt ccaccggctg 300

gaggagtcct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggact ccaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa ctccgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgcctccg gcgtggacgc caaggccatc ctgtccgccc ggctgtccaa gtcccggcgg 660

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tacatcgacg gcggcgcctc ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg ctccatcccc caccagatcc acctgggcga gctgcacgcc 1260

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gagcacatcg ccaacctggc cggctccccc gccatcaaga agggcatcct gcagaccgtg 2220

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gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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gtgctggacg ccaccctgat ccaccagtcc atcaccggcc tgtacgagac ccggatcgac 4080

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tcccccaaga agaagcggaa ggtggactcc ggc 4173

<210> 355
<211> 4173
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 355
gacaagaagt acagcatcgg cctggacatc ggcaccaaca gcgtgggctg ggccgtgatc 60

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agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc      180
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ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg      480
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gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc      600
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gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tcagcaagga gagcatcctg cccaagcgga acagcgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg acagccccac cgtggcctac 3420

agcgtgctgg tggtggccaa ggtggagaag ggcaagagca agaagctgaa gagcgtgaag 3480

gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacaccag caccaaggag 4020

gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080

ctgagccagc tgggcggcga cggcagcggc agccccaaga agaagcggaa ggtggacggc 4140

agccccaaga agaagcggaa ggtggacagc ggc 4173

<210> 356
<211> 4101
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 ORF using low A/U codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 356
gacaagaagt acagcatcgg cctggacatc ggcaccaaca gcgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc cagcaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttcagcaa cgagatggcc aaggtggacg acagcttctt ccaccggctg 300

gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa cagcgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgccagcg gcgtggacgc caaggccatc ctgagcgccc ggctgagcaa gagccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctga gcctgggcct gaccccaac ttcaagagca acttcgacct ggccgaggac 780

gccaagctgc agctgagcaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgagcga cgccatcctg 900

ctgagcgaca tcctgcgggt gaacaccgag atcaccaagg ccccccctgag cgccagcatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccaga gcaagaacgg ctacgccggc 1080

tacatcgacg gcggcgccag ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg cagcatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caacagccgg 1380

ttcgcctgga tgacccggaa gagcgaggag accatcaccc cctggaactt cgaggaggtg 1440

gtggacaagg gcgccagcgc ccagagcttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac agcctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgagc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgacagcgt ggagatcagc 1740

ggcgtggagg accggttcaa cgccagcctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgagccgga agctgatcaa cggcatccgg gacaagcaga gcggcaagac catcctggac 2040

ttcctgaaga gcgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgacagc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgagcg gccagggcga cagcctgcac 2160

gagcacatcg ccaacctggc cggcagcccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaacagccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggcagccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

atgtacgtgg accaggagct ggacatcaac cggctgagcg actacgacgt ggaccacatc 2520

gtgccccaga gcttcctgaa ggacgacagc atcgacaaca aggtgctgac ccggagcgac 2580

aagaaccggg gcaagagcga caacgtgccc agcgaggagg tggtgaagaa gatgaagaac 2640

tactggcggc agctgctgaa cgccaagctg atcacccagc ggaagttcga caacctgacc 2700

aaggccgagc ggggcggcct gagcgagctg gacaaggccg gcttcatcaa gcggcagctg 2760

gtggagaccc ggcagatcac caagcacgtg gcccagatcc tggacagccg gatgaacacc 2820

aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagagcaag 2880

ctggtgagcg acttccggaa ggacttccag ttctacaagg tgcgggagat caacaactac 2940

caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

cccaagctgg agagcgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg 3060

atcgccaaga gcgagcagga gatcggcaag gccaccgcca agtacttctt ctacagcaac 3120

atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tcagcaagga gagcatcctg cccaagcgga acagcgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg acagccccac cgtggcctac 3420

agcgtgctgg tggtggccaa ggtggagaag ggcaagagca agaagctgaa gagcgtgaag 3480

gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacaccag caccaaggag 4020

gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080

ctgagccagc tgggcggcga c 4101

<210> 357
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A/U codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 357
gacaagaagt acagcatcgg cctggccatc ggcaccaaca gcgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc cagcaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttcagcaa cgagatggcc aaggtggacg acagcttctt ccaccggctg 300

gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa cagcgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgccagcg gcgtggacgc caaggccatc ctgagcgccc ggctgagcaa gagccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctga gcctgggcct gaccccaac ttcaagagca acttcgacct ggccgaggac 780

gccaagctgc agctgagcaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgagcga cgccatcctg 900

ctgagcgaca tcctgcgggt gaacaccgag atcaccaagg ccccccctgag cgccagcatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccaga gcaagaacgg ctacgccggc 1080

tacatcgacg gcggcgccag ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg cagcatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caacagccgg 1380

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gtggacaagg gcgccagcgc ccagagcttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac agcctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgagc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgacagcgt ggagatcagc 1740

ggcgtggagg accggttcaa cgccagcctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgagccgga agctgatcaa cggcatccgg gacaagcaga gcggcaagac catcctggac 2040

ttcctgaaga gcgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgacagc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgagcg gccagggcga cagcctgcac 2160

gagcacatcg ccaacctggc cggcagcccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaacagccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggcagccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

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gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacaccag caccaaggag 4020

gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080

ctgagccagc tgggcggcga cggcggcggc agccccaaga agaagcggaa ggtg 4134

<210> 358
<211> 4173
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A/U codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 358
gacaagaagt acagcatcgg cctggccatc ggcaccaaca gcgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc cagcaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttcagcaa cgagatggcc aaggtggacg acagcttctt ccaccggctg 300

gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa cagcgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgccagcg gcgtggacgc caaggccatc ctgagcgccc ggctgagcaa gagccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctga gcctgggcct gaccccaac ttcaagagca acttcgacct ggccgaggac 780

gccaagctgc agctgagcaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgagcga cgccatcctg 900

ctgagcgaca tcctgcgggt gaacaccgag atcaccaagg ccccctgag cgccagcatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccaga gcaagaacgg ctacgccggc 1080

tacatcgacg gcggcgccag ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg cagcatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caacagccgg 1380

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aagcagctga aggaggacta cttcaagaag atcgagtgct tcgacagcgt ggagatcagc 1740

ggcgtggagg accggttcaa cgccagcctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgagccgga agctgatcaa cggcatccgg gacaagcaga gcggcaagac catcctggac 2040

ttcctgaaga gcgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgacagc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgagcg gccagggcga cagcctgcac 2160

gagcacatcg ccaacctggc cggcagcccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaacagccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggcagccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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tactggcggc agctgctgaa cgccaagctg atcacccagc ggaagttcga caacctgacc 2700

aaggccgagc ggggcggcct gagcgagctg gacaaggccg gcttcatcaa gcggcagctg 2760

gtggagaccc ggcagatcac caagcacgtg gcccagatcc tggacagccg gatgaacacc 2820

aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagagcaag 2880

ctggtgagcg acttccggaa ggacttccag ttctacaagg tgcgggagat caacaactac 2940

caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

cccaagctgg agagcgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg 3060

atcgccaaga gcgagcagga gatcggcaag gccaccgcca agtacttctt ctacagcaac 3120

atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

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cggaagaagg actgggaccc caagaagtac ggcggcttcg acagccccac cgtggcctac 3420

agcgtgctgg tggtggccaa ggtggagaag ggcaagagca agaagctgaa gagcgtgaag 3480

gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacaccag caccaaggag 4020

gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080

ctgagccagc tgggcggcga cggcagcggc agccccaaga agaagcggaa ggtggacggc 4140

agccccaaga agaagcggaa ggtggacagc ggc 4173

<210> 359
<211> 4101
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Cas9 nickase ORF using low A/U codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 359
gacaagaagt acagcatcgg cctggccatc ggcaccaaca gcgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc cagcaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttcagcaa cgagatggcc aaggtggacg acagcttctt ccaccggctg 300

gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa cagcgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

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aacgccagcg gcgtggacgc caaggccatc ctgagcgccc ggctgagcaa gagccggcgg      660
ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg      720
atcgccctga gcctgggcct gacccccaac ttcaagagca acttcgacct ggccgaggac      780
gccaagctgc agctgagcaa ggacacctac gacgacgacc tggacaacct gctggcccag      840
atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgagcga cgccatcctg      900
ctgagcgaca tcctgcgggt gaacaccgag atcaccaagg ccccccctgag cgccagcatg      960
atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag     1020
cagctgcccg agaagtacaa ggagatcttc ttcgaccaga gcaagaacgg ctacgccggc     1080
tacatcgacg gcggcgccag ccaggaggag ttctacaagt tcatcaagcc catcctggag     1140
aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag     1200
cagcggacct tcgacaacgg cagcatcccc caccagatcc acctgggcga gctgcacgcc     1260
atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag     1320
aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caacagccgg     1380
ttcgcctgga tgacccggaa gagcgaggag accatcaccc cctggaactt cgaggaggtg     1440
gtggacaagg gcgccagcgc ccagagcttc atcgagcgga tgaccaactt cgacaagaac     1500
ctgcccaacg agaaggtgct gcccaagcac agcctgctgt acgagtactt caccgtgtac     1560
aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgagc     1620
ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg     1680
aagcagctga aggaggacta cttcaagaag atcgagtgct tcgacagcgt ggagatcagc     1740
ggcgtggagg accggttcaa cgccagcctg ggcacctacc acgacctgct gaagatcatc     1800
aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg     1860
accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac     1920
ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg     1980
ctgagccgga agctgatcaa cggcatccgg gacaagcaga gcggcaagac catcctggac     2040
ttcctgaaga gcgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgacagc     2100
ctgaccttca aggaggacat ccagaaggcc caggtgagcg gccagggcga cagcctgcac     2160
gagcacatcg ccaacctggc cggcagcccc gccatcaaga agggcatcct gcagaccgtg     2220
aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc     2280
gagatggccc gggagaacca gaccacccag aagggccaga agaacagccg ggagcggatg     2340
aagcggatcg aggagggcat caaggagctg ggcagccaga tcctgaagga gcaccccgtg     2400
gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac     2460
atgtacgtgg accaggagct ggacatcaac cggctgagcg actacgacgt ggaccacatc     2520
gtgccccaga gcttcctgaa ggacgacagc atcgacaaca aggtgctgac ccggagcgac     2580
aagaaccggg gcaagagcga caacgtgccc agcgaggagg tggtgaagaa gatgaagaac     2640
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tactggcggc agctgctgaa cgccaagctg atcacccagc ggaagttcga caacctgacc 2700

aaggccgagc ggggcggcct gagcgagctg gacaaggccg gcttcatcaa gcggcagctg 2760

gtggagaccc ggcagatcac caagcacgtg gcccagatcc tggacagccg gatgaacacc 2820

aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagagcaag 2880

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caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

cccaagctgg agagcgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg 3060

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atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tcagcaagga gagcatcctg cccaagcgga acagcgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg acagccccac cgtggcctac 3420

agcgtgctgg tggtggccaa ggtggagaag ggcaagagca agaagctgaa gagcgtgaag 3480

gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacaccag caccaaggag 4020

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ctgagccagc tgggcggcga c 4101

<210> 360
<211> 4134
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A/U codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 360
gacaagaagt acagcatcgg cctggccatc ggcaccaaca gcgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc cagcaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttcagcaa cgagatggcc aaggtggacg acagcttctt ccaccggctg 300

gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa cagcgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgccagcg gcgtggacgc caaggccatc ctgagcgccc ggctgagcaa gagccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctga gcctgggcct gacccccaac ttcaagagca acttcgacct ggccgaggac 780

gccaagctgc agctgagcaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgagcga cgccatcctg 900

ctgagcgaca tcctgcgggt gaacaccgag atcaccaagg ccccctgag cgccagcatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccaga gcaagaacgg ctacgccggc 1080

tacatcgacg gcggcgccag ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg cagcatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caacagccgg 1380

ttcgcctgga tgacccggaa gagcgaggag accatcaccc cctggaactt cgaggaggtg 1440

gtggacaagg gcgccagcgc ccagagcttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac agcctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgagc 1620

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aagcagctga aggaggacta cttcaagaag atcgagtgct tcgacagcgt ggagatcagc 1740

ggcgtggagg accggttcaa cgccagcctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgagccgga agctgatcaa cggcatccgg gacaagcaga gcggcaagac catcctggac 2040

ttcctgaaga gcgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgacagc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgagcg gccagggcga cagcctgcac 2160

gagcacatcg ccaacctggc cggcagcccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaacagccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggcagccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

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gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

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gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080

ctgagccagc tgggcggcga cggcggcggc agccccaaga agaagcggaa ggtg 4134

<210> 361
<211> 4173
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic: dCas9 ORF using low A/U codons of Table 4, with two C-terminal NLS sequences (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 361

gacaagaagt acagcatcgg cctggccatc ggcaccaaca gcgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc cagcaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttcagcaa cgagatggcc aaggtggacg acagcttctt ccaccggctg 300

gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa cagcgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgccagcg gcgtggacgc caaggccatc ctgagcgccc ggctgagcaa gagccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctga gcctgggcct gaccccaac ttcaagagca acttcgacct ggccgaggac 780

gccaagctgc agctgagcaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgagcga cgccatcctg 900

ctgagcgaca tcctgcgggt gaacaccgag atcaccaagg ccccccctgag cgccagcatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccaga gcaagaacgg ctacgccggc 1080

tacatcgacg gcggcgccag ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg cagcatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caacagccgg 1380

ttcgcctgga tgacccggaa gagcgaggag accatcaccc cctggaactt cgaggaggtg 1440

gtggacaagg gcgccagcgc ccagagcttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac agcctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgagc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgacagcgt ggagatcagc 1740

ggcgtggagg accggttcaa cgccagcctg ggcacctacc acgacctgct gaagatcatc 1800

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accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgagccgga agctgatcaa cggcatccgg gacaagcaga gcggcaagac catcctggac 2040

ttcctgaaga gcgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgacagc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgagcg gccagggcga cagcctgcac 2160

gagcacatcg ccaacctggc cggcagcccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaacagccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggcagccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

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aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagagcaag 2880

ctggtgagcg acttccggaa ggacttccag ttctacaagg tgcgggagat caacaactac 2940

caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

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ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tcagcaagga gagcatcctg cccaagcgga acagcgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg acagccccac cgtggcctac 3420

agcgtgctgg tggtggccaa ggtggagaag ggcaagagca agaagctgaa gagcgtgaag 3480

gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacaccag caccaaggag 4020

gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080

ctgagccagc tgggcggcga cggcagcggc agccccaaga agaagcggaa ggtggacggc 4140

agccccaaga agaagcggaa ggtggacagc ggc 4173

<210> 362
<211> 4101
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: dCas9 ORF using low A/U codons of Table 4 (no NLS and no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 362
gacaagaagt acagcatcgg cctggccatc ggcaccaaca gcgtgggctg ggccgtgatc 60

accgacgagt acaaggtgcc cagcaagaag ttcaaggtgc tgggcaacac cgaccggcac 120

agcatcaaga agaacctgat cggcgccctg ctgttcgaca gcggcgagac cgccgaggcc 180

acccggctga agcggaccgc ccggcggcgg tacacccggc ggaagaaccg gatctgctac 240

ctgcaggaga tcttcagcaa cgagatggcc aaggtggacg acagcttctt ccaccggctg 300

gaggagagct tcctggtgga ggaggacaag aagcacgagc ggcaccccat cttcggcaac 360

atcgtggacg aggtggccta ccacgagaag taccccacca tctaccacct gcggaagaag 420

ctggtggaca gcaccgacaa ggccgacctg cggctgatct acctggccct ggcccacatg 480

atcaagttcc ggggccactt cctgatcgag ggcgacctga accccgacaa cagcgacgtg 540

gacaagctgt tcatccagct ggtgcagacc tacaaccagc tgttcgagga gaaccccatc 600

aacgccagcg gcgtggacgc caaggccatc ctgagcgccc ggctgagcaa gagccggcgg 660

ctggagaacc tgatcgccca gctgcccggc gagaagaaga acggcctgtt cggcaacctg 720

atcgccctga gcctgggcct gaccccaac ttcaagagca acttcgacct ggccgaggac 780

gccaagctgc agctgagcaa ggacacctac gacgacgacc tggacaacct gctggcccag 840

atcggcgacc agtacgccga cctgttcctg gccgccaaga acctgagcga cgccatcctg 900

ctgagcgaca tcctgcgggt gaacaccgag atcaccaagg ccccctgag cgccagcatg 960

atcaagcggt acgacgagca ccaccaggac ctgaccctgc tgaaggccct ggtgcggcag 1020

cagctgcccg agaagtacaa ggagatcttc ttcgaccaga gcaagaacgg ctacgccggc 1080

tacatcgacg gcggcgccag ccaggaggag ttctacaagt tcatcaagcc catcctggag 1140

aagatggacg gcaccgagga gctgctggtg aagctgaacc gggaggacct gctgcggaag 1200

cagcggacct tcgacaacgg cagcatcccc caccagatcc acctgggcga gctgcacgcc 1260

atcctgcggc ggcaggagga cttctacccc ttcctgaagg acaaccggga gaagatcgag 1320

aagatcctga ccttccggat cccctactac gtgggccccc tggcccgggg caacagccgg 1380

ttcgcctgga tgacccggaa gagcgaggag accatcaccc cctggaactt cgaggaggtg 1440

gtggacaagg gcgccagcgc ccagagcttc atcgagcgga tgaccaactt cgacaagaac 1500

ctgcccaacg agaaggtgct gcccaagcac agcctgctgt acgagtactt caccgtgtac 1560

aacgagctga ccaaggtgaa gtacgtgacc gagggcatgc ggaagcccgc cttcctgagc 1620

ggcgagcaga agaaggccat cgtggacctg ctgttcaaga ccaaccggaa ggtgaccgtg 1680

aagcagctga aggaggacta cttcaagaag atcgagtgct tcgacagcgt ggagatcagc 1740

ggcgtggagg accggttcaa cgccagcctg ggcacctacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaggag aacgaggaca tcctggagga catcgtgctg 1860

accctgaccc tgttcgagga ccgggagatg atcgaggagc ggctgaagac ctacgcccac 1920

ctgttcgacg acaaggtgat gaagcagctg aagcggcggc ggtacaccgg ctggggccgg 1980

ctgagccgga agctgatcaa cggcatccgg gacaagcaga gcggcaagac catcctggac 2040

ttcctgaaga gcgacggctt cgccaaccgg aacttcatgc agctgatcca cgacgacagc 2100

ctgaccttca aggaggacat ccagaaggcc caggtgagcg gccagggcga cagcctgcac 2160

gagcacatcg ccaacctggc cggcagcccc gccatcaaga agggcatcct gcagaccgtg 2220

aaggtggtgg acgagctggt gaaggtgatg ggccggcaca agcccgagaa catcgtgatc 2280

gagatggccc gggagaacca gaccacccag aagggccaga agaacagccg ggagcggatg 2340

aagcggatcg aggagggcat caaggagctg ggcagccaga tcctgaagga gcaccccgtg 2400

gagaacaccc agctgcagaa cgagaagctg tacctgtact acctgcagaa cggccgggac 2460

atgtacgtgg accaggagct ggacatcaac cggctgagcg actacgacgt ggacgccatc 2520

gtgccccaga gcttcctgaa ggacgacagc atcgacaaca aggtgctgac ccggagcgac 2580

aagaaccggg gcaagagcga caacgtgccc agcgaggagg tggtgaagaa gatgaagaac 2640

tactggcggc agctgctgaa cgccaagctg atcacccagc ggaagttcga caacctgacc 2700

aaggccgagc ggggcggcct gagcgagctg gacaaggccg gcttcatcaa gcggcagctg 2760

gtggagaccc ggcagatcac caagcacgtg gcccagatcc tggacagccg gatgaacacc 2820

aagtacgacg agaacgacaa gctgatccgg gaggtgaagg tgatcaccct gaagagcaag 2880

ctggtgagcg acttccggaa ggacttccag ttctacaagg tgcgggagat caacaactac 2940

caccacgccc acgacgccta cctgaacgcc gtggtgggca ccgccctgat caagaagtac 3000

cccaagctgg agagcgagtt cgtgtacggc gactacaagg tgtacgacgt gcggaagatg 3060

atcgccaaga gcgagcagga gatcggcaag gccaccgcca agtacttctt ctacagcaac 3120

atcatgaact tcttcaagac cgagatcacc ctggccaacg gcgagatccg gaagcggccc 3180

ctgatcgaga ccaacggcga gaccggcgag atcgtgtggg acaagggccg ggacttcgcc 3240

accgtgcgga aggtgctgag catgccccag gtgaacatcg tgaagaagac cgaggtgcag 3300

accggcggct tcagcaagga gagcatcctg cccaagcgga acagcgacaa gctgatcgcc 3360

cggaagaagg actgggaccc caagaagtac ggcggcttcg acagccccac cgtggcctac 3420

agcgtgctgg tggtggccaa ggtggagaag ggcaagagca agaagctgaa gagcgtgaag 3480

gagctgctgg gcatcaccat catggagcgg agcagcttcg agaagaaccc catcgacttc 3540

ctggaggcca agggctacaa ggaggtgaag aaggacctga tcatcaagct gcccaagtac 3600

agcctgttcg agctggagaa cggccggaag cggatgctgg ccagcgccgg cgagctgcag 3660

aagggcaacg agctggccct gcccagcaag tacgtgaact tcctgtacct ggccagccac 3720

tacgagaagc tgaagggcag ccccgaggac aacgagcaga agcagctgtt cgtggagcag 3780

cacaagcact acctggacga gatcatcgag cagatcagcg agttcagcaa gcgggtgatc 3840

ctggccgacg ccaacctgga caaggtgctg agcgcctaca acaagcaccg ggacaagccc 3900

atccgggagc aggccgagaa catcatccac ctgttcaccc tgaccaacct gggcgccccc 3960

gccgccttca agtacttcga caccaccatc gaccggaagc ggtacaccag caccaaggag 4020

gtgctggacg ccaccctgat ccaccagagc atcaccggcc tgtacgagac ccggatcgac 4080

ctgagccagc tgggcggcga c 4101

<210> 363
<211> 3306
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Nme Cas9 ORF using low A codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 363
gccgccttca agcccaactc catcaactac atcctgggcc tggacatcgg catcgcctcc 60

gtgggctggg ccatggtgga gatcgacgag gaggagaacc ccatccggct gatcgacctg 120

ggcgtgcggg tgttcgagcg ggccgaggtg cccaagaccg gcgactccct ggccatggcc 180

cggcggctgg cccggtccgt gcggcggctg acccggcggc gggcccaccg gctgctgcgg 240

acccggcggc tgctgaagcg ggagggcgtg ctgcaggccg ccaacttcga cgagaacggc 300

ctgatcaagt ccctgcccaa cacccctgg cagctgcggg ccgccgccct ggaccggaag 360

ctgacccccc tggagtggtc cgccgtgctg ctgcacctga tcaagcaccg gggctacctg 420

tcccagcgga agaacgaggg cgagaccgcc gacaaggagc tgggcgccct gctgaagggc 480

gtggccggca acgcccacgc cctgcagacc ggcgacttcc ggaccccgc cgagctggcc 540

ctgaacaagt tcgagaagga gtccggccac atccggaacc agcggtccga ctactcccac 600

accttctccc ggaaggacct gcaggccgag ctgatcctgc tgttcgagaa gcagaaggag 660

ttcggcaacc cccacgtgtc cggcggcctg aaggagggca tcgagaccct gctgatgacc 720

cagcggcccg ccctgtccgg cgacgccgtg cagaagatgc tgggccactg caccttcgag 780

cccgccgagc ccaaggccgc caagaacacc tacaccgccg agcggttcat ctggctgacc 840

aagctgaaca acctgcggat cctggagcag ggctccgagc ggcccctgac cgacaccgag 900

cgggccaccc tgatggacga gccctaccgg aagtccaagc tgacctacgc ccaggcccgg 960

aagctgctgg gcctggagga caccgccttc ttcaagggcc tgcggtacgg caaggacaac 1020

gccgaggcct ccaccctgat ggagatgaag gcctaccacg ccatctcccg ggccctggag 1080

aaggagggcc tgaaggacaa gaagtccccc ctgaacctgt cccccgagct gcaggacgag 1140

atcggcaccg ccttctccct gttcaagacc gacgaggaca tcaccggccg gctgaaggac 1200

cggatccagc ccgagatcct ggaggccctg ctgaagcaca tctccttcga caagttcgtg 1260

cagatctccc tgaaggccct gcggcggatc gtgcccctga tggagcaggg caagcggtac 1320

gacgaggcct gcgccgagat ctacggcgac cactacggca agaagaacac cgaggagaag 1380

atctacctgc cccccatccc cgccgacgag atccggaacc ccgtggtgct gcgggccctg 1440

tcccaggccc ggaaggtgat caacggcgtg gtgcggcggt acggctcccc cgcccggatc 1500

cacatcgaga ccgcccggga ggtgggcaag tccttcaagg accggaagga gatcgagaag 1560

cggcaggagg agaaccggaa ggaccgggag aaggccgccg ccaagttccg ggagtacttc 1620

cccaacttcg tgggcgagcc caagtccaag gacatcctga agctgcggct gtacgagcag 1680

cagcacggca agtgcctgta ctccggcaag gagatcaacc tgggccggct gaacgagaag 1740

ggctacgtgg agatcgacca cgccctgccc ttctcccgga cctgggacga ctccttcaac 1800

aacaaggtgc tggtgctggg ctccgagaac cagaacaagg gcaaccagac cccctacgag 1860

tacttcaacg gcaaggacaa ctcccgggag tggcaggagt tcaaggcccg ggtggagacc 1920

tcccggttcc cccggtccaa gaagcagcgg atcctgctgc agaagttcga cgaggacggc 1980

ttcaaggagc ggaacctgaa cgacacccgg tacgtgaacc ggttcctgtg ccagttcgtg 2040

gccgaccgga tgcggctgac cggcaagggc aagaagcggg tgttcgcctc caacggccag 2100

atcaccaacc tgctgcgggg cttctggggc ctgcggaagg tgcgggccga gaacgaccgg 2160

caccacgccc tggacgccgt ggtggtggcc tgctccaccg tggccatgca gcagaagatc 2220

acccggttcg tgcggtacaa ggagatgaac gccttcgacg gcaagaccat cgacaaggag 2280

accggcgagg tgctgcacca gaagacccac ttcccccagc cctgggagtt cttcgcccag 2340

gaggtgatga tccgggtgtt cggcaagccc gacggcaagc ccgagttcga ggaggccgac 2400

accctggaga agctgcggac cctgctggcc gagaagctgt cctcccggcc cgaggccgtg 2460

cacgagtacg tgacccccct gttcgtgtcc cgggccccca accggaagat gtccggccag 2520

ggccacatgg agaccgtgaa gtccgccaag cggctggacg agggcgtgtc cgtgctgcgg 2580

gtgcccctga cccagctgaa gctgaaggac ctggagaaga tggtgaaccg ggagcgggag 2640

cccaagctgt acgaggccct gaaggcccgg ctggaggccc acaaggacga ccccgccaag 2700

gccttcgccg agcccttcta caagtacgac aaggccggca accggaccca gcaggtgaag 2760

gccgtgcggg tggagcaggt gcagaagacc ggcgtgtggg tgcggaacca caacggcatc 2820

gccgacaacg ccaccatggt gcgggtggac gtgttcgaga agggcgacaa gtactacctg 2880

gtgcccatct actcctggca ggtggccaag ggcatcctgc ccgaccgggc cgtggtgcag 2940

ggcaaggacg aggaggactg gcagctgatc gacgactcct tcaacttcaa gttctccctg 3000

caccccaacg acctggtgga ggtgatcacc aagaaggccc ggatgttcgg ctacttcgcc 3060

tcctgccacc ggggcaccgg caacatcaac atccggatcc acgacctgga ccacaagatc 3120

ggcaagaacg gcatcctgga gggcatcggc gtgaagaccg ccctgtcctt ccagaagtac 3180

cagatcgacg agctgggcaa ggagatccgg ccctgccggc tgaagaagcg gccccccgtg 3240

cggtccggca agcggaccgc cgacggctcc gagttcgagt cccccaagaa gaagcggaag 3300

gtggag 3306

<210> 364
<211> 3306
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Nme Cas9 ORF using low A/U codons of Table 4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 364
gccgccttca agcccaacag catcaactac atcctgggcc tggacatcgg catcgccagc 60

gtgggctggg ccatggtgga gatcgacgag gaggagaacc ccatccggct gatcgacctg 120

ggcgtgcggg tgttcgagcg ggccgaggtg cccaagaccg gcgacagcct ggccatggcc 180

cggcggctgg cccggagcgt gcggcggctg acccggcggc gggcccaccg gctgctgcgg 240

acccggcggc tgctgaagcg ggagggcgtg ctgcaggccg ccaacttcga cgagaacggc 300

ctgatcaaga gcctgcccaa cacccctgg cagctgcggg ccgccgccct ggaccggaag 360

ctgacccccc tggagtggag cgccgtgctg ctgcacctga tcaagcaccg gggctacctg 420

agccagcgga agaacgaggg cgagaccgcc gacaaggagc tgggcgccct gctgaagggc 480

gtggccggca acgcccacgc cctgcagacc ggcgacttcc ggacccccgc cgagctggcc 540

ctgaacaagt tcgagaagga gagcggccac atccggaacc agcggagcga ctacagccac 600

accttcagcc ggaaggacct gcaggccgag ctgatcctgc tgttcgagaa gcagaaggag 660

ttcggcaacc cccacgtgag cggcggcctg aaggagggca tcgagaccct gctgatgacc 720

cagcggcccg ccctgagcgg cgacgccgtg cagaagatgc tgggccactg caccttcgag 780

cccgccgagc ccaaggccgc caagaacacc tacaccgccg agcggttcat ctggctgacc 840

aagctgaaca acctgcggat cctggagcag ggcagcgagc ggcccctgac cgacaccgag 900

cgggccaccc tgatggacga gccctaccgg aagagcaagc tgacctacgc ccaggcccgg 960

aagctgctgg gcctggagga caccgccttc ttcaagggcc tgcggtacgg caaggacaac 1020

gccgaggcca gcaccctgat ggagatgaag gcctaccacg ccatcagccg ggccctggag 1080

aaggagggcc tgaaggacaa gaagagcccc ctgaacctga gccccgagct gcaggacgag 1140

atcggcaccg ccttcagcct gttcaagacc gacgaggaca tcaccggccg gctgaaggac 1200

cggatccagc ccgagatcct ggaggccctg ctgaagcaca tcagcttcga caagttcgtg 1260

cagatcagcc tgaaggccct gcggcggatc gtgccctga tggagcaggg caagcggtac 1320

gacgaggcct gcgccgagat ctacggcgac cactacggca agaagaacac cgaggagaag 1380

atctacctgc cccccatccc cgccgacgag atccggaacc ccgtggtgct gcgggccctg 1440

agccaggccc ggaaggtgat caacggcgtg gtgcggcggt acggcagccc cgcccggatc 1500

cacatcgaga ccgcccggga ggtgggcaag agcttcaagg accggaagga gatcgagaag 1560

cggcaggagg agaaccggaa ggaccgggag aaggccgccg ccaagttccg ggagtacttc 1620

cccaacttcg tgggcgagcc caagagcaag gacatcctga agctgcggct gtacgagcag 1680

cagcacggca agtgcctgta cagcggcaag gagatcaacc tgggccggct gaacgagaag 1740

ggctacgtgg agatcgacca cgccctgccc ttcagccgga cctgggacga cagcttcaac 1800

aacaaggtgc tggtgctggg cagcgagaac cagaacaagg gcaaccagac cccctacgag 1860

tacttcaacg gcaaggacaa cagccgggag tggcaggagt tcaaggcccg ggtggagacc 1920

agccggttcc cccggagcaa gaagcagcgg atcctgctgc agaagttcga cgaggacggc 1980

ttcaaggagc ggaacctgaa cgacacccgg tacgtgaacc ggttcctgtg ccagttcgtg 2040

gccgaccgga tgcggctgac cggcaagggc aagaagcggg tgttcgccag caacggccag 2100

atcaccaacc tgctgcgggg cttctggggc ctgcggaagg tgcgggccga gaacgaccgg 2160

caccacgccc tggacgccgt ggtggtggcc tgcagcaccg tggccatgca gcagaagatc 2220

acccggttcg tgcggtacaa ggagatgaac gccttcgacg gcaagaccat cgacaaggag 2280

accggcgagg tgctgcacca gaagacccac ttcccccagc cctgggagtt cttcgcccag 2340

gaggtgatga tccgggtgtt cggcaagccc gacggcaagc ccgagttcga ggaggccgac 2400

accctggaga agctgcggac cctgctggcc gagaagctga gcagccggcc cgaggccgtg 2460

cacgagtacg tgacccccct gttcgtgagc cgggccccca accggaagat gagcggccag 2520

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gtgcccctga cccagctgaa gctgaaggac ctggagaaga tggtgaaccg ggagcgggag 2640

cccaagctgt acgaggccct gaaggcccgg ctggaggccc acaaggacga ccccgccaag 2700

gccttcgccg agcccttcta caagtacgac aaggccggca accggaccca gcaggtgaag 2760

gccgtgcggg tggagcaggt gcagaagacc ggcgtgtggg tgcggaacca caacggcatc 2820

gccgacaacg ccaccatggt gcgggtggac gtgttcgaga agggcgacaa gtactacctg 2880

gtgcccatct acagctggca ggtggccaag ggcatcctgc ccgaccgggc cgtggtgcag 2940

ggcaaggacg aggaggactg gcagctgatc gacgacagct tcaacttcaa gttcagcctg 3000

caccccaacg acctggtgga ggtgatcacc aagaaggccc ggatgttcgg ctacttcgcc 3060

agctgccacc ggggcaccgg caacatcaac atccggatcc acgacctgga ccacaagatc 3120

ggcaagaacg gcatcctgga gggcatcggc gtgaagaccg ccctgagctt ccagaagtac 3180

cagatcgacg agctgggcaa ggagatccgg ccctgccggc tgaagaagcg gccccccgtg 3240

cggagcggca agcggaccgc cgacggcagc gagttcgaga gccccaagaa gaagcggaag 3300

gtggag 3306

<210> 365
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS1 (no start or stop codons; suitable for inclusion in fusion protein coding

sequence)

<400> 365

gacaagaagt acagcatcgg actggacatc ggaacaaaca gcgtcggatg ggcagtcatc 60

acagacgaat acaaggtccc gagcaagaag ttcaaggtcc tgggaaacac agacagacac 120

agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

gacaagctgt tcatccagct ggtccagaca tacaaccagc tgttcgaaga aaacccgatc 600

aacgcaagcg gagtcgacgc aaaggcaatc ctgagcgcaa gactgagcaa gagcagaaga 660

ctggaaaacc tgatcgcaca gctgccggga gaaaagaaga acggactgtt cggaaacctg 720

atcgcactga gcctgggact gacaccgaac ttcaagagca acttcgacct ggcagaagac 780

gcaaagctgc agctgagcaa ggacacatac gacgacgacc tggacaacct gctggcacag 840

atcggagacc agtacgcaga cctgttcctg gcagcaaaga acctgagcga cgcaatcctg 900

ctgagcgaca tcctgagagt caacacagaa atcacaaagg caccgctgag cgcaagcatg 960

atcaagagat acgacgaaca ccaccaggac ctgacactgc tgaaggcact ggtcagacag 1020

cagctgccgg aaaagtacaa ggaaatcttc ttcgaccaga gcaagaacgg atacgcagga 1080

tacatcgacg gaggagcaag ccaggaagaa ttctacaagt tcatcaagcc gatcctggaa 1140

aagatggacg gaacagaaga actgctggtc aagctgaaca gagaagacct gctgagaaag 1200

cagagaacat tcgacaacgg aagcatcccg caccagatcc acctgggaga actgcacgca 1260

atcctgagaa gacaggaaga cttctacccg ttcctgaagg acaacagaga aaagatcgaa 1320

aagatcctga cattcagaat cccgtactac gtcggaccgc tggcaagagg aaacagcaga 1380

ttcgcatgga tgacaagaaa gagcgaagaa acaatcacac cgtggaactt cgaagaagtc 1440

gtcgacaagg gagcaagcgc acagagcttc atcgaaagaa tgacaaactt cgacaagaac 1500

ctgccgaacg aaaaggtcct gccgaagcac agcctgctgt acgaatactt cacagtctac 1560

aacgaactga caaaggtcaa gtacgtcaca gaaggaatga gaaagccggc attcctgagc 1620

ggagaacaga agaaggcaat cgtcgacctg ctgttcaaga caaacagaaa ggtcacagtc 1680

aagcagctga aggaagacta cttcaagaag atcgaatgct tcgacagcgt cgaaatcagc 1740

ggagtcgaag acagattcaa cgcaagcctg ggaacatacc acgacctgct gaagatcatc 1800

aaggacaagg acttcctgga caacgaagaa aacgaagaca tcctggaaga catcgtcctg 1860

acactgacac tgttcgaaga cagagaaatg atcgaagaaa gactgaagac atacgcacac 1920

ctgttcgacg acaaggtcat gaagcagctg aagagaagaa gatacacagg atggggaaga 1980

ctgagcagaa agctgatcaa cggaatcaga gacaagcaga gcggaaagac aatcctggac 2040

ttcctgaaga gcgacggatt cgcaaacaga aacttcatgc agctgatcca cgacgacagc 2100

ctgacattca aggaagacat ccagaaggca caggtcagcg gacagggaga cagcctgcac 2160

gaacacatcg caaacctggc aggaagcccg gcaatcaaga agggaatcct gcagacagtc 2220

aaggtcgtcg acgaactggt caaggtcatg ggaagacaca agccggaaaa catcgtcatc 2280

gaaatggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaatg 2340

aagagaatcg aagaaggaat caaggaactg ggaagccaga tcctgaagga acacccggtc 2400

gaaaacacac agctgcagaa cgaaaagctg tacctgtact acctgcagaa cggaagagac 2460

atgtacgtcg accaggaact ggacatcaac agactgagcg actacgacgt cgaccacatc 2520

gtcccgcaga gcttcctgaa ggacgacagc atcgacaaca aggtcctgac aagaagcgac 2580

aagaacagag gaaagagcga caacgtcccg agcgaagaag tcgtcaagaa gatgaagaac 2640

tactggagac agctgctgaa cgcaaagctg atcacacaga gaaagttcga caacctgaca 2700

aaggcagaga gaggaggact gagcgaactg gacaaggcag gattcatcaa gagacagctg 2760

gtcgaaacaa gacagatcac aaagcacgtc gcacagatcc tggacagcag aatgaacaca 2820

aagtacgacg aaaacgacaa gctgatcaga gaagtcaagg tcatcacact gaagagcaag 2880

ctggtcagcg acttcagaaa ggacttccag ttctacaagg tcagagaaat caacaactac 2940

caccacgcac acgacgcata cctgaacgca gtcgtcggaa cagcactgat caagaagtac 3000

ccgaagctgg aaagcgaatt cgtctacgga gactacaagg tctacgacgt cagaaagatg 3060

atcgcaaaga gcgaacagga aatcggaaag gcaacagcaa agtacttctt ctacagcaac 3120

atcatgaact tcttcaagac agaaatcaca ctggcaaacg gagaaatcag aaagagaccg 3180

ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

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acaggaggat tcagcaagga aagcatcctg ccgaagagaa acagcgacaa gctgatcgca 3360

agaaagaagg actgggaccc gaagaagtac ggaggattcg acagcccgac agtcgcatac 3420

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tacgaaaagc tgaagggaag cccggaagac aacgaacaga agcagctgtt cgtcgaacag 3780

cacaagcact acctggacga aatcatcgaa cagatcagcg aattcagcaa gagagtcatc 3840

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atcagagaac aggcagaaaa catcatccac ctgttcacac tgacaaacct gggagcaccg 3960

gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

ctgagccagc tgggaggaga cggaggagga agcctggcag caaagagaag cagaacaaca 4140

<210> 366
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS2 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 366
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acagacgaat acaaggtccc gagcaagaag ttcaaggtcc tgggaaacac agacagacac 120

agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

gacaagctgt tcatccagct ggtccagaca tacaaccagc tgttcgaaga aaacccgatc 600

aacgcaagcg gagtcgacgc aaaggcaatc ctgagcgcaa gactgagcaa gagcagaaga 660

ctggaaaacc tgatcgcaca gctgccggga gaaaagaaga acggactgtt cggaaacctg 720

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atcaagagat acgacgaaca ccaccaggac ctgacactgc tgaaggcact ggtcagacag 1020

cagctgccgg aaaagtacaa ggaaatcttc ttcgaccaga gcaagaacgg atacgcagga 1080

tacatcgacg gaggagcaag ccaggaagaa ttctacaagt tcatcaagcc gatcctggaa 1140

aagatggacg gaacagaaga actgctggtc aagctgaaca gagaagacct gctgagaaag 1200

cagagaacat tcgacaacgg aagcatcccg caccagatcc acctgggaga actgcacgca 1260

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aagatcctga cattcagaat cccgtactac gtcggaccgc tggcaagagg aaacagcaga 1380

ttcgcatgga tgacaagaaa gagcgaagaa acaatcacac cgtggaactt cgaagaagtc 1440

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ctgccgaacg aaaaggtcct gccgaagcac agcctgctgt acgaatactt cacagtctac 1560

aacgaactga caaaggtcaa gtacgtcaca gaaggaatga gaaagccggc attcctgagc 1620

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aaggacaagg acttcctgga caacgaagaa aacgaagaca tcctggaaga catcgtcctg 1860

acactgacac tgttcgaaga cagagaaatg atcgaagaaa gactgaagac atacgcacac 1920

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ctgagcagaa agctgatcaa cggaatcaga gacaagcaga gcggaaagac aatcctggac 2040

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gaaatggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaatg 2340

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gaaaacacac agctgcagaa cgaaaagctg tacctgtact acctgcagaa cggaagagac 2460

atgtacgtcg accaggaact ggacatcaac agactgagcg actacgacgt cgaccacatc 2520

gtcccgcaga gcttcctgaa ggacgacagc atcgacaaca aggtcctgac aagaagcgac 2580

aagaacagag gaaagagcga caacgtcccg agcgaagaag tcgtcaagaa gatgaagaac 2640

tactggagac agctgctgaa cgcaaagctg atcacacaga gaaagttcga caacctgaca 2700

aaggcagaga gaggaggact gagcgaactg gacaaggcag gattcatcaa gagacagctg 2760

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aagtacgacg aaaacgacaa gctgatcaga gaagtcaagg tcatcacact gaagagcaag 2880

ctggtcagcg acttcagaaa ggacttccag ttctacaagg tcagagaaat caacaactac 2940

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ccgaagctgg aaagcgaatt cgtctacgga gactacaagg tctacgacgt cagaaagatg 3060

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atcatgaact tcttcaagac agaaatcaca ctggcaaacg gagaaatcag aaagagaccg 3180

ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

acagtcagaa aggtcctgag catgccgcag gtcaacatcg tcaagaagac agaagtccag 3300

acaggaggat tcagcaagga aagcatcctg ccgaagagaa acagcgacaa gctgatcgca 3360

agaaagaagg actgggaccc gaagaagtac ggaggattcg acagcccgac agtcgcatac 3420

agcgtcctgg tcgtcgcaaa ggtcgaaaag ggaaagagca agaagctgaa gagcgtcaag 3480

gaactgctgg gaatcacaat catggaaaga agcagcttcg aaaagaaccc gatcgacttc 3540

ctggaagcaa agggatacaa ggaagtcaag aaggacctga tcatcaagct gccgaagtac 3600

agcctgttcg aactggaaaa cggaagaaag agaatgctgg caagcgcagg agaactgcag 3660

aagggaaacg aactggcact gccgagcaag tacgtcaact tcctgtacct ggcaagccac 3720

tacgaaaagc tgaagggaag cccggaagac aacgaacaga agcagctgtt cgtcgaacag 3780

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atcagagaac aggcagaaaa catcatccac ctgttcacac tgacaaacct gggagcaccg 3960

gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

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<210> 367
<211> 4143
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 367
gacaagaagt acagcatcgg actggacatc ggaacaaaca gcgtcggatg ggcagtcatc 60

acagacgaat acaaggtccc gagcaagaag ttcaaggtcc tgggaaacac agacagacac 120

agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

gacaagctgt tcatccagct ggtccagaca tacaaccagc tgttcgaaga aaacccgatc 600

aacgcaagcg gagtcgacgc aaaggcaatc ctgagcgcaa gactgagcaa gagcagaaga 660

ctggaaaacc tgatcgcaca gctgccggga gaaaagaaga acggactgtt cggaaacctg 720

atcgcactga gcctgggact gacaccgaac ttcaagagca acttcgacct ggcagaagac 780

gcaaagctgc agctgagcaa ggacacatac gacgacgacc tggacaacct gctggcacag 840

atcggagacc agtacgcaga cctgttcctg gcagcaaaga acctgagcga cgcaatcctg 900

ctgagcgaca tcctgagagt caacacagaa atcacaaagg caccgctgag cgcaagcatg 960

atcaagagat acgacgaaca ccaccaggac ctgacactgc tgaaggcact ggtcagacag 1020

cagctgccgg aaaagtacaa ggaaatcttc ttcgaccaga gcaagaacgg atacgcagga 1080

tacatcgacg gaggagcaag ccaggaagaa ttctacaagt tcatcaagcc gatcctggaa 1140

aagatggacg gaacagaaga actgctggtc aagctgaaca gagaagacct gctgagaaag 1200

cagagaacat tcgacaacgg aagcatcccg caccagatcc acctgggaga actgcacgca 1260

atcctgagaa gacaggaaga cttctacccg ttcctgaagg acaacagaga aaagatcgaa 1320

aagatcctga cattcagaat cccgtactac gtcggaccgc tggcaagagg aaacagcaga 1380

ttcgcatgga tgacaagaaa gagcgaagaa acaatcacac cgtggaactt cgaagaagtc 1440

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ctgccgaacg aaaaggtcct gccgaagcac agcctgctgt acgaatactt cacagtctac 1560

aacgaactga caaaggtcaa gtacgtcaca gaaggaatga gaaagccggc attcctgagc 1620

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aaggacaagg acttcctgga caacgaagaa aacgaagaca tcctggaaga catcgtcctg 1860

acactgacac tgttcgaaga cagagaaatg atcgaagaaa gactgaagac atacgcacac 1920

ctgttcgacg acaaggtcat gaagcagctg aagagaagaa gatacacagg atggggaaga 1980

ctgagcagaa agctgatcaa cggaatcaga gacaagcaga gcggaaagac aatcctggac 2040

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gaaatggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaatg 2340

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gaaaacacac agctgcagaa cgaaaagctg tacctgtact acctgcagaa cggaagagac 2460

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gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

ctgagccagc tgggaggaga cggaggagga agcccggcac cggcaaagag agaaagaaca 4140

aca 4143

<210> 368
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS4 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 368
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acagacgaat acaaggtccc gagcaagaag ttcaaggtcc tgggaaacac agacagacac 120

agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

gacaagctgt tcatccagct ggtccagaca tacaaccagc tgttcgaaga aaacccgatc 600

aacgcaagcg gagtcgacgc aaaggcaatc ctgagcgcaa gactgagcaa gagcagaaga 660

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gaaatggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaatg 2340

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gaaaacacac agctgcagaa cgaaaagctg tacctgtact acctgcagaa cggaagagac 2460

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ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

acagtcagaa aggtcctgag catgccgcag gtcaacatcg tcaagaagac agaagtccag 3300

acaggaggat tcagcaagga aagcatcctg ccgaagagaa acagcgacaa gctgatcgca 3360

agaaagaagg actgggaccc gaagaagtac ggaggattcg acagcccgac agtcgcatac 3420

agcgtcctgg tcgtcgcaaa ggtcgaaaag ggaaagagca agaagctgaa gagcgtcaag 3480

gaactgctgg gaatcacaat catggaaaga agcagcttcg aaaagaaccc gatcgacttc 3540

ctggaagcaa agggatacaa ggaagtcaag aaggacctga tcatcaagct gccgaagtac 3600

agcctgttcg aactggaaaa cggaagaaag agaatgctgg caagcgcagg agaactgcag 3660

aagggaaacg aactggcact gccgagcaag tacgtcaact tcctgtacct ggcaagccac 3720

tacgaaaagc tgaagggaag cccggaagac aacgaacaga agcagctgtt cgtcgaacag 3780

cacaagcact acctggacga aatcatcgaa cagatcagcg aattcagcaa gagagtcatc 3840

ctggcagacg caaacctgga caaggtcctg agcgcataca acaagcacag agacaagccg 3900

atcagagaac aggcagaaaa catcatccac ctgttcacac tgacaaacct gggagcaccg 3960

gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

ctgagccagc tgggaggaga cggaggagga agccaggcag caaagagacc gagaacaaca 4140

<210> 369
<211> 4140
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS5 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 369
gacaagaagt acagcatcgg actggacatc ggaacaaaca gcgtcggatg ggcagtcatc 60

acagacgaat acaaggtccc gagcaagaag ttcaaggtcc tgggaaacac agacagacac 120

agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

gacaagctgt tcatccagct ggtccagaca tacaaccagc tgttcgaaga aaacccgatc 600

aacgcaagcg gagtcgacgc aaaggcaatc ctgagcgcaa gactgagcaa gagcagaaga 660

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ctgagcgaca tcctgagagt caacacagaa atcacaaagg caccgctgag cgcaagcatg 960

atcaagagat acgacgaaca ccaccaggac ctgacactgc tgaaggcact ggtcagacag 1020

cagctgccgg aaaagtacaa ggaaatcttc ttcgaccaga gcaagaacgg atacgcagga 1080

tacatcgacg gaggagcaag ccaggaagaa ttctacaagt tcatcaagcc gatcctggaa 1140

aagatggacg gaacagaaga actgctggtc aagctgaaca gagaagacct gctgagaaag 1200

cagagaacat tcgacaacgg aagcatcccg caccagatcc acctgggaga actgcacgca 1260

atcctgagaa gacaggaaga cttctacccg ttcctgaagg acaacagaga aaagatcgaa 1320

aagatcctga cattcagaat cccgtactac gtcggaccgc tggcaagagg aaacagcaga 1380

ttcgcatgga tgacaagaaa gagcgaagaa acaatcacac cgtggaactt cgaagaagtc 1440

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aacgaactga caaaggtcaa gtacgtcaca gaaggaatga gaaagccggc attcctgagc 1620

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acactgacac tgttcgaaga cagagaaatg atcgaagaaa gactgaagac atacgcacac 1920

ctgttcgacg acaaggtcat gaagcagctg aagagaagaa gatacacagg atggggaaga 1980

ctgagcagaa agctgatcaa cggaatcaga gacaagcaga gcggaaagac aatcctggac 2040

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ctgacattca aggaagacat ccagaaggca caggtcagcg gacagggaga cagcctgcac 2160

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gaaatggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaatg 2340

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gaaaacacac agctgcagaa cgaaaagctg tacctgtact acctgcagaa cggaagagac 2460

atgtacgtcg accaggaact ggacatcaac agactgagcg actacgacgt cgaccacatc 2520

gtcccgcaga gcttcctgaa ggacgacagc atcgacaaca aggtcctgac aagaagcgac 2580

aagaacagag gaaagagcga caacgtcccg agcgaagaag tcgtcaagaa gatgaagaac 2640

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aaggcagaga gaggaggact gagcgaactg gacaaggcag gattcatcaa gagacagctg 2760

gtcgaaacaa gacagatcac aaagcacgtc gcacagatcc tggacagcag aatgaacaca 2820

aagtacgacg aaaacgacaa gctgatcaga gaagtcaagg tcatcacact gaagagcaag 2880

ctggtcagcg acttcagaaa ggacttccag ttctacaagg tcagagaaat caacaactac 2940

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ccgaagctgg aaagcgaatt cgtctacgga gactacaagg tctacgacgt cagaaagatg 3060

atcgcaaaga gcgaacagga aatcggaaag gcaacagcaa agtacttctt ctacagcaac 3120

atcatgaact tcttcaagac agaaatcaca ctggcaaacg gagaaatcag aaagagaccg 3180

ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

acagtcagaa aggtcctgag catgccgcag gtcaacatcg tcaagaagac agaagtccag 3300

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atcagagaac aggcagaaaa catcatccac ctgttcacac tgacaaacct gggagcaccg 3960

gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

ctgagccagc tgggaggaga cggaggagga agcagagcag caaagagacc gagaacaaca 4140

<210> 370
<211> 4146
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS6 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 370
gacaagaagt acagcatcgg actggacatc ggaacaaaca gcgtcggatg ggcagtcatc 60

acagacgaat acaaggtccc gagcaagaag ttcaaggtcc tgggaaacac agacagacac 120

agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

gacaagctgt tcatccagct ggtccagaca tacaaccagc tgttcgaaga aaacccgatc 600

aacgcaagcg gagtcgacgc aaaggcaatc ctgagcgcaa gactgagcaa gagcagaaga 660

ctggaaaacc tgatcgcaca gctgccggga gaaaagaaga acggactgtt cggaaacctg 720

atcgcactga gcctgggact gacaccgaac ttcaagagca acttcgacct ggcagaagac 780

gcaaagctgc agctgagcaa ggacacatac gacgacgacc tggacaacct gctggcacag 840

atcggagacc agtacgcaga cctgttcctg gcagcaaaga acctgagcga cgcaatcctg 900

ctgagcgaca tcctgagagt caacacagaa atcacaaagg caccgctgag cgcaagcatg 960

atcaagagat acgacgaaca ccaccaggac ctgacactgc tgaaggcact ggtcagacag 1020

cagctgccgg aaaagtacaa ggaaatcttc ttcgaccaga gcaagaacgg atacgcagga 1080

tacatcgacg gaggagcaag ccaggaagaa ttctacaagt tcatcaagcc gatcctggaa 1140

aagatggacg gaacagaaga actgctggtc aagctgaaca gagaagacct gctgagaaag 1200

cagagaacat tcgacaacgg aagcatcccg caccagatcc acctgggaga actgcacgca 1260

atcctgagaa gacaggaaga cttctacccg ttcctgaagg acaacagaga aaagatcgaa 1320

aagatcctga cattcagaat cccgtactac gtcggaccgc tggcaagagg aaacagcaga 1380

ttcgcatgga tgacaagaaa gagcgaagaa acaatcacac cgtggaactt cgaagaagtc 1440

gtcgacaagg gagcaagcgc acagagcttc atcgaaagaa tgacaaactt cgacaagaac 1500

ctgccgaacg aaaaggtcct gccgaagcac agcctgctgt acgaatactt cacagtctac 1560

aacgaactga caaaggtcaa gtacgtcaca gaaggaatga gaaagccggc attcctgagc 1620

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aaggacaagg acttcctgga caacgaagaa aacgaagaca tcctggaaga catcgtcctg 1860

acactgacac tgttcgaaga cagagaaatg atcgaagaaa gactgaagac atacgcacac 1920

ctgttcgacg acaaggtcat gaagcagctg aagagaagaa gatacacagg atggggaaga 1980

ctgagcagaa agctgatcaa cggaatcaga gacaagcaga gcggaaagac aatcctggac 2040

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gaaaacacac agctgcagaa cgaaaagctg tacctgtact acctgcagaa cggaagagac 2460

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ctggtcagcg acttcagaaa ggacttccag ttctacaagg tcagagaaat caacaactac 2940

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ccgaagctgg aaagcgaatt cgtctacgga gactacaagg tctacgacgt cagaaagatg 3060

atcgcaaaga gcgaacagga aatcggaaag gcaacagcaa agtacttctt ctacagcaac 3120

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ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

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atcagagaac aggcagaaaa catcatccac ctgttcacac tgacaaacct gggagcaccg 3960

gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

ctgagccagc tgggaggaga cggaggagga agcgcagcag caaagagaag ctggagcatg 4140

gcagca 4146

<210> 371

<211> 4146
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS7 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 371
gacaagaagt acagcatcgg actggacatc ggaacaaaca gcgtcggatg ggcagtcatc 60

acagacgaat acaaggtccc gagcaagaag ttcaaggtcc tgggaaacac agacagacac 120

agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

gacaagctgt tcatccagct ggtccagaca tacaaccagc tgttcgaaga aaacccgatc 600

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atcaagagat acgacgaaca ccaccaggac ctgacactgc tgaaggcact ggtcagacag 1020

cagctgccgg aaaagtacaa ggaaatcttc ttcgaccaga gcaagaacgg atacgcagga 1080

tacatcgacg gaggagcaag ccaggaagaa ttctacaagt tcatcaagcc gatcctggaa 1140

aagatggacg gaacagaaga actgctggtc aagctgaaca gagaagacct gctgagaaag 1200

cagagaacat tcgacaacgg aagcatcccg caccagatcc acctgggaga actgcacgca 1260

atcctgagaa gacaggaaga cttctacccg ttcctgaagg acaacagaga aaagatcgaa 1320

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aaggacaagg acttcctgga caacgaagaa aacgaagaca tcctggaaga catcgtcctg 1860

acactgacac tgttcgaaga cagagaaatg atcgaagaaa gactgaagac atacgcacac 1920

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gaaatggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaatg 2340

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gaaaacacac agctgcagaa cgaaaagctg tacctgtact acctgcagaa cggaagagac 2460

atgtacgtcg accaggaact ggacatcaac agactgagcg actacgacgt cgaccacatc 2520

gtcccgcaga gcttcctgaa ggacgacagc atcgacaaca aggtcctgac aagaagcgac 2580

aagaacagag gaaagagcga caacgtcccg agcgaagaag tcgtcaagaa gatgaagaac 2640

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ccgaagctgg aaagcgaatt cgtctacgga gactacaagg tctacgacgt cagaaagatg 3060

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ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

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acaggaggat tcagcaagga aagcatcctg ccgaagagaa acagcgacaa gctgatcgca 3360

agaaagaagg actgggaccc gaagaagtac ggaggattcg acagcccgac agtcgcatac 3420

agcgtcctgg tcgtcgcaaa ggtcgaaaag ggaaagagca agaagctgaa gagcgtcaag 3480

gaactgctgg gaatcacaat catggaaaga agcagcttcg aaaagaaccc gatcgacttc 3540

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agcctgttcg aactggaaaa cggaagaaag agaatgctgg caagcgcagg agaactgcag 3660

aagggaaacg aactggcact gccgagcaag tacgtcaact tcctgtacct ggcaagccac 3720

tacgaaaagc tgaagggaag cccggaagac aacgaacaga agcagctgtt cgtcgaacag 3780

cacaagcact acctggacga aatcatcgaa cagatcagcg aattcagcaa gagagtcatc 3840

ctggcagacg caaacctgga caaggtcctg agcgcataca acaagcacag agacaagccg 3900

atcagagaac aggcagaaaa catcatccac ctgttcacac tgacaaacct gggagcaccg 3960

gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

ctgagccagc tgggaggaga cggaggagga agcgcagcag caaagagagt ctggagcatg 4140

gcattc 4146

<210> 372
<211> 4146
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS8 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 372
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agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

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atcaagagat acgacgaaca ccaccaggac ctgacactgc tgaaggcact ggtcagacag 1020

cagctgccgg aaaagtacaa ggaaatcttc ttcgaccaga gcaagaacgg atacgcagga 1080

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aacgaactga caaaggtcaa gtacgtcaca gaaggaatga gaaagccggc attcctgagc 1620

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ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

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agaaagaagg actgggaccc gaagaagtac ggaggattcg acagcccgac agtcgcatac 3420

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gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

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gcattc 4146

<210> 373
<211> 4143
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS9 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

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agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

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tacatcgacg gaggagcaag ccaggaagaa ttctacaagt tcatcaagcc gatcctggaa 1140

aagatggacg gaacagaaga actgctggtc aagctgaaca gagaagacct gctgagaaag 1200

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ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

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gcagcattca agtacttcga cacaacaatc gacagaaaga gatacacaag cacaaaggaa 4020

gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

ctgagccagc tgggaggaga cggaggagga agcgcagcag caaagagaaa gtacttcgca 4140

gca 4143

<210> 374
<211> 4143
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS10 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> 374
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agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

acaagactga agagaacagc aagaagaaga tacacaagaa gaaagaacag aatctgctac 240

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gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

atcgtcgacg aagtcgcata ccacgaaaag tacccgacaa tctaccacct gagaaagaag 420

ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

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tactggagac agctgctgaa cgcaaagctg atcacacaga gaaagttcga caacctgaca 2700

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ctgatcgaaa caaacggaga aacaggagaa atcgtctggg acaagggaag agacttcgca 3240

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gtcctggacg caacactgat ccaccagagc atcacaggac tgtacgaaac aagaatcgac 4080

ctgagccagc tgggaggaga cggaggagga agcagagcag caaagagaaa ggcattcgca 4140

gca 4143

<210> 375
<211> 4143
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic: Open reading frame for Cas9 with NLS11 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

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agcatcaaga agaacctgat cggagcactg ctgttcgaca gcggagaaac agcagaagca 180

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ctgcaggaaa tcttcagcaa cgaaatggca aaggtcgacg acagcttctt ccacagactg 300

gaagaaagct tcctggtcga agaagacaag aagcacgaaa gacacccgat cttcggaaac 360

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ctggtcgaca gcacagacaa ggcagacctg agactgatct acctggcact ggcacacatg 480

atcaagttca gaggacactt cctgatcgaa ggagacctga acccggacaa cagcgacgtc 540

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ugggccugac ccccaacuuc aagagcaacu ucgaccuggc cgaggacgcc aagcugcagc   840

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<210> 377
<211> 4405
<212> RNA
<213> Artificial Sequence

<220>
<223> Synthetic: mRNA transcript with XBG UTRs and Cas9 ORF with low A codons of Table 4

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<210> 385
<211> 4517
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<220>
<223> Synthetic: mRNA transcript with ORF encoding Cas9 with HiBiT tag,
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<211> 1381
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence for Cas9 with NLS1

<400> 386

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20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

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His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
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Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670
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Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940
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Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960


Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975


Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990


Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020


Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035


Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050


Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065


Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080


Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095


Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110


Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125


Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140


Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155


Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170


Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185


Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200
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Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                 1210                 1215


Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                 1225                 1230


Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                 1240                 1245


Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                 1255                 1260


His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                 1270                 1275


Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                 1285                 1290


Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                 1300                 1305


Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                 1315                 1320


Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                 1330                 1335


Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                 1345                 1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
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Gly Gly  Gly Ser Leu Ala Ala  Lys Arg Ser Arg Thr  Thr
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<212>  PRT
<213>  Artificial Sequence

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45
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Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320
```

```
Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
```

```
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880
```

```
Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
               885                 890                 895


Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910


Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925


Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940


Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960


Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
               965                 970                 975


Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990


Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020


Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035


Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050


Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065


Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080


Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095


Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110


Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125


Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140
```

```
Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Gly  Gly Ser Gln Ala Ala  Lys Arg Ser Arg Thr  Thr
    1370                1375                1380
```

<210> 388
<211> 1382
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence for Cas9 with NLS3

<400> 388

```
Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15


Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45


Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60


Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80


Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95


Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110


His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125


His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140


Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160


Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175


Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190


Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205


Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220


Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240


Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255
```

```
Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525
```

```
Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
```

```
               805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080
```

```
Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                 1090                 1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                 1105                 1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                 1120                 1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                 1135                 1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                 1150                 1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                 1165                 1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                 1180                 1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                 1195                 1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                 1210                 1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                 1225                 1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                 1240                 1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                 1255                 1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                 1270                 1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                 1285                 1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                 1300                 1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                 1315                 1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                 1330                 1335
```

```
Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365


Gly Gly  Gly Ser Pro Ala Pro  Ala Lys Arg Glu Arg  Thr Thr
    1370                1375                1380


<210>  389
<211>  1381
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic: Amino acid sequence for Cas9 with NLS4

<400>  389

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15


Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45


Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60


Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80


Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95


Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110


His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125


His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140


Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160


Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175


Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190
```

```
Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460
```

```
Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735
```

```
Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
```

```
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275
```

```
Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290


Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305


Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320


Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335


Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365


Gly Gly  Gly Ser Gln Ala Ala  Lys Arg Pro Arg Thr  Thr
    1370                1375                1380
```

<210> 390
<211> 1381
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence for Cas9 with NLS5

<400> 390

```
Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15


Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45


Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60


Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80


Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95


Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110


His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
```

```
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400
```

```
Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670
```

```
Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940
```

```
Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960


Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975


Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990


Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020


Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035


Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050


Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065


Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080


Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095


Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110


Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125


Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140


Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155


Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170


Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185


Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200


Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
```

```
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Gly  Gly Ser Arg Ala Ala  Lys Arg Pro Arg Thr  Thr
    1370                1375                1380


<210>  391
<211>  1383
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic: Amino acid sequence for Cas9 with NLS6

<400>  391

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45
```

```
Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
```

```
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605
```

```
Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880
```

```
Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140
```

```
Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Gly  Gly Ser Ala Ala Ala  Lys Arg Ser Trp Ser  Met Ala Ala
    1370                1375                1380
```

<210> 392
<211> 1383
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence for Cas9 with NLS7

<400> 392

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1   5   10   15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20   25   30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35   40   45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50   55   60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65   70   75   80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85   90   95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100   105   110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115   120   125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130   135   140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145   150   155   160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165   170   175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180   185   190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195   200   205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210   215   220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225   230   235   240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245   250   255

```
Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
```

```
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815
```

```
Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080
```

```
Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                 1090                 1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                 1105                 1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                 1120                 1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                 1135                 1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                 1150                 1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                 1165                 1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                 1180                 1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                 1195                 1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                 1210                 1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                 1225                 1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                 1240                 1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                 1255                 1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                 1270                 1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                 1285                 1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                 1300                 1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                 1315                 1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                 1330                 1335
```

```
Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365


Gly Gly  Gly Ser Ala Ala Ala  Lys Arg Val Trp Ser  Met Ala Phe
    1370                1375                1380


<210>  393
<211>  1383
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic: Amino acid sequence for Cas9 with NLS8

<400>  393

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15


Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45


Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60


Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80


Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95


Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110


His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125


His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140


Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160


Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175


Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190
```

```
Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460
```

```
Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
```

```
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020
```

```
Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275
```

```
Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290


Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305


Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320


Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335


Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365


Gly Gly  Gly Ser Ala Ala Ala  Lys Arg Ser Trp Ser  Met Ala Phe
    1370                1375                1380
```

<210> 394
<211> 1382
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic: Amino acid sequence for Cas9 with NLS9

<400> 394

```
Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15


Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45


Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60


Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80


Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95


Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110


His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125
```

```
His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400
```

```
Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670
```

```
Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
```

```
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215
```

```
Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230


Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245


Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260


His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275


Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290


Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305


Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320


Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335


Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350


Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365


Gly Gly  Gly Ser Ala Ala Ala  Lys Arg Lys Tyr Phe  Ala Ala
    1370                1375                1380


<210>  395
<211>  1382
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic: Amino acid sequence for Cas9 with NLS10

<400>  395

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15


Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45


Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
```

```
    50                  55                  60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335
```

```
Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605
```

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Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880
```

```
Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895


Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910


Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925


Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940


Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960


Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975


Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990


Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005


Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020


Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035


Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050


Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065


Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080


Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095


Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110


Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125


Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140


Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
```

```
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Gly  Gly Ser Arg Ala Ala  Lys Arg Lys Ala Phe  Ala Ala
    1370                1375                1380
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<210> 396
<211> 1382
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic: Amino acid sequence for Cas9 with NLS11

<400> 396

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Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15


Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30


Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45


Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60


Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80


Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95


Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110


His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125


His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140


Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160


Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175


Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190


Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205


Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220


Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240


Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255


Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
```

```
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540
```

```
Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815
```

```
Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080
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Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                 1090                 1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                 1105                 1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                 1120                 1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                 1135                 1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                 1150                 1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                 1165                 1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                 1180                 1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                 1195                 1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                 1210                 1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                 1225                 1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                 1240                 1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                 1255                 1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                 1270                 1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                 1285                 1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                 1300                 1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                 1315                 1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                 1330                 1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
```

```
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Gly  Gly Ser Arg Ala Ala  Lys Arg Lys Tyr Phe  Ala Val
    1370                1375                1380
```