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WO-A2-2014/144592
US-A1- 2014 273 232
M. JINEK ET AL: "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity", SCIENCE, vol. 337, no. 6096, 17 August 2012 (2012-08-17), pages 816-821, XP055229606, US ISSN: 0036-8075, DOI: 10.1126/science.1225829 cited in the application -& M. JINEK ET AL: "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity (Supplementary Material)", SCIENCE, vol. 337, no. 6096, 28 June 2012 (2012-06-28), XP055067747, US ISSN: 0036-8075, DOI: 10.1126/science.1225829
REGINA CENCIC ET AL: "Protospacer Adjacent Motif (PAM)-Distal Sequences Engage CRISPR Cas9 DNA Target Cleavage", PLOS ONE, vol. 9, no. 10, 2 October 2014 (2014-10-02), page e109213, XP055175713, DOI: 10.1371/journal.pone.0109213
MEGHDAD RAHDAR ET AL: "Synthetic CRISPR RNA-Cas9-guided genome editing in human cells",

Fortsættes ...

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, 16 November 2015 (2015-11-16), XP055264777, US ISSN: 0027-8424, DOI: 10.1073/pnas.1520883112
AYAL HENDEL ET AL: "Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells", NATURE BIOTECHNOLOGY, vol. 33, no. 9, 29 June 2015 (2015-06-29), pages 985-989, XP055233915, US ISSN: 1087-0156, DOI: 10.1038/nbt.3290

DESCRIPTION

SEQUENCE LISTING

[0001] The instant application contains a Sequence Listing that has been submitted in ASCII format via EFS-Web. The ASCII copy, created on December 18, 2015, is named IDT01-008-US_ST25.txt, and is 177,163 bytes in size.

FIELD OF THE INVENTION

[0002] This disclosure pertains to modified compositions for use in CRISPR systems, and their methods of use.

BACKGROUND OF THE INVENTION

[0003] The use of clustered regularly interspaced short palindromic repeats (CRISPR) and associated Cas proteins (CRISPR-Cas system) for site-specific DNA cleavage has shown great potential for a number of biological applications. CRISPR is used for genome editing; the genome-scale-specific targeting of transcriptional repressors (CRISPRi) and activators (CRISPRa) to endogenous genes; and other applications of RNA-directed DNA targeting with Cas enzymes.

[0004] CRISPR-Cas systems are native to bacteria and Archaea to provide adaptive immunity against viruses and plasmids. There are three classes of CRISPR-Cas systems that could potentially be adapted for research and therapeutic reagents, but Type-II CRISPR systems have a desirable characteristic in utilizing a single CRISPR associated (Cas) nuclease (specifically Cas9) in a complex with the appropriate guide RNAs - either a 2-part RNA system similar to the natural complex in bacteria comprising a CRISPR-activating RNA:trans-activating crRNA (crRNA:tracrRNA) pair or an artificial chimeric single-guide-RNA (sgRNA) - to mediate double-stranded cleavage of target DNA. In mammalian systems, these RNAs have been introduced by transfection of DNA cassettes containing RNA Pol III promoters (such as U6 or H1) driving RNA transcription, viral vectors, and single-stranded RNA following *in vitro* transcription (see Xu, T., et al., Appl Environ Microbiol, 2014. 80(5): p. 1544-52).

[0005] In the CRISPR-Cas9 system, using, for example, the system present in *Streptococcus pyogenes* as an example (*S.py.* or Spy), native crRNAs are about 42 bp long, containing a 5'-region of about 20 bases complementary to a target sequence (also referred to as a protospacer sequence) and a 3' region typically about 22 bases long that corresponds to a complementary region of the tracrRNA sequence. The native tracrRNAs are about 85-90 bases long, having a 5'-region containing the region complementary to the crRNA as well as about a 10-base region 5'-upstream. The remaining 3' region of the tracrRNA includes secondary structures (herein

referred to as the "tracrRNA 3'-tail").

[0006] Jinek et al. extensively investigated the portions of the crRNA and tracrRNA that are required for proper functioning of the CRISPR-Cas9 system (Science, 2012. 337(6096): p. 816-21). They devised a truncated crRNA:tracrRNA fragment that could still function in CRISPR-Cas9 wherein the crRNA was the wild type 42 nucleotides and the tracrRNA was truncated to 75 nucleotides. They also developed an embodiment wherein the crRNA and tracrRNA are attached with a linker loop, forming a single guide RNA (sgRNA), which varies between 99-123 nucleotides in different embodiments. The configuration of the native 2-part crRNA:tracrRNA complex is shown in FIG. 1 and the 99 nucleotide embodiment of the artificial sgRNA single guide is shown in FIG. 2.

[0007] At least two groups have elucidated the crystal structure of *Streptococcus pyogenes* Cas9 (SpyCas9). In Jinek, M., et al., the structure did not show the nuclease in complex with either a guide RNA or target DNA. They carried out molecular modeling experiments to reveal predictive interactions between the protein in complex with RNA and DNA (Science, 2014. 343, p. 1215, DOI: 10.1126/science/1247997).

[0008] In Nishimasu, H., et al., the crystal structure of SpyCas9 is shown in complex with sgRNA and its target DNA at 2.5 angstrom resolution (Cell, 2014. 156(5): p. 935-49). The crystal structure identified two lobes to the Cas9 enzyme: a recognition lobe (REC) and a nuclease lobe (NUC). The sgRNA:target DNA heteroduplex (negatively charged) sits in the positively charged groove between the two lobes. The REC lobe, which shows no structural similarity with known proteins and therefore likely a Cas9-specific functional domain, interacts with the portions of the crRNA and tracrRNA that are complementary to each other.

[0009] Another group, Briner et al. (Mol Cell, 2014. 56(2): p. 333-9), identified and characterized the six conserved modules within native crRNA:tracrRNA duplexes and sgRNA.

[0010] US2014273232 (A1) discloses vectors and vector systems, some of which encode one or more components of a CRISPR complex, as well as methods for the design and use of such vectors. Also provided are methods of directing CRISPR complex formation in eukaryotic cells and methods for selecting specific cells by introducing precise mutations utilizing the CRISPR-Cas system.

[0011] WO2014144592 (A2) discloses methods for increasing specificity of RNA-guided genome editing, e.g., editing using CRISPR/Cas9 systems, using truncated guide RNAs (tru-gRNAs).

[0012] WO2014065596 (A1) relates to a composition for cleaving a target DNA in eukaryotic cells or organisms comprising a guide RNA specific for the target DNA and Cas protein-encoding nucleic acid or Cas protein, and use thereof.

[0013] WO2014124226 (A1) discloses compositions and methods for selectively reducing the amount of antibiotic resistant and/or virulent bacteria in a mixed bacteria population, or for reducing any other type of unwanted bacteria in a mixed bacteria population. The compositions

and methods involve targeting bacteria that are differentiated from other members of the population by at least one unique clustered regularly interspaced short palindromic repeats (CRISPR) targeted DNA sequence.

[0014] Regina Cencic et al, "Protospacer Adjacent Motif (PAM)-Distal Sequences Engage CRISPR Cas9 DNA Target Cleavage", PLOS ONE, (20141002), vol. 9, no. 10, teach using chromatin immunoprecipitation followed by sequencing (ChIP-seq), to delineate the genome-wide binding panorama of catalytically inactive Cas9 directed by two different single guide (sg) RNAs targeting the Trp53 locus. Interactions between the 5' end of the guide and PAM-distal target sequences are found necessary to efficiently engage Cas9 nucleolytic activity, providing an explanation for why off-target editing is significantly lower than expected from ChIP-seq data.

[0015] The CRISPR-Cas9 system is utilized in genomic engineering as follows: a portion of the crRNA hybridizes to a target sequence, a portion of the tracrRNA hybridizes to a portion of the crRNA, and the Cas9 nuclease binds to the entire construct and directs cleavage. The Cas9 contains two domains homologous to endonucleases HNH and RuvC, wherein the HNH domain cleaves the DNA strand complementary to the crRNA and the RuvC-like domain cleaves the noncomplementary strand. This results in a double-stranded break in the genomic DNA. When repaired by non-homologous end joining (NHEJ) the break is typically shifted by 1 or more bases, leading to disruption of the natural DNA sequence and in many cases leading to a frameshift mutation if the event occurs in the coding exon of a protein-encoding gene. The break by also be repaired by homology dependent recombination (HDR), which permits insertion of new genetic material via experimental manipulation into the cut site created by Cas9 cleavage.

[0016] Some of the current methods for guide RNA delivery into mammalian cells include transfection of double-stranded DNA (dsDNA) containing RNA Pol III promoters for endogenous transcription, viral delivery, transfection of RNAs as *in vitro* transcription (IVT) products, or microinjection of IVT products. There are disadvantages to each of these methods. Unmodified exogenous RNA introduced into mammalian cells is known to initiate the innate immune response via recognition by Toll-like Receptors (TLRs), RIG-I, OASI and others receptors that recognize pathogen-associated molecular patterns (PAMPs). However, in most published studies, RNA which has been *in vitro* transcribed (IVT) by a T7 RNA polymerase is delivered to the cells. This type of RNA payload has been shown to be a trigger for the innate immune response. The alternative delivery methods described above each have their own disadvantages as well. For example, dsDNA cassettes can lead to integration, guide RNA transcription driven endogenously by a RNA Pol II promoter can persist constitutively, and the amount of RNA transcribed is uncontrollable.

[0017] RNA is quickly degraded by nucleases present in serum and in cells. Unmodified CRISPR RNA triggers (crRNAs, tracrRNAs, and sgRNAs) made by IVT methods or chemical synthesis are quickly degraded during delivery or after delivery to mammalian cells. Greater activity would be realized if the RNA was chemically modified to gain nuclease resistance. The most potent degradative activity present in serum and in cells is a 3'-exonuclease (Eder et al., Antisense Research and Development 1:141-151, 1991). Thus "end blocking" a synthetic oligonucleotide often improves nuclease stability. Chemical modification of single-stranded antisense

oligonucleotides (ASOs) and double-stranded small interfering RNAs (siRNAs) has been well studied and successful approaches are in practice today (for reviews, see: Kurreck, Eur. J. Biochem., 270:1628-1644, 2003; Behlke, Oligonucleotides, 18:305-320, 2008; Lennox et al., Gene Therapy, 18:1111-1120, 2011). It is therefore desirable to devise chemical modification strategies for use with the RNA components of CRISPR/Cas. While the basic toolbox of chemical modifications available is well known to those with skill in the art, the effects that site-specific modification have on the interaction of a RNA species and an effector protein are not easily predicted and effective modification patterns usually must be empirically determined. In some cases, sequence of the RNA may influence the effectiveness of a modification pattern, requiring adjustment of the modification pattern employed for different sequence contexts, making practical application of such methods more challenging.

[0018] There is therefore a need to modify the guide RNA to reduce its toxicity to cells and to extend lifespan and functionality in mammalian cells while still performing their intended purpose in the CRISPR-Cas system. The methods and compositions described herein provide RNA and modified RNA oligonucleotides for use in a CRISPR-Cas system.

SUMMARY OF THE INVENTION

[0019] In a first embodiment, the instant invention provides an isolated tracrRNA comprising a chemically-modified form of one of SEQ ID NOs.:2, 18, 30-33 and 36-39, wherein the isolated tracrRNA displays activity in a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) endonuclease system, wherein the chemically-modified form of one of SEQ ID NOs.:2, 18, 30-33 and 36-39 comprises a chemically-modified nucleotide having an end-modifying group selected from a group consisting of a propanediol (C3) spacer, N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine ("ZEN"), and an inverted-dT residue

[0020] In a second embodiment, the instant invention provides an isolated crRNA comprising a chemically-modified form of formula (I):

5'-X-Z-3' (I),

wherein X represents sequences comprising a target-specific protospacer domain comprising from 17 nucleotides to 20 nucleotides and Z represents sequences comprising a tracrRNA-binding domain comprising from 12 nucleotides to 19 nucleotides,
wherein the isolated crRNA displays activity in a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) endonuclease system¬,
wherein the chemically-modified form of formula (I) comprises a chemically-modified nucleotide having an end-modifying group selected from the group consisting of a propanediol (C3) spacer, N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine ("ZEN"), and an inverted-dT residue

[0021] In a third embodiment, the instant invention provides an isolated tracrRNA selected from the group consisting of SEQ ID NOs.:11-13, 17, 87-106, 110-114, 116, 117, 126, 129, 130, 131,

132, 134, 136, 144, 145, and 147-156, 159, 160, 449 and 451, wherein the isolated tracrRNA is active in a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein endonuclease system.

[0022] In a fourth embodiment, the instant invention provides an isolated crRNA selected from the group consisting of SEQ ID NOs.: 14, 164, 166-169, 171, 174-178, 186, 187, 189-207, 212-214, 221-224, 226-228, 233-249, 252, 256-259, 264-271, 291-374, 376, 377, 379-384, 430-437, and 445-448, wherein the isolated crRNA is active in a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein endonuclease system.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023]

FIG. 1 [reference] is an illustration of a wild-type (WT) natural 2-part crRNA:tracrRNA complex with a 42 base unmodified crRNA (SEQ ID No. 46_) and an 89 base unmodified tracrRNA (SEQ ID No. 18). Lowercase letters represent RNA.

FIG. 2 [reference] is an illustration of a 99 base artificial single-guide RNA (SEQ ID NO: 428_) (sgRNA) that fuses the crRNA and tracrRNA elements into a single sequence through the addition of a new hairpin loop. Lowercase letters represent RNA.

FIG. 3 [reference] shows an alignment of the full-length and truncated tracrRNA species studied in Example 2. Sequences are RNA and are shown 5'-3'. Alignment is based upon the 89 base WT tracrRNA sequence at the top (SEQ ID No. 18). Internal gaps represent sites of internal truncation/deletion. Uppercase letters represent RNA.

FIG. 4 [reference] shows an alignment of the full-length and truncated crRNA and tracrRNA species studied in Example 3. Alignment is based upon the 42 base WT crRNA (SEQ ID No. 46) and 89 base WT tracrRNA (SEQ ID No. 18) sequences at the top of their respective groupings. The 20 base 5'-domain in the crRNAs is sequence-specific and targets human *HPRT1.* The 3'-domain in underlined and binds to a region towards the 5'-end of the tracrRNA. The 5'-domain in the tracrRNA is underlined that binds the 3'-end of the crRNA. Uppercase letters represent RNA.

FIG. 5 [reference] is an illustration of a truncated 2-part crRNA:tracrRNA complex with a 36 base crRNA (SEQ ID No. 48) and a 67 base tracrRNA (SEQ ID No. 2). Lowercase letters represent RNA.

FIG. 6 [invention] is a schematic showing structure of one embodiment of an optimized truncated and chemically-modified tracrRNA (SEQ ID No. 134). Length is 67 bases. RNA is lower case and 2'OMe RNA is uppercase. Phosphorothioate (PS) internucleotide linkages are indicated by "*". Residues which lead to substantial loss of function when converted from RNA to 2'OMe RNA are identified by large arrows and residues which lead to a moderate loss of function when converted from RNA to 2'OMe RNA are identified by small arrows.

FIG. 7 [invention] is a schematic showing structure of one embodiment of an optimized truncated

and chemically-modified crRNA (SEQ ID No. 239). Length is 36 bases. RNA is lower case and 2'OMe RNA is uppercase. Phosphorothioate (PS) internucleotide linkages are indicated by "*". Residues which lead to substantial loss of function when converted from RNA to 2'OMe RNA are identified by large arrows and residues which lead to a moderate loss of function when converted from RNA to 2'OMe RNA are identified by small arrows. The 5'-end 20 base protospacer target-specific guide domain is indicated, which in this case is sequence specific to the human *HPRT1* gene. The 3'-end 16 base tracrRNA binding domain is indicated.

FIG. 8 [invention] is a schematic showing structure of one embodiment of the optimized truncated/modified crRNA:tracrRNA complex as employed in Example 8. The crRNA is positioned at the top with the 5'-protospacer domain 20 base underlined, which in this case is specific for target human *HPRT1* site 38285; the 3'-end is the 16 base tracrRNA binding domain. The tracrRNA is aligned below. RNA is lower case, 2'OMe RNA is uppercase, and "*" indicates a phosphorothioate internucleotide linkage modification. This figure shows the complex formed by crRNA SEQ ID No. 178 and tracrRNA SEQ ID No. 100.

FIG. 9 [invention] is a schematic showing structure of one embodiment of the optimized truncated/modified crRNA:tracrRNA complex that is highly modified. The crRNA is positioned at the top with the 5'-protospacer domain 20 base underlined, which in this case is specific for target human *HPRT1* site 38285; the 3'-end is the 16 base tracrRNA binding domain. The tracrRNA is aligned below. RNA is lower case, 2'OMe RNA is uppercase, and "*" indicates a phosphorothioate internucleotide linkage modification. This figure shows the complex formed by crRNA SEQ ID No. 446 and tracrRNA SEQ ID No. 134.

FIG. 10 [invention] is a schematic showing the crRNA modification patterns employed in Example 10. Oligonucleotide sequences (SEQ ID NOS 429-439, respectively, in order of appearance) are shown 5'-3'. Lowercase = RNA; Underlined = 2'-O-methyl RNA; C3 = C3 spacer (propanediol modifier); * = phosphorothioate internucleotide linkage; ZEN = napthyl-azo modifier. The 5'-target specific protospacer domain is indicated. Bases are indicated by "N" in this domain as sequence is different for each target site, although the modification pattern employed remains constant. The 3'-universal tracrRNA binding domain is indicated. Modification patterns are numbered for reference between Table 10 and FIG. 10.

FIG. 11 [invention] is a plot of the data in Table 10 showing the functional gene editing observed using the T7E1 assay in mammalian cells using crRNAs made with 11 different modification patterns tested at 12 different sites in the human *HPRT1* gene. All crRNA variants were paired with an optimized, modified tracrRNA (SEQ ID No. 100).

FIG. 12 [invention] is a schematic showing structure of one embodiment of the optimized truncated/modified crRNA:tracrRNA complex that is highly modified using crRNA Mod Pattern 6 that is universal and can be applied in any sequence context. The crRNA (SEQ ID NO: 440) is positioned at the top with the 5'-protospacer domain 20 base underlined (N-bases); the 3'-end is the 16 base tracrRNA binding domain. The tracrRNA is aligned below (SEQ ID No. 134). RNA is lower case, 2'OMe RNA is uppercase, and "*" indicates a phosphorothioate internucleotide linkage modification.

FIG. 13 [invention] shows a plot of RT-qPCR data from HEK-Cas9 cells transfected with different

CRISPR gRNAs showing relative expression levels of IFIT1 and IFITM1, 2 genes involved in interferon signaling pathways.

DETAILED DESCRIPTION OF THE INVENTION

[0024] Aspects of this disclosure relate to modified compositions for use in CRISPR systems, and their methods of use.

[0025] The term "oligonucleotide," as used herein, refer to polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), and to any other type of polynucleotide which is an N glycoside of a purine or pyrimidine base (a single nucleotide is also referred to as a "base" or "residue"). There is no intended distinction in length between the terms "nucleic acid", "oligonucleotide" and "polynucleotide", and these terms can be used interchangeably. These terms refer only to the primary structure of the molecule. Thus, these terms include double- and single-stranded DNA, as well as double- and single-stranded RNA. For use in the present disclosure, an oligonucleotide also can comprise nucleotide analogs in which the base, sugar or phosphate backbone is modified as well as non-purine or non-pyrimidine nucleotide analogs. An oligonucleotide may comprise ribonucleotides, deoxyribonucleotides, modified nucleotides (e.g., nucleotides with 2' modifications, synthetic base analogs, etc.) or combinations thereof.

[0026] Compositions of the present disclosure include any modification that potentially reduces activation of the innate immune system. Modifications can be placed or substituted at a conventional phosphodiester linkage, at the ribose sugar, or at the nucleobase of RNA. Such compositions could include, for example, a modified nucleotide such as 2'-O-methly-modified RNAs.

[0027] More broadly, the term "modified nucleotide" refers to a nucleotide that has one or more modifications to the nucleoside, the nucleobase, pentose ring, or phosphate group. For example, modified nucleotides exclude ribonucleotides containing adenosine monophosphate, guanosine monophosphate, uridine monophosphate, and cytidine monophosphate and deoxyribonucleotides containing deoxyadenosine monophosphate, deoxyguanosine monophosphate, deoxythymidine monophosphate, and deoxycytidine monophosphate. Modifications include those naturally occurring that result from modification by enzymes that modify nucleotides, such as methyltransferases. Modified nucleotides also include synthetic or non-naturally occurring nucleotides. Modifications also include base analogs and universal bases. Synthetic or non-naturally occurring modifications in nucleotides include those with 2' modifications, e.g., 2'-O-alkyl (including 2'-O-methyl), 2'-fluoro, 2'-methoxyethoxy, 2'-allyl, 2'-O-[2-(methylamino)-2-oxoethyl], 4'-thio, bicyclic nucleic acids, 4'-CH2-O-2'-bridge, 4'-(CH2)2-O-2'-bridge, 2'-LNA, and 2'-O-(N-methylcarbamate) or those comprising base analogs. Such modified groups are described, e.g., in Eckstein et al., U.S. Pat. No. 5,672,695 and Matulic-Adamic et al., U.S. Pat. No. 6,248,878.

[0028] The use of 2'-O-methyl has been documented in siRNA literature (See Behlke, M.A., Oligonucleotides, 2008. 18(4): p. 305-19) as well as in mRNA delivery (see Sahin, U. et al., Nat Rev Drug Discov, 2014. 13(10): p. 759-80). Sahin et al., describes modifications of mRNA therapeutics that extend beyond 2'-OMe modification and "non-immunogenic" mRNA.

[0029] The term "ribonucleotide" encompasses natural and synthetic, unmodified and modified ribonucleotides. Modifications include changes to the sugar moiety, to the base moiety and/or to the linkages between ribonucleotides in the oligonucleotide.

[0030] The term "Cas9 protein" encompasses wild-type and mutant forms of Cas9 having biochemical and biological activity when combined with a suitable guide RNA (for example sgRNA or dual crRNA:tracrRNA compositions) to form an active CRISPR-Cas endonuclease system. This includes orthologs and Cas9 variants having different amino acid sequences from the *Streptococcus pyogenese* Cas9 employed as example in the present disclosure.

[0031] The term "length-modified," as that term modifies RNA, refers to a shortened or truncated form of a reference RNA lacking nucleotide sequences or an elongated form of a reference RNA including additional nucleotide sequences.

[0032] The term "chemically-modified," as that term modifies RNA, refers to a form of a reference RNA containing a chemically-modified nucleotide or a non-nucleotide chemical group covalently linked to the RNA. Chemically-modified RNA, as described herein, generally refers to synthetic RNA prepared using oligonucleotide synthesis procedures wherein modified nucleotides are incorporated during synthesis of an RNA oligonucleotide. However, chemically-modified RNA also includes synthetic RNA oligonucleotides modified with suitable modifying agents post-synthesis.

[0033] Applicants have discovered novel crRNA and tracrRNA oligonucleotide compositions that display robust activity in the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) endonuclease system. The oligonucleotide compositions include length-modified forms of crRNA and tracrRNA, as well as chemically-modified forms of crRNA and tracrRNA. The length-modified forms of crRNA and tracrRNA enable one to prepare active forms of these RNAs with cost-effective and efficient oligonucleotide synthesis protocols routinely available. The chemically-modified forms of crRNA and tracrRNA provide one with active agents tunable with certain specific properties, such as improved stability in cellular and *in vivo* contexts. The length-modified forms of crRNA and tracrRNA can also include modifications, thereby enabling access to a broad range of compositions having activity in CRISPR-Cas endonuclease system contexts. These oligonucleotide compositions and their properties in the CRISPR-Cas endonuclease system are described below.

Length-modified forms of crRNA and tracrRNA

[0034] FIG.1 depicts a representation of the wild-type *S. pyogenes* crRNA:tracrRNA complex, wherein an exemplary isolated crRNA (SEQ ID No. 46) is paired with an isolated tracrRNA (SEQ

ID No. 18). In a first aspect of the instant disclosure, an isolated tracrRNA including a length-modified form of SEQ ID NO.:18 is provided. The isolated tracrRNA displays activity in the CRISPR-Cas endonuclease system. In one respect, the isolated tracrRNA includes a length-modified form of SEQ ID NO.:18 nucleotide having deleted sequence information. In some aspects of the instant disclosure, the length-modified form of SEQ ID NO.:18 includes shortened or truncated forms of SEQ ID NO.:18, wherein SEQ ID NO.:18 can be shortened by 1 to 20 nucleotides at the 5'-end and by 1-10 nucleotides at the 3'-end. Such shortened or truncated forms of SEQ ID NO.:18 retain activity when paired with a functionally competent crRNA in the CRISPR-Cas endonuclease system. Where shortening of the 5'-end of the tracrRNA is performed and extends into sequence that pairs with the 3'-end of the crRNA, improved activity may be obtained using chemical modification that enhance binding affinity in these domains. Where shortening of the 3'-end of the crRNA is performed and extends into sequence that pairs with the 5'-end of the tracrRNA, improved activity may be obtained using chemical modification that enhance binding affinity in these domains. Preferred examples according to the instant disclosure of a length-modified form of SEQ ID NO.:18 having a shortened or truncated form include SEQ ID NOs:2, 30-33 and 36-39. A highly preferred example of a length-modified form of SEQ ID NO.:18 having a shortened or truncated form includes SEQ ID NO:2. For each of the foregoing exemplary length-modified forms of SEQ ID NO.: 18 having a shortened or truncated form, SEQ ID NOs.:2, 30-33 and 36- 39 according to the instant disclosure can consist of chemically non-modified nucleotides.

[0035] According to the first embodiment of the instant invention, an isolated tracrRNA comprising a chemically-modified form of one of SEQ ID NOs.:2, 18, 30-33 and 36-39, wherein the isolated tracrRNA displays activity in a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) endonuclease system, wherein the chemically-modified form of one of SEQ ID NOs.:2, 18, 30-33 and 36-39 comprises a chemically-modified nucleotide having an end-modifying group selected from a group consisting of a propanediol (C3) spacer, N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine ("ZEN"), and an inverted-dT residue.

[0036] Particularly preferred are isolated tracrRNA , wherein the isolated tracrRNA is selected from the group consisting of SEQ ID NOs.: 97, 99, 104, 105, 130, 131, and 132.
The third embodiment of the invention is directed to an isolated crRNA selected from the group consisting of SEQ ID NOs.: 14, 164, 166-169, 171, 174-178, 186, 187, 189-207, 212-214, 221-224, 226-228, 233-249, 252, 256-259, 264-271, 291-374, 376, 377, 379-384, 430-437, and 445-448, wherein the isolated crRNA is active in a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein endonuclease system.

[0037] In a second aspect of the instant disclosure, an isolated crRNA comprising a length-modified form of formula (I) is provided:

5'-X-Z-3' (I),

wherein X represents sequences including a target-specific protospacer domain, and Z represents sequences including a tracrRNA-binding domain.

[0038] The target-specific protospacer domain (X domain of formula (I)) typically includes about twenty nucleotides having complementarity to a region of DNA targeted by the CRISPR-Cas endonuclease system. The tracrRNA-binding domain (the Z domain of formula (I)) typically includes about 20 nucleotides in most CRISPR endonuclease systems (in the native *S.py.* version, this domain is 22 nucleotides). The isolated crRNA displays activity in the CRISPR-Cas endonuclease system.

[0039] In one respect, the isolated crRNA includes a length-modified form of formula (I) having deleted sequence information. In some aspects of the instant disclosure, the length-modified form of formula (I) includes shortened or truncated forms of formula (I), wherein formula (I) can be shortened by 1-8 nucleotides at the 3'-end of the Z domain. The length-modified form of formula (I) can be shortened at the 5-end of the X-domain to accommodate a target-specific protospacer domain having 17, 18, 19 or 20 nucleotides. Highly preferred examples of such length-modified form of formula (I) include target-specific protospacer domain having 19 or 20 nucleotides. The exemplary length-modified forms of formula (I) having a shortened or truncated form with a target-specific protospacer (X-domain) of 17-20 nucleotides in length and/or lacking 1-8 nucleotides at the 3'-end of the Z-domain can consist of chemically non-modified nucleotides.

[0040] Such shortened or truncated forms of formula (I) according to the instant disclosure retain activity when paired with a competent tracrRNA in the CRISPR-Cas endonuclease system. Preferred spects of isolated crRNA of formula (I) having a length-modified form of formula (I) can include chemically non-modified nucleotides and chemically modified nucleotides.

Chemically-modified forms of crRNA and tracrRNA

[0041] In a third aspect of the instant disclosure, an isolated tracrRNA including a chemically-modified nucleotide or a non-nucleotide chemical modifier is provided. The isolated tracrRNA displays activity in the CRISPR-Cas endonuclease system. In one respect, the isolated tracrRNA includes a chemically-modified nucleotide having a modification selected from a group consisting of a ribose modification, an end-modifying group, and internucleotide modifying linkages. Exemplary ribose modifications include 2'O-alkyl (e.g., 2'OMe), 2'F, bicyclic nucleic acid, and locked nucleic acid (LNA). Exemplary end-modifying groups include a propanediol (C3) spacer and napthyl-azo modifier (N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine, or "ZEN"), and an inverted-dT residue. Exemplary internucleotide modifying linkages include phosphorothioate modification. In one respect, the isolated tracrRNA having a chemically-modified form include SEQ ID NO.:46 and length-modified forms thereof, such as shortened or truncated forms of SEQ ID NO.:46. Preferred shortened or truncated forms of SEQ ID NO.:46 having a chemically-modified nucleotide include SEQ ID NOs:2, 30-33 and 36-39 having a chemically-modified nucleotide. Yet other examples of isolated tracrRNA having a chemically-modified nucleotide with robust activity in the CRISPR-Cas endonuclease system are presented in the Examples.

[0042] In a fourth aspect of the instant disclosure, an isolated crRNA including a chemically-modified nucleotide is provided. The isolated crRNA displays activity in the CRISPR-Cas

endonuclease system. In one respect, the isolated crRNA includes a chemically-modified nucleotide having a modification selected from a group consisting of a ribose modification, an end-modifying group, and internucleotide modifying linkage. Exemplary ribose modifications include 2'O-alkyl (e.g., 2'OMe), 2'F, bicyclic nucleic acid, and locked nucleic acid (LNA). Exemplary end-modifying groups include a propanediol (C3) spacer and napthyl-azo modifier (N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine, or "ZEN"), and an inverted-dT residue. Exemplary internucleotide modifying linkages include phosphorothioate modification. In one respect, the isolated crRNA having a chemically-modified form include crRNA of formula (I) and length-modified forms thereof.

[0043] More in particular, according to the second embodiment of the instant invention, an isolated crRNA comprising a chemically-modified form of formula (I):

5'-X-Z-3' (I)

is provided,
wherein X represents sequences comprising a target-specific protospacer domain comprising from 17 nucleotides to 20 nucleotides and Z represents sequences comprising a tracrRNA-binding domain comprising from 12 nucleotides to 19 nucleotides, wherein the isolated crRNA displays activity in a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) endonuclease system, wherein the chemically-modified form of formula (I) comprises a chemically-modified nucleotide having an end-modifying group selected from the group consisting of a propanediol (C3) spacer, N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine ("ZEN"), and an inverted-dT residue.

[0044] Particularly preferred is isolated crRNA, wherein the chemically-modified form of formula (I) is selected from SEQ ID NOs.: 163, 179-181, 235-237, 240-243, 256, 257, 268-271, 385-406, 438 and 439.

[0045] According to the fourth embodiment of the instant invention, an isolated crRNA selected from the group consisting of SEQ ID NOs.: 14, 164, 166-169, 171, 174-178, 186, 187, 189-207, 212-214, 221-224, 226-228, 233-249, 252, 256-259, 264-271, 291-374, 376, 377, 379-384, 430-437, and 445-448, wherein the isolated crRNA is active in a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein endonuclease system is provided.

[0046] Preferred shortened or truncated forms of crRNA of formula (I) having a chemically-modified nucleotide according to the instant disclosure include SEQ ID NOs.:429-439, with SEQ ID NOs. 438 and 439 being preferred by the instant invention. Highly preferred examples of an isolated crRNA according to the invention having a chemically-modified nucleotide include SEQ ID NOs.:434 and 435. These particular isolated crRNA species represent "universal" crRNAs having a chemically-modified nucleotide showing high activity when combined with a competent tracrRNA in the CRISPR-Cas endonuclease system. Yet other examples of isolated crRNA having a chemically-modified nucleotide with robust activity in the CRISPR-Cas endonuclease system are presented in the Examples.

[0047] The foregoing isolated, length-modified and chemically-modified of crRNA and tracrRNA preferably include chemical modifications at the 2'-OH groups (for example, 2'OMe, 2'F, bicyclic nucleic acid, locked nucleic acid, among others) and end-blocking modifications (for example, ZEN, C3 spacer, inverted-dT). Use of both types of general modifications provides isolated, length-modified and chemically-modified of crRNA and tracrRNA with biochemical stability and immunologic tolerance for isolated, length-modified and chemically-modified of crRNA and tracrRNA in biological contexts.

[0048] The foregoing isolated, length-modified and chemically-modified of crRNA and tracrRNA can be mixed in different combinations to form active crRNA:tracrRNA as the guide RNA for Cas9. For example, an isolated, length-modified tracrRNA can be combined with an isolated chemically-modified crRNA to form an active crRNA:tracrRNA as the guide RNA for Cas9. The Examples provide illustrations of different combinations of isolated, length-modified and chemically-modified of crRNA and tracrRNA resulting in active crRNA:tracrRNA as the guide RNA for Cas9.

[0049] The extent to which one needs particular chemically-modified nucleotides included in one (or both) of the isolated, length-modified and chemically-modified crRNA and tracrRNA depends upon the application for which the resultant active crRNA:tracrRNA serves as the guide RNA for Cas9. In certain biochemical assays of the CRISPR-Cas endonuclease system, particularly where nucleases can be minimized or absent, one may not need extensively chemically-modified crRNA and tracrRNA to effect robust activity of the resultant guide RNA for Cas9 of the CRISPR-Cas endonuclease system. This is attributed to the fact that chemically-modified nucleotides that confer resistance to nucleases are not necessary when nucleases are minimal or absent. In certain biological *(in vivo)* contexts, wherein a mixture including crRNA and tracrRNA is delivered to cells inside carrier vehicles, such as liposome nanoparticles, the isolated length-modified and chemically-modified crRNA and tracrRNA may require less extensive chemically-modified nucleotides than mixtures of crRNA and tracrRNA delivered directly into the blood stream or injected into organ systems as isolated, "naked," RNA mixtures. The extent of chemical modification present in chemically-modified crRNA and tracrRNA can dictate the half-life of the relevant RNA molecules *in vivo* (that is, in the relevant biological context, such as, for example, in the blood stream or inside cells). Accordingly, the modification profile of chemically-modified crRNA and tracrRNA can be used to fine tune the biochemical and biological activity of the resultant crRNA:tracrRNA duplexes as a guide RNA for Cas9 in the CRISPR-Cas endonuclease system.

[0050] Although the prior art focuses on the structure of Cas9 as it interacts with a sgRNA, the disclosed design patterns described herein contemplate the aforementioned crRNA:tracrRNA dual RNA systems. A single strand guide RNA offers several benefits, such as simplicity of a therapeutic design. However, standard solid phase phosphoramidite RNA synthesis shows diminishing yields for oligonucleotides as length increases and this problem becomes more apparent as length exceeds 60-70 bases. This precludes robust, cost-effective synthesis of some tracrRNAs as well as the chimeric sgRNA, especially at larger scales needed for some commercial or therapeutic applications. For this reason, the disclosure contemplates aspects of not only sgRNA, but also alternate dual crRNA:tracrRNA as the guide RNA for Cas9. However, an

isolated guide RNA having robust activity when combined with Cas9 in the CRISPR-Cas endonuclease system can be engineered by linkage or synthesis of appropriate crRNA and tracrRNA as an artificial, unimolecular sgRNA based upon the isolated, length-modified and chemically-modified forms of crRNA and tracrRNA provided herein. Long single guides of this type may be obtained by direct synthesis or by post-synthetic chemical conjugation of shorter strands.

[0051] The design of length-modified and chemically-modified tracrRNA compositions addresses the potential synthetic issues associated with tracrRNA oligonucleotides that are >80 nucleotides in length. The coupling efficiency of 2'-OMe-modified RNA monomers (effectively containing a protecting group on the 2'-OH) is greater than RNA monomer coupling. Incorporating 2'-OMe modified RNAs provides some advantages. First, it allows for longer oligonucleotides to be synthesized as either full 2'-OMe or RNA/2'-OMe mixed oligonucleotides. Secondly, the methods and compositions of the disclosure lead to synthesis and transfection of crRNA:tracrRNA that can evade detection by the immune system. It is well known that exogenous, unmodified RNAs trigger an innate immune response in mammalian cells as well as whole animals. Using 2'OMe-modified oligonucleotides can confer RNA stability to nucleases (a third advantage) as well as reduce cell death and toxicity associated with immunogenic triggers. These advantages are not unique to 2'-OMe modification, per se, as the other disclosed modified nucleotides having different chemical moieties (for example, 2'F, other 2'O-alkyls, LNA, and other bicyclic nucleotides) can offer similar benefits and advantages in terms of conferring resistance to nucleases.

[0052] In another embodiment of the instant invention, the tracrRNA portion according to the instant invention complementary to the crRNA according to the instant invention contains at least one modified nucleotide, and in a further embodiment the tracrRNA portion according to the invention complementary to the crRNA according to the invention is comprised of more than 10% modified residues, and in a further embodiment the tracrRNA portion according to the invention not complementary to the crRNA according to the invention is comprised of more than 50% modified residues, and in a further embodiment the tracrRNA portion according to the invention not complementary to the crRNA according to the invention is comprised of more than 90% modified residues.

[0053] In another aspect, the crRNA portion is unmodified and the tracrRNA portion is comprised of at least one modified nucleotide. In a further aspect the crRNA portion is unmodified and the tracrRNA portion is comprised of more than 10% modified bases.

[0054] In another aspect of the instant disclosure, an isolated crRNA of formula (I) is designed with modifications that are empirically determined. As depicted in FIGs. 7 and 10, the 12 nucleotides at the 3'-end of the Z domain (the tracrRNA-binding domain) and the 10-12 nucleotides at the 5'-end of the X domain (within the protospacer domain) represent universal nucleotides amenable to substitution with chemically-modified nucleotides, wherein the resultant RNAs retain robust activity in the CRISPR-Cas endonuclease system. Yet other nucleotides within the 5'-end of the Z domain (the tracrRNA-binding domain) are intolerant to substitution with chemically-modified nucleotides (FIG. 7). Yet the ability of other sites within an isolated crRNA of formula (I) to accept chemically-modified nucleotides and retain activity in the CRISPR-Cas

endonuclease system is largely determined empirically. The tracrRNA binding domain (Z domain) of the crRNA is constant (i.e., sequence does not change as target site varies), so the modifications patterns described herein are universal to all crRNAs regardless of target site and can be broadly applied. The protospacer (X domain) of the crRNA varies with target, and the tolerance of some of the base positions within this domain to chemical modification vary with sequence context and, if maximal chemical modification of a site is desired, may benefit from empiric optimization. However, some of the residues within the target-specific protospacer (X) domain can be modified without consideration to sequence context. The 10-12 residues at the 5'-end of this domain can be substituted with 2'-modified residues with the expectation that full activity of the modified crRNA will be maintained. The remaining 8-10 bases towards the 3'-end of the protospacer (X) domain may tolerate modification or may not, depending on sequence context. One sequence context where 17 out of the 20 bases of the protospacer (X) domain can be modified while maintaining full activity are shown in FIG. 7. Sites were modification compromised activity are indicated.

[0055] The applications of Cas9-based tools are many and varied. They include, but are not limited to: plant gene editing, yeast gene editing, rapid generation of knockout/knockin animal lines, generating an animal model of disease state, correcting a disease state, inserting a reporter gene, and whole genome functional screening.

[0056] The utility of the present disclosure is further expanded by including mutant versions of Cas enzymes, such as a D10A and H840a double mutant of Cas9 as a fusion protein with transcriptional activators (CRISPRa) and repressors (CRISPRi) (see Xu, T., et al., Appl Environ Microbiol, 2014. 80(5): p. 1544-52). The Cas9-sgRNA complex also can be used to target single-stranded mRNA as well (see O'Connell, M.R., et al., Nature, 516:263, 2014). In the same way as targeting dsDNA, crRNA:tracrRNA can be used with a PAMmer DNA oligonucleotide to direct Cas9 cleavage to the target mRNA or use it in the mRNA capture assay described by O'Connell.

[0057] By utilizing an approach to deliver synthetic RNA oligonucleotides for CRISPR/Cas9 applications, it is possible to 1) use mass spectroscopy to confirm discrete RNA sequences, 2) selectively insert 2'-OMe modified RNAs in well-tolerated locations to confer stability and avoid immunogenicity yet retain functional efficacy, 3) specifically control the amount of RNA that is introduced into cells for a controlled transient effect, and 4) eliminate concern over introducing dsDNA that would be endogenously transcribed to RNA but could also become substrate in either homology-directed repair pathway or in non-homologous end joining resulting in an integration event. These integration events can lead to long term undesired expression of crRNA or tracrRNA elements. Further, integration can disrupt other genes in a random and unpredictable fashion, changing the genetic material of the cell in undesired and potentially deleterious ways. The present disclosure is therefore desirable as a means to introduce transient expression of elements of the CRISPR pathway in cells in a way which is transient and leaves no lasting evidence or change in the genome outside of whatever alteration is intended as directed by the crRNA guide.

CRISPR-Cas endonuclease systems

[0058] A competent CRISPR-Cas endonuclease system includes a ribonucleoprotein (RNP) complex formed with isolated Cas9 protein and isolated guide RNA selected from one of a dual crRNA:tracrRNA combination and a chimeric sgRNA. In some aspects, isolated length-modified and/or chemically-modified forms of crRNA and tracrRNA are combined with purified Cas9 protein (for example, SEQ ID NOs.:407-410), an isolated mRNA encoding Cas9 protein (for example, SEQ ID NO.:413), or a gene encoding Cas9 protein (for example, SEQ ID NOs.: 411 and 412) in an expression vector. In certain assays, isolated length-modified and/or chemically-modified forms of crRNA and tracrRNA can be introduced into cell lines that stably express Cas9 protein from an endogenous expression cassette encoding the Cas9 gene. In other assays, a mixture of length-modified and/or chemically-modified forms of crRNA and tracrRNA in combination with either Cas9 mRNA or Cas9 protein can be introduced into cells.

EXAMPLE 1

[0059] This example illustrates functioning of chemically modified and truncated guide RNAs in an *in vitro* Cas9 DNA cleavage assay.

[0060] CrRNA and tracrRNA oligonucleotides were synthesized having various chemical modifications and truncations relative to the WT sequences as indicated (Table 1).

Table 1: In vitro biochemical studies of cleavage of HPRT1 target DNA by Cas9 endonuclease with various crRNA and tracrRNA pairs.

cr/tracrRNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage
1A	1	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
1B	1	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	3	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
1C	1	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	4	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
2A	5	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	+++
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	

cr/tracrRNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage
2B	5	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	6	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuuuuuu	89	
2C	5	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	4	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu	67	
3A	7	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu	67	
3B	7	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	6	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuuuuuu	89	
3C	7	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	4	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu	67	
4A	8	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu	67	
4B	8	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	6	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuuuuuu	89	
4C	8	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	4	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu	67	
5A	9	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu	67	

cr/tracrRNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage
5B	9	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	6	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
5C	9	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	4	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
6A	10	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
6B	10	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	6	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
6C	10	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	4	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
1G	1	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	11	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
1K	1	uuauauccaacacuucgugguuuuagagcuaugcu	35	++
	12	agcauagcaaguuaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
1L	1	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	13	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
	14	uuauauccaacacuucgugguuuuagagcuaugcu	35	

cr/tracrRNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage
14A	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	+++
14G	14	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	11	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
14K	14	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	12	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
14L	14	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	13	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
15A	15	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
15G	15	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	11	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
15K	15	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	12	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
15L	15	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	13	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
16A	16	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
	16	uuauauccaacacuucgugguuuuagagcuaugcu	35	

cr/tracrRNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage
16G	11	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	+++
16K	16	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	12	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
16L	16	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	13	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
1H	1	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	17	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
2D	5	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	+++
	18	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
2E	5	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	+++
	19	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
2F	5	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	+++
	20	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
21	5	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	++
	21	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
7A	22	uuauauccaacacuucgugguuuuagagcuaugcu	35	-
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	

cr/tracrRNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage
9A	23	uuauauccaacacuucgugguuuuagagcuaugcu	35	+++
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
10A	24	*uuauauccaacacuucgugguuuuagagcuaugcu*	35	+++
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcuuu	67	
3D	7	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	18	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
4D	8	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	18	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
8D	25	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	-
	18	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
13D	26	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	+++
	18	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	
131	26	uuauauccaacacuucgugguuuuagagcuaugcuguuuug	41	+++
	21	guuggaaccauucaaaacagcauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuu uuuuu	89	

Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA, Underlined = 2'-O-methyl RNA, Italics = 2'-fluoro RNA. Lengths of the RNA oligonucleotides are indicated (bases). The relative efficiency of cleavage of the DNA target by recombinant Cas9 with each of the crRNA:tracrRNA pairs as visualized by agarose gel electrophoresis is indicated with "+++" indicating complete cleavage, "++" and "+" indicating intermediate levels of cleavage, and "-" indicating no cleavage.

[0061] The crRNAs contained a 19 base protospacer guide sequence matching a site in the human *HPRT1* gene adjacent to a suitable 'NGG" PAM site. A 938 base pair region from the human *HPRT1* gene was cloned into the pCR-Blunt vector (Life Technologies). The plasmid was linearized by digestion with the restriction endonuclease XmaI (New England BioLabs) prior to use in the Cas9 cleavage assay. Sequence of the *HPRT1* target fragment is shown below. The target PAM site is indicated in bold font and the protospacer guide sequence binding site is underlined.

HPRT1 target sequence. SEQ ID No. 27.

GAATGTTGTGATAAAAGGTGATGCTCACCTCTCCCACACCCTTTTATAGTTTAGGGATTGTATTTCCAAGG
TTTCTAGACTGAGAGCCCTTTTCATCTTTGCTCATTGACACTCTGTACCCATTAATCCTCCTTATTAGCTC
CCCTTCAATGGACACATGGGTAGTCAGGGTGCAGGTCTCAGAACTGTCCTTCAGGTTCCAGGTGATCAACC
AAGTGCCTTGTCTGTAGTGTCAACTCATTGCTGCCCCTTCCTAGTAATCCCCATAATTTAGCTCTCCATTT
CATAGTCTTTCCTTGGGTGTGTTAAAAGTGACCATGGTACACTCAGCACGGATGAAATGAAACAGTGTTTA
GAAACGTCAGTCTTCTCTTTTGTAATGCCCTGTAGTCTCTCTGTATGTTATATGTCACATTTTGTAATTAA
CAGCTTGCTGGTGAAAAGGA**CCC**CACGAAGTGTTGGATATAAGCCAGACTGTAAGTGAATTACTTTTTTTG
TCAATCATTTAACCATCTTTAACCTAAAAGAGTTTTATGTGAAATGGCTTATAATTGCTTAGAGAATATTT
GTAGAGAGGCACATTTGCCAGTATTAGATTTAAAAGTGATGTTTTCTTTATCTAAATGATGAATTATGATT
CTTTTTAGTTGTTGGATTTGAAATTCCAGACAAGTTTGTTGTAGGATATGCCCTTGACTATAATGAATACT
TCAGGGATTTGAATGTAAGTAATTGCTTCTTTTTCTCACTCATTTTTCAAAACACGCATAAAAATTTAGGA
AAGAGAATTGTTTTCTCCTTCCAGCACCTCATAATTTGAACAGACTGATGGTTCCCATTAGTCACATAAAG
CTGTAGTCTAGTACAGACGTCCTTAGAACTGGAACCTGGCCAGGCTAGGGTGACACTTCTTGTTGGCTGAA
ATAGTTGAACAGCTT

[0062] The crRNA and tracrRNA pairs were tested for the ability to direct degradation of a linearized plasmid DNA containing a cloned fragment of the human *HPRT1* gene by recombinant Spy Cas9 (New England BioLabs). The crRNA:tracrRNA were annealed in Duplex Buffer (30 mM HEPES pH 7.5, 100 mM potassium acetate) at 150 nM concentration. Spy Cas9 (15 nM) was preincubated with the crRNA:tracrRNA for 10 min at 37°C at a 1:1 molar ratio. Subsequently, 3 nM of the linearized target plasmid was added and incubated at 37°C for 1 hour. The reaction products were separated on a 1% agarose gel at 125 V for 1 hour. Bands were visualized by GelRed (Biotium) post-staining according to the manufacturer's protocol. The gel was imaged on a UV-transilluminator and results are summarized in Table 1 above.

[0063] Native wild-type (WT) CRISPR RNAs have a 19-20 base protospacer domain (guide, which binds to a target nucleic acid) at the 5'-end and a 22 base domain at the 3'-end that binds to the tracrRNA. Thus WT crRNAs are 41-42 bases long. The WT tracrRNA is 89 bases long. We observed that a WT type crRNA:tracrRNA pair supported full cleavage of the target DNA (cr/tracrRNA pair 2D). We additionally observed that a truncated version of the reagents with a 35 base crRNA (19 base protospacer and 16 base tracrRNA binding domain) paired with a 67 base tracrRNA supported full cleavage of the target RNA (cr/tracrRNA pair 1A). The crRNA tracrRNA binding region was truncated 6 bases at the 3'-end (SEQ ID No. 1). The tracrRNA was truncated at both ends (SEQ ID No. 2). Pairwise combinations of the short crRNA with the long tracrRNA showed cleavage as well as the long crRNA with the short tracrRNA (pair 2A). These findings are significant as it permits use of shorter RNA components to direct Cas9 target recognition and cleavage. Shorter RNA oligonucleotides are less expensive and less difficult for chemical synthesis, requiring less purification and giving higher yields than longer RNA oligonucleotides.

[0064] Some elements of the native crRNA and tracrRNA (Fig. 1) were deleted to make a

functional sgRNA (Fig. 2). However, the amount of duplex nucleic acid binding the crRNA to the tracrRNA in the sgRNA is limited to 11 base pairs, which is typically too short for duplex formation in biological salt conditions. The complex is stable in sgRNA format due to the unimolecular hairpin structure, however the same sequences split into 2 RNAs would be unstable. It was therefore unclear what length of duplex domain was needed to make a minimal yet functional 2-molecule (2-part) CRISPR complex, or if this complex would function to direct target cleavage by Cas9. The present example demonstrates that having as little of 15 bases base paired permits a function 2-part crRNA:tracrRNA complex that is competent to direct Cas9 nuclease activity against a target complementary to the crRNA protospacer domain (SEQ ID Nos. 1 and 2).

[0065] Complete chemical modification of the crRNA with 2'OMe RNA was not tolerated (pair 3A and pair 5A). Further, complete 2'OMe modification of the 22 base tracrRNA binding domain of the crRNA did not support target cleavage (pair 4A, pair 6A) and complete 2'OMe modification of the protospacer guide domain did not support cleavage (pair 7A). Complete chemical modification of the tracrRNA with 2'OMe RNA was also not tolerated (pair 1B, 1C and pair 2B, 2C).

[0066] Importantly, some highly 2'OMe-modified versions of both CRISPR RNA species did support cleavage. Pair 1K shows high cleavage activity with a tracrRNA having 29 2'OMe residues at the 3'-end (SEQ ID No. 11). Pair 1L shows high cleavage activity with 9 2'OMe residues at the 5'-end and 29 2'OMe residues at the 3'-end (SEQ ID No. 13). Thus 38 out of 67 RNA residues in the short version of the tracrRNA can be converted to 2'OMe RNA (57%) with no loss of activity in an *in vitro* cleavage assay.

[0067] Pair 14A demonstrates that 11 bases at the 3'-end of the crRNA (50% of the 22 base tracrRNA binding domain) can be modified with 2'OMe RNA and support target cleavage (SEQ ID No. 14). The modified crRNA retains full activity when paired with the modified tracrRNA (pair 14L, Seq ID Nos. 13 and 14). Modification of 11 bases towards the 5'-end of the crRNA (in the guide, protospacer domain, bases 2-12) supports target cleavage (pair 15A) and this modification is also functional when paired with the modified tracrRNA (pair 15L, SEQ ID Nos. 13 and 15). The 2'OMe modifications towards the 5'-end and 3'-end of the crRNA can be combined (SEQ ID No. 16) such that 22 out of 35 residues are modified (63%) and still support cleavage (pair 16A), even when paired with the modified tracrRNA (pair 16L, SEQ ID Nos. 13 and 16).

[0068] The crRNA:tracrRNA pairs mentioned above all employed 2'OMe RNA as the modifier. Additional studies showed that 2'F modification was also tolerated by Cas9 and enabled cleavage of a target DNA. Pair 9A employs a crRNA with 2'F modification at all pyrimidine bases (SEQ ID No. 23) and this design supported complete target cleavage. Likewise complete 2'F modification of the crRNA supported complete target cleavage (pair 10A, SEQ ID No. 24). Combined use of 2'OMe and 2'F modifications may permit complete modification of both the crRNA and tracrRNA. The studies in the present example utilized *in vitro* biochemical analyses. Performance may vary in the context of mammalian gene editing where the sequences have to function in the milieu of a cell nucleus.

EXAMPLE 2

[0069] This example demonstrates functioning of truncated tracrRNAs to direct genome editing by the Spy Cas9 nuclease in mammalian cells.

[0070] **Both** functional Cas9 nuclease and the RNA triggers (a single sgRNA or dual crRNA:tracrRNA pair) must be present in the nucleus of mammalian cells for CRISPR genome editing to take place. Transfection of large plasmid vectors expressing Cas9 is inefficient and adds variability to experimental results. In order to accurately assess the impact that changes in length and chemical composition of the crRNA and tracrRNA have in mammalian cells in the absence of other variables, a cell line that stably expresses Spy Cas9 was constructed.

[0071] A HEK293 cell line having constitutive expression of SpyCas9 (human codon-optimized) with stable vector integration and selection under G418 was developed as described below. Human optimized Spy Cas9 was ligated into a pcDNA3.1 expression vector (Life Technologies) and transfected into HEK293 cells using Lipofectamine2000 (Life Technologies). The transfected cells were allowed to grow for 2 days before being placed under selective pressure using Neomycin. After 7 days, cells were plated to single colonies using limiting dilution techniques. Monoclonal colonies were screened for Cas9 activity and the clone having highest level of expression was used for future studies. A single copy integration event for Cas9 was determined using droplet digital PCR (ddPCR). Western blot using an anti-Cas9 antibody showed low but constant expression of Cas9 protein. This cell line is henceforth referred to as "HEK-Cas9".

[0072] The HEK-Cas9 cell line was employed in subsequent studies. In a reverse transfection format, anti-*HPRT1* crRNA:tracrRNA complexes were mixed with Lipofectamine RNAiMAX (Life Technologies) and transfected into the HEK-Cas9 cells. Transfections were done with 40,000 cells per well in 96 well plate format. RNAs were introduced at a final concentration of 30 nM in 0.75 µl of the lipid reagent. Cells were incubated at 37°C for 48 hours. Genomic DNA was isolated using QuickExtract solution (Epicentre). Genomic DNA was amplified with KAPA HiFi DNA Polymerase (Roche) and primers targeting the HPRT region of interest (HPRT forward primer: AAGAATGTTGTGATAAAAGGTGATGCT (SEQ ID No. 28); HPRT reverse primer: ACACATCCATGGGACTTCTGCCTC (SEQ ID No. 29)). PCR products were melted and re-annealed in NEB buffer 2 (New England BioLabs) to allow for heteroduplex formation followed by digestion with 2 units of T7 endonuclease 1 (T7EI; New England BioLabs) for 1 hour at 37°C. The digested products were visualized on a Fragment Analyzer (Advanced Analytics). Percent cleavage of targeted DNA was calculated as the average molar concentration of the cut products / (average molar concentration of the cut products + molar concentration of the uncut band) × 100.

[0073] TracrRNAs (Table 2) were synthesized having deletions at the 5'-end, 3'-end, internal or combinations thereof. The tracrRNAs were complexed with unmodified truncated anti-*HPRT1* crRNA SEQ ID No. 1 (Table 1) which has a 19 base protospacer domain targeting *HPRT1* at the 5'-end and a 16 base tracrRNA binding domain at the 3'-end. The paired crRNA:tracrRNA RNA oligonucleotides were transfected into the HEK-Cas9 cells and processed as described above. Relative gene editing activities were assessed by comparing cleavage rates in the *HPRT1* gene

using the T7EI mismatch endonuclease cleavage assay with quantitative measurement of products done using the Fragment Analyzer. A representation of the wild-type S. pyogenes crRNA:tracrRNA complex is shown in FIG. 1 (which pairs crRNA SEQ ID No. 46 with tracrRNA SEQ ID No. 18). The relative location of deletions in the tracrRNA tested in this example are shown in sequence alignment format in FIG. 3.

Table 2: Effect of length truncations in the tracrRNA on efficiency of gene editing in mammalian cells by Cas9 endonuclease.

SEQ ID No.	tracrRNA Sequence 5'-3'	Cleavage (%)	Length	Truncation positions
18	guuggaaccauucaaaacagcauagcaaguua aaauaaggcuaguccguuaucaacuugaaaaa guggcaccgagucggugcuuuuuuu	38	89	-
30	caaaacagcauagcaaguuaaaauaaggcuag uccguuaucaacuugaaaaaguggcaccgagu cggugcuuuu	26	74	5' - 12 bases 3' - 3 bases
31	aacagcauagcaaguuaaaauaaggcuagucc guuaucaacuugaaaaaguggcaccgagucgg ugcuuu	32	70	5' - 15 bases 3' - 4 bases
2	agcauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggugc uuu	57	67	5' - 18 bases 3' - 4 bases
32	agcauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggugc u	47	65	5' - 18 bases 3' - 6 bases
33	cauagcaaguuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgagucggugcu	27	63	5' - 20 bases 3' - 6 bases
34	agcauagcaaguuaaaauaguuaucaacuuga aaaaguggcaccgagucggugcu	0	55	5' - 18 bases Int - 10 bases 3' - 6 bases
				5' - 18 bases

SEQ ID No.	tracrRNA Sequence 5'-3'	Cleavage (%)	Length	Truncation positions
35	agcauagcaaguuaaaauaaacuugaaaaagu ggcaccgagucggugcu	0	49	Int - 16 bases
				3' - 6 bases
36	agcauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggugc	53	64	5' - 18 bases
				3' - 7 bases
37	agcauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggug	56	63	5' - 18 bases
				3' - 8 bases
38	agcauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggu	56	62	5' - 18 bases
				3' - 9 bases
39	agcauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucgg	53	61	5' - 18 bases
				3' - 10 bases
40	agcauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaagugccgagucgg	5	58	5' - 18 bases
				Int - 3 bases
				3' - 10 bases
41	agcauagcaaguuaaaauaaggcuaguccaac uugaaaaaguggcaccgagucggugcu	0	59	5' - 18 bases
				Int - 6 bases
				3' - 6 bases
42	agcauagcaaguuaaaauaaggcuaguccaac uugaaaaaguggcaccgagucgg	0	55	5' - 18 bases
				Int - 6 bases
				3' - 10 bases
				5' - 18 bases

SEQ ID No.	tracrRNA Sequence 5'-3'	Cleavage (%)	Length	Truncation positions
43	agcauagcaaguuaaaauaaggcuaguccaac uugaaaaagugccgagucgg	0	52	Int - 9 bases 3' - 10 bases
44	agcauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaagug	0	49	5' - 18 bases 3' - 22 bases
45	agcauagcaaguuaaaauaaggcuaguccguu aucagcaccgagucggugcu	0	52	5' - 18 bases Int - 13 bases 3' - 6 bases
427	agcauagcaaguuaaaauaaggcuaguccguc aacuugaaaaaguggcaccgagucggugcuuu	4	64	5' - 18 bases Int - 3 bases 3' - 4 bases

Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA. Lengths of the RNA oligonucleotides are indicated (bases). The number of RNA residues removed in truncation studies at the 5'-end, 3'-end, and internal (int) are indicated. The relative functional activity of each species is indicated by the % cleavage in a T7EI heteroduplex assay.

[0074] This example demonstrates that for purposes of gene editing in mammalian cells that the tracrRNA can tolerate significant deletions from both the 5'-end and 3'-end and retain full functionality. Deletion of 18 bases from the 5'-end was well tolerated. Deletion of 20 bases from the 5'-end led to reduced activity, possibly due to lower affinity of binding of the crRNA. It is possible that this reduced length or even shorter might be functional if Tm-enhancing modifications were employed to stabilize the short duplex forming region. Deletion of up to 10 bases from the 3'-end was well tolerated. Additional deletions resulted in loss of activity. Internal deletions that disrupted hairpin elements or spacing between hairpin elements were not functional.

[0075] In summary, this example demonstrates that truncation of the tracrRNA from the 89 base length of the wild-type (WT, SEQ ID No. 18) to a 67 base length (SEQ ID No. 2) or to a 62 base length (SEQ ID No. 38), or to a 61 base length (SEQ ID No. 39) retained high functional activity. Use of shortened tracrRNAs of this kind will be less costly and easier to manufacture by chemical methods than the WT 89 base RNA. Some of the truncated species (SEQ ID No. 2, SEQ ID No.

38, and SEQ ID No. 39) showed increased functional activity over the 89 base WT tracrRNA. Therefore in addition to being less costly and easier to manufacture by chemical methods, the shortened tracrRNAs of the present disclosure showed improved activity.

EXAMPLE 3

[0076] Examples 1 and 2 demonstrated that crRNA:tracrRNA complexes shorter than the WT lengths of 42 and 89 bases, respectively, can show higher functional activity in mammalian gene editing. The present example shows further optimization of the lengths of these RNA species.

[0077] A series of crRNAs and tracrRNAs (Table 3) were synthesized having different lengths as indicated. Truncations were made at the 3'-end of the crRNA, the 5'-end of the tracrRNA, and/or the 3'-end of the tracrRNA. The crRNAs and tracrRNA were paired as indicated in Table 3. The crRNAs all employed a 20 base protospacer domain targeting *HPRT1* at the 5'-end and variable length 3'-ends (tracrRNA binding domains). An alignment of the crRNA and tracrRNA sequences studied in this example is shown in FIG. 4 to make clear the positions of truncations relative to each functional domain.

[0078] The paired crRNA:tracrRNA RNA oligonucleotides were transfected into the HEK-Cas9 cells and processed as described in Example 2. Relative gene editing activities were assessed by comparing cleavage rates in the *HPRT1* gene using the T7EI mismatch endonuclease cleavage assay with quantitative measurement of products done using the Fragment Analyzer. Results are shown in Table 3. The relative location of deletions are shown in sequence alignment format in FIG. 4.

Table 3: Effect of length truncations in both the crRNA and tracrRNA on efficiency of gene editing in mammalian cells by Cas9 endonuclease.

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
42/89	46	cuuauauccaacacuucgugguuuuagagcuaugcu guuuug	42	25
	18	guuggaaccauucaaaacagcauagcaaguuaaaau aaggcuaguccguuaucaacuugaaaaaguggcacc gagucggugcuuuuuuu	89	
39/89	47	cuuauauccaacacuucgugguuuuagagcuaugcu guu	39	31
	18	guuggaaccauucaaaacagcauagcaaguuaaaau aaggcuaguccguuaucaacuugaaaaaguggcacc gagucggugcuuuuuuu	89	

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
36/89	48	cuuauauccaacacuucgugguuuuagagcuaugcu	36	38
	18	guuggaaccauucaaaacagcauagcaaguuaaaau aaggcuaguccguuaucaacuugaaaaaguggcacc gagucggugcuuuuuuu	89	
34/89	49	cuuauauccaacacuucgugguuuuagagcuaug	34	21
	18	guuggaaccauucaaaacagcauagcaaguuaaaau aaggcuaguccguuaucaacuugaaaaaguggcacc gagucggugcuuuuuuu	89	
42/74	46	cuuauauccaacacuucgugguuuuagagcuaugcu guuuug	42	35
	50	caaaacagcauagcaaguuaaaauaaggcuaguccg uuaucaacuugaaaaaguggcaccgagucggugcuu uu	74	
39/74	47	cuuauauccaacacuucgugguuuuagagcuaugcu guu	39	34
	50	caaaacagcauagcaaguuaaaauaaggcuaguccg uuaucaacuugaaaaaguggcaccgagucggugcuu uu	74	
36/74	48	cuuauauccaacacuucgugguuuuagagcuaugcu	36	26
	50	caaaacagcauagcaaguuaaaauaaggcuaguccg uuaucaacuugaaaaaguggcaccgagucggugcuu uu	74	
34/74	49	cuuauauccaacacuucgugguuuuagagcuaug	34	20
	50	caaaacagcauagcaaguuaaaauaaggcuaguccg uuaucaacuugaaaaaguggcaccgagucggugcuu uu	74	
42/70	46	cuuauauccaacacuucgugguuuuagagcuaugcu guuuug	42	55
	51	aacagcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuuu	70	

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
39/70	47	cuuauauccaacacuucgugguuuuagagcuaugcu guu	39	48
	51	aacagcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuuu	70	
36/70	48	cuuauauccaacacuucgugguuuuagagcuaugcu	36	32
	51	aacagcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuuu	70	
34/70	49	cuuauauccaacacuucgugguuuuagagcuaug	34	9
	51	aacagcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuuu	70	
42/67	46	cuuauauccaacacuucgugguuuuagagcuaugcu guuuug	42	36
	2	agcauagcaaguuaaaauaaggcuaguccguuauca acuugaaaaaguggcaccgagucggugcuuu	67	
39/67	47	cuuauauccaacacuucgugguuuuagagcuaugcu guu	39	41
	2	agcauagcaaguuaaaauaaggcuaguccguuauca acuugaaaaaguggcaccgagucggugcuuu	67	
36/67	48	cuuauauccaacacuucgugguuuuagagcuaugcu	36	57
	2	agcauagcaaguuaaaauaaggcuaguccguuauca acuugaaaaaguggcaccgagucggugcuuu	67	
34/67	49	cuuauauccaacacuucgugguuuuagagcuaug	34	44
	2	agcauagcaaguuaaaauaaggcuaguccguuauca acuugaaaaaguggcaccgagucggugcuuu	67	
42/65	46	cuuauauccaacacuucgugguuuuagagcuaugcu guuuug	42	50

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
	52	agcauagcaaguuaaaauaaggcuaguccguuauca acuugaaaaaguggcaccgagucggugcu	65	
39/65	47	cuuauauccaacacuucgugguuuuagagcuaugcu guu	39	46
	52	agcauagcaaguuaaaauaaggcuaguccguuauca acuugaaaaaguggcaccgagucggugcu	65	
36/65	48	cuuauauccaacacuucgugguuuuagagcuaugcu	36	47
	52	agcauagcaaguuaaaauaaggcuaguccguuauca acuugaaaaaguggcaccgagucggugcu	65	
34/65	49	cuuauauccaacacuucgugguuuuagagcuaug	34	16
	52	agcauagcaaguuaaaauaaggcuaguccguuauca acuugaaaaaguggcaccgagucggugcu	65	
42/63	46	cuuauauccaacacuucgugguuuuagagcuaugcu guuuug	42	6
	53	cauagcaaguuaaaauaaggcuaguccguuaucaac uugaaaaaguggcaccgagucggugcu	63	
39/63	47	cuuauauccaacacuucgugguuuuagagcuaugcu guu	39	13
	53	cauagcaaguuaaaauaaggcuaguccguuaucaac uugaaaaaguggcaccgagucggugcu	63	
36/63	48	cuuauauccaacacuucgugguuuuagagcuaugcu	36	28
	53	cauagcaaguuaaaauaaggcuaguccguuaucaac uugaaaaaguggcaccgagucggugcu	63	
34/63	49	cuuauauccaacacuucgugguuuuagagcuaug	34	33
	53	cauagcaaguuaaaauaaggcuaguccguuaucaac uugaaaaaguggcaccgagucggugcu	63	

Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA. Lengths of the RNA oligonucleotides are indicated (bases). The relative functional activity of each crRNA:tracrRNA pair is indicated by the % cleavage in a T7EI heteroduplex assay.

[0079] All of the compounds studied directed CRISPR/Cas editing at the HPRT1 locus in HEK-Cas9 cells. Efficiency varied widely from 6% to 57%. The most effective crRNA+tracrRNA combination was the 36 base crRNA (SEQ ID No. 48) with the 67mer tracrRNA (SEQ ID No. 2). A schematic representation of the truncated, optimized crRNA:tracrRNA complex is shown in FIG. 5. In this case the tracrRNA binding domain of the crRNA was truncated to 16 bases from the WT 22 base sequence (3'-end). This hybridizes to the crRNA binding domain at the 5'-end of the tracrRNA. The tracrRNA was truncated 18 bases at the 5'-end and 4 bases at the 3'-end to product the active 67 base product. For this pair, a blunt end is formed upon hybridization of the 3'-end of the crRNA with the 5'-end of the tracrRNA. Other versions also showed high activity, including the 42 base (WT) crRNA (SEQ ID No. 46) paired with the 70 base tracrRNA (SEQ ID No. 51).

[0080] The shortest crRNA tested was 34 bases in length (SEQ ID No. 49) and, in general, showed lower activity than the longer variants. The shorter duplex domain formed between this variant and the tracrRNA has reduced binding affinity (Tm) compared to the 36 base crRNA variant and that 34 base complex was less stable at 37°C. Use of chemical modification that increase binding affinity (Tm), such as 2'OMe RNA, 2'F RNA, or LNA residues, will increase stability of this short duplex domain and will lead to improved activity, permitting use of very short crRNAs of this design. Extensive use of Tm-enhancing modifications will permit use of even shorter tracrRNA binding domains in the crRNA, such as 13 base, or 12 base, or 11 base, or 10 base, or 9 base, or 8 base or shorter, depending on the kind and number of modified residues employed.

EXAMPLE 4

[0081] Examples 1, 2, and 3 demonstrated that crRNA:tracrRNA complexes shorter than the WT lengths of 42 and 89 bases, respectively, can show higher functional activity in mammalian gene editing. In those examples, all truncations were made in the universal domains of the RNAs. The present example tests the effects that truncations have on the target-specific protospacer domain of the guide crRNA.

[0082] A series of crRNAs (Table 4) were synthesized having protospacer domain lengths of 20, 19, 18, or 17 bases as indicated. Truncations were made at the 5'-end of the crRNA, using a 16mer universal tracrRNA binding sequence at the 3'-end. The crRNAs were paired with an unmodified 67mer tracrRNA (SEQ ID No. 2). The crRNAs targeted 4 different sites in the same exon of the human *HPRT1* gene.

[0083] The paired crRNA:tracrRNA RNA oligonucleotides were transfected into the HEK-Cas9 cells and processed as described in Example 2. Relative gene editing activities were assessed by

comparing cleavage rates in the *HPRT1* gene using the T7EI mismatch endonuclease cleavage assay with quantitative measurement of products done using the Fragment Analyzer. Results are shown in Table 4.

Table 4: Effect of length truncations in the 5'-protospacer domain of the crRNA on efficiency of gene editing in mammalian cells by Cas9 endonuclease.

SEQ ID No.	crRNA Sequence 5'-3'	Length Protospacer domain	Cleavage (%)	*HPRT1* site
48	cuuauauccaacacuucguggguuuuagagc uaugcu	20	64	38285
1	uuauauccaacacuucguggguuuuagagcu augcu	19	62	
54	uauauccaacacuucguggguuuuagagcua ugcu	18	57	
55	auauccaacacuucguggguuuuagagcuau gcu	17	42	
56	aauuauggggauuacuaggaguuuuagagc uaugcu	20	78	38087
57	auuauggggauuacuaggaguuuuagagcu augcu	19	81	
58	uuauggggauuacuaggaguuuuagagcua ugcu	18	82	
59	uauggggauuacuaggaguuuuagagcuau gcu	17	82	
60	auuucacauaaaacucuuuuguuuuagagc uaugcu	20	52	38358
61	uuucacauaaaacucuuuuguuuuagagcu augcu	19	30	

SEQ ID No.	crRNA Sequence 5'-3'	Length Protospacer domain	Cleavage (%)	*HPRT1* site
62	uucacauaaaacucuuuuguuuuagagcua ugcu	18	12	
63	ucacauaaaacucuuuuguuuuagagcuau gcu	17	0	
64	uccauuucauagucuuuccuguuuuagagc uaugcu	20	70	38094
65	ccauuucauagucuuuccuguuuuagagcu augcu	19	71	
66	cauuucauagucuuuccuguuuuagagcua ugcu	18	52	
67	auuucauagucuuuccuguuuuagagcuau gcu	17	0	

Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA. The target-specific protospacer domain is underlined and length is indicated (bases). The relative functional activity of each species is indicated by the % cleavage in a T7EI heteroduplex assay.

[0084] Of the 4 sites studied, one (site 38087) showed high activity for all 4 crRNAs with no changes seen as the protospacer domain was shortened. Site 38285 similar efficacy for the 20 and 19 base protospacer crRNAs (SEQ ID Nos. 48 and 1), a slight decrease for the 18 base version (SEQ ID No. 54), and a large decrease for the 17 base version (SEQ ID No. 55). Site 38094 showed similar efficacy for the 20 and 19 base protospacer crRNAs (SEQ ID Nos. 64 and 65), a moderate decrease for the 18 base version (SEQ ID No. 66), and no activity for the 17 base version (SEQ ID No. 67). Site 38358 showed good activity for the 20 base version (SEQ ID No. 60), lower activity for the 19 base version (SEQ ID No. 61), even lower activity for the 18 base version (SEQ ID No. 62) and no activity for the 17 base version (SEQ ID No. 63).

[0085] The use of shortened 17 base protospacer guide domains can lower the occurrence of undesired off-target events compared to the wild-type 20 base domain (Fu et al., Nature Biotechnol., 32:279, 2014). We observe that on-target efficacy varies in a sequence-context specific fashion and that 20 base and 19 base protospacer guide domains are generally effective but that activity begins to decrease when 18 base protospacer domains are used and activity

significantly decreases when 17 base protospacer domains are used. Therefore, to maintain desired on-target efficiency, use of 20 and 19 base target-specific protospacer guide domains are employed herein. Significant truncation of the protospacer guide domain in many cases lowers on-target cleavage of a DNA target by the Cas9 endonuclease. Use of chemical modifications that enhance Tm (increase binding affinity of the protospacer target-specific domain of the crRNA to the target DNA sequence) may permit use of shorter sequences such that a 17 base protospacer guide may show similar on-target efficacy as an unmodified 20 base protospacer guide domain.

EXAMPLE 5

[0086] This example demonstrates that truncated crRNA:tracrRNA complex show improved gene editing activity at multiple sites. The prior examples studied efficacy of the truncated RNAs as triggers of CRISPR gene editing in mammalian cells at a single site in the human *HRPT1* gene. Site/sequence specific effects may exist. The present example demonstrates improved performance of the truncated species at 12 sites in an exon of the human *HPRT1* gene.

[0087] A series of crRNAs (Table 5) were synthesized having a protospacer domain lengths of 20 bases specific to 12 sites in the human HPRT1 gene with a 16mer universal tracrRNA binding sequence at the 3'-end. The crRNAs were paired with an unmodified 67mer tracrRNA (SEQ ID No. 2). The same 12 sites were studied using WT length crRNA:tracrRNA complexes wherein the crRNA comprised a 20 base protospacer guide paired with a 22mer universal tracrRNA binding sequence at the 3'-end complexed with the WT 89mer tracrRNA (SEQ ID No. 18).

[0088] The paired crRNA:tracrRNA RNA oligonucleotides were transfected into the HEK-Cas9 cells and processed as described in Example 2. Relative gene editing activities were assessed by comparing cleavage rates in the *HPRT1* gene using the T7EI mismatch endonuclease cleavage assay with quantitative measurement of products done using the Fragment Analyzer. Results are shown in Table 5.

Table 5: Effect of length truncations in both the crRNA and tracrRNA on efficiency of gene editing in mammalian cells by Cas9 endonuclease.

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
38094 short	64	uccauuucauagucuuuccuguuuuagagcuaugcu	36	55
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu	67	
	68	uccauuucauagucuuuccuguuuuagagcuaugcuguuuug	42	

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
38094 long	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuuu	89	31
38231 short	69	uuuuguaauuaacagcuugcguuuuagagcuau gcu	36	7
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
38231 long	70	uuuuguaauuaacagcuugcguuuuagagcuau gcuguuuug	42	0
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38371 short	71	cuuagagaauauuuguagaggguuuuagagcuau gcu	36	57
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
38371 long	72	cuuagagaauauuuguagaggguuuuagagcuau gcuguuuug	42	27
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38509 short	73	uugacuauaaugaauacuucguuuuagagcuau gcu	36	56
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
	74	uugacuauaaugaauacuucguuuuagagcuau gcuguuuug	42	

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
38509 long				7
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38574 short	75	caaaacacgcauaaaaauuuguuuuagagcuau gcu	36	58
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
38574 long	76	caaaacacgcauaaaaauuuguuuuagagcuau gcuguuuug	42	22
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38087 short	56	aauuauggggauuacuaggaguuuuagagcuau gcu	36	60
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
38087 long	77	aauuauggggauuacuaggaguuuuagagcuau gcuguuuug	42	53
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38133 short	78	ggucacuuuuaacacacccaguuuuagagcuau gcu	36	53
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
38133 long	79	ggucacuuuuaacacacccaguuuuagagcuau gcuguuuug	42	37
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38285 short	48	cuuauauccaacacuucguggguuuuagagcuau gcu	36	38
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
38285 long	46	cuuauauccaacacuucguggguuuuagagcuau gcuguuuug	42	8
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38287 short	80	ggcuuauauccaacacuucgguuuuagagcuau gcu	36	48
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
38287 long	81	ggcuuauauccaacacuucgguuuuagagcuau gcuguuuug	42	6
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38358 short	60	auuucacauaaaacucuuuuguuuuagagcuau gcu	36	42
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Length	Cleavage %
38358 long	82	auuucacauaaaacucuuuuguuuuagagcuau gcuguuuug	42	8
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38636 short	83	ucaaauuaugaggugcuggaguuuuagagcuau gcu	36	26
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
38636 long	84	ucaaauuaugaggugcuggaguuuuagagcuau gcuguuuug	42	16
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	
38673 short	85	uacagcuuuaugugacuaauguuuuagagcuau gcu	36	45
	2	agcauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcuu u	67	
38673 long	86	uacagcuuuaugugacuaauguuuuagagcuau gcuguuuug	42	32
	18	guuggaaccauucaaaacagcauagcaaguuaa aauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuuuuuu	89	

Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA. Lengths of the RNA oligonucleotides are indicated (bases). The relative functional activity of each crRNA:tracrRNA pair is indicated by the % cleavage in a T7EI heteroduplex assay.

[0089] The relative efficiency of CRISPR mediated gene editing in the HEK-Cas9 cells varied with sequence context. However, in all cases the shorter optimized RNA guides (36mer crRNA and

67mer tracrRNA) showed higher efficiency than the WT RNAs (42mer crRNA and 89mer tracrRNA). Use of the shortened, optimized guide RNAs improve Cas9 cleavage of targeted DNAs relative to the WT RNAs, improving the gene editing rates.

EXAMPLE 6

[0090] Example 1 described chemical modification patterns that functioned with Cas9 in an *in vitro* biochemical target DNA cleavage assay. This example demonstrates functioning of chemically modified tracrRNAs to direct genome editing by the Spy Cas9 nuclease in mammalian cells. Optimal modification patterns differ between *in vitro* and *in vivo* use.

[0091] A series of tracrRNAs (Table 6) were synthesized having a variety of chemical modifications, including: the ribose modifications 2'OMe RNA and LNA; the end-modifying groups propanediol spacer and napthyl-azo modifier (N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine, or "ZEN"); and select internucleotide linkages with phosphorothioate modifications. See: Lennox et al., Molecular Therapy Nucleic Acids 2:e117 2013 for structure of the napthyl-azo modified and use of the napthyl-azo modifier and propanediol modifier for use as end-groups to block exonuclease attack. The tracrRNAs listed in Table 6 were complexed with unmodified truncated anti-*HPRT1* crRNA SEQ ID No. 1 (Table 1) which has a 19 base protospacer domain targeting *HPRT1* at the 5'-end and a 16 base tracrRNA binding domain at the 3'-end. The paired crRNA:tracrRNA RNA oligonucleotides were transfected into the HEK-Cas9 cells and processed as described above. Relative gene editing activities were assessed by comparing cleavage rates in the *HPRT1* gene using the T7EI mismatch endonuclease cleavage assay with quantitative measurement of products done using the Fragment Analyzer.

Table 6: Optimization of tracrRNA oligonucleotide modification patterns in mammalian cells.

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	65
4	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	0
11	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	56
17	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	12
12	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	20

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
13	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	64
87	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	61
88	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	60
89	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	64
90	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcu	60
91	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcu	61
92	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcu	59
93	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcu	57
94	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcu	57
95	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcu	62
96	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcu	62
97	C3-agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugcuuu-C3	53

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
98	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	58
99	C3-agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugcuuu-C3	20
100	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	63
101	a*g*c*auagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugc*u*u*u	55
102	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu	39
103	a*g*c*auagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugc*u*u*u	54
104	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu-ZEN	55
105	ZEN-agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcuuu-ZEN	23
106	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucg*g*u	58
107	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	8
108	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	0
109	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	11
110	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	61

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
111	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	61
112	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	62
113	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	62
114	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	61
115	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	14
116	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	60
117	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	60
118	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	15
119	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	0
120	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	7
121	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	14
122	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	11

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
123	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	0
124	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	0
125	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	0
126	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	64
127	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	0
128	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	0
129	+a*+g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagu ggcaccgagucggugcu*+t*+t	57
130	C3-agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugcuuu-InvT	
131	C3-agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggu-C3	58
132	C3-agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggu-InvT	59
133	agcauagca*a*g*u*u*a*a*a*a*u*a*a*g*g*c*u*a*g*u*c*c*g* u*u*a*u*c*a*a*cuugaaaaaguggcaccgagucggugcuuu	0
134	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	58

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
135	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u	19
136	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucg*g*u	54
137	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucg*g*u	13
138	a*g*cauagcaagTTAAAATAAGGCTAGTCCGTTaucaacuugaaaaaguggcaccgagucggugcu*u*u	0
139	a*g*cauagcaagTTAAAATAAGgcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u	0
140	a*g*cauagcaaguuaaaauaagGCTAGTCCGTTaucaacuugaaaaaguggcaccgagucggugcu*u*u	0
141	a*g*cauagcaag*uuaaaauaaggcuaguccguu*aucaacuugaaaaaguggcaccgagucggugcu*u*u	0
142	a*g*cauagcaag*uuaaaauaag*gcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u	4
143	a*g*cauagcaaguuaaaauaag*gcuaguccguu*aucaacuugaaaaaguggcaccgagucggugcu*u*u	0
144	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u	52
145	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u	63
146	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u	0
147	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u	62

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
148	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	57
149	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	47
150	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	61
151	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	61
152	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	61
153	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	61
154	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	50
155	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	46
156	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	59
157	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	2
158	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	18
159	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	50

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
160	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	58
161	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	14
162	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaagugg caccgagucggugcu*u*u	8

Oligonucleotide sequences are shown 5'-3'. Uppercase = DNA; Lowercase = RNA; Underlined = 2'-O-methyl RNA; Italics = 2'-fluoro RNA; +a, +c, +t, +g = LNA; C3 = C3 spacer (propanediol modifier); * = phosphorothioate internucleotide linkage; ZEN - napthyl-azo modifier; Inv-dT = inverted-dT. The relative functional activity of each species is indicated by the % cleavage in a T7EI heteroduplex assay.

[0092] Modification is usually necessary for synthetic nucleic acids to function well in an intracellular environment due to the presence of exonucleases and endonucleases that degrade unmodified oligonucleotides. A wide range of modifications have been described that confer nuclease resistance to oligonucleotides. The precise combination and order of modifications employed that works well for a given application can vary with sequence context and the nature of the protein interactions required for biological function. Extensive prior work has been done relating to chemical modification of antisense oligonucleotides (which interact with RNase HI) and siRNAs (which interact with DICER, AGO2, and other proteins). It is expected that chemical modification will improve function of the CRISPR crRNA:tracrRNA complex. However, it is not possible to predict what modifications and/or pattern of modifications will be compatible with functional complexation of the synthetic RNAs with Cas9. The present disclosure defines minimal, moderate, and extensive chemical modification patterns for the tracrRNA that retain high levels of function to direct Cas9 mediated gene editing in mammalian cells.

[0093] The results in Table 6 demonstrate that extensive modification is tolerated throughout the 5' and 3' end domains of the tracrRNA. Modification of the internal domains of the tracrRNA showed reduced activity, likely due to altered structure of the folded RNA and/or blocking of protein contact points with the 2'-OH of key RNA residues by the 2'OMe modification. For example, compound SEQ ID No. 100 has 39/67 residues modified with 2'OMe RNA (58%) and retains full activity compared with the unmodified sequence. SEQ ID No. 134 has 46/67 residues modified with 2'OMe RNA (69%) and retains near full activity compared with the unmodified sequence (FIG. 6). SEQ ID No. 134 is a truncated 67mer variant of the tracrRNA. Using SEQ ID No. 134 as a model, modification of 11 sequential residues in the 5'-domain with 2'OMe RNA was tolerated with no loss of activity. Modification of 35 sequential residues in the 3'-domain with 2'OMe RNA was tolerated with not loss of activity. Of note, the two hairpin structures present in the 3'-domain are necessary for function as deletion of either of these features results in loss of

activity (Example 2, FIG. 3), yet both of these domains can be completely modified with 2'OMe RNA without compromising function. Note that both SEQ ID Nos. 134 and 100 also have phosphorothioate (PS) modified internucleotide linkages at the 5'- and 3'-ends, which provides additional protection against exonuclease attack.

[0094] Specific residues were identified that led to large reductions or complete loss of activity when modified. Using the 67 base tracrRNA (for example, SEQ ID No. 134) as reference, starting from the 5'-end of the sequence substitution of 2'OMe RNA for the natural RNA at residues U12, A15, G26, U27, G30, U31, and U32 led to substantial loss of activity (FIG. 6). Specific residues were also identified that led to smaller yet significant reductions in activity when modified. Using the 67 base tracrRNA (for example, SEQ ID No. 134) as reference, starting from the 5'-end of the sequence substitution of 2'OMe RNA for the natural RNA at residues U13, U18, C23, U24, and C28 led to reduced activity (FIG. 6). This study was performed using 2'OMe RNA. Use of other modifications, such as 2'F, LNA, DNA, etc. at these positions may be better tolerated. The central 21 residue domain of unmodified RNA in SEQ ID No. 134 was modified with 2'-F RNA either completely (SEQ ID No. 141) or partially (SEQ ID Nos. 142 and 143). These variants were not functional. The central 21 residue domain of unmodified RNA in SEQ ID No. 134 was modified with DNA either completely (SEQ ID No. 138) or partially (SEQ ID Nos. 139 and 140). These variants were not functional. Modification of isolated residues in this domain may work, however large continuous blocks of modification in this domain reduce activity of the tracrRNA.

[0095] To further investigate which individual residues can be modified using 2'OMe RNA within the central domain of the tracrRNA, a single base modification 2'OMe RNA 'walk' was done (SEQ ID Nos. 144-162). Within this series, modification as residues A14, A19, A20, G21, G22, A25, and C29 showed no loss of activity and are candidates for modification.

[0096] Antisense oligonucleotides are often made using complete PS modification, where every internucleotide linkage is phosphorothioate modified. This extensive level of modification is possible because the protein effector molecule RNase HI (which mediates ASO-directed mRNA degradation) tolerates the PS modification in the ASO when forming a functional substrate/enzyme complex. On the other hand, siRNAs do not tolerate full PS modification; extensive PS modification disrupts productive interaction with the effector protein AGO2 (which mediates siRNA-directed mRNA degradation). Extensive PS modification of the tracrRNA in the internal RNA loops disrupts functional interaction with Cas9 (Seq ID No. 133; 29 PS modifications). Limited PS end-modification can be done with no loss of activity (SEQ ID Nos. 98 and 101; 2-3 PP linkages on each end). Less extensive PS modification may be tolerated in the central domain. In particular, RNase cleavage mapping (where incubation of the tracrRNA in a series of serum or cell extract dilutions are used to find the sites that are most sensitive to RNase attack) may be used to identify critical sites where PS modification of only one or a few linkages may stabilize the RNA without disrupting function.

[0097] There are applications where the PS modification contributes to chemical toxicity. In this case use of other methods to block exonuclease attack are desirable. Options include end-modifiers such as inverted-dT or abasic groups such as dSpacer, C3 spacer (propanediol), ZEN (napthyl-azo modifier), and others. Placement of such end-modifying groups can eliminate the

need for terminal PS internucleotide linkages.

EXAMPLE 7

[0098] Example 1 described chemical modification patterns that functioned with Cas9 in an *in vitro* biochemical target DNA cleavage assay. This example demonstrates functioning of chemically modified crRNAs to direct genome editing by the Spy Cas9 nuclease in mammalian cells. Optimal modification patterns differ between *in vitro* and *in vivo* use.

[0099] A series of crRNAs (Table 7) were synthesized having a variety of chemical modifications, including: the ribose modifications 2'OMe RNA, 2'F, and LNA; the end-modifying groups propanediol spacer and napthyl-azo modifier (N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine, or "ZEN"), and an inverted-dT residue; and select internucleotide linkages with phosphorothioate modifications. See: Lennox et al., Molecular Therapy Nucleic Acids 2:e117 2013 for structure of the napthyl-azo modified and use of the napthyl-azo modifier and propanediol modifier for use as end-groups to block exonuclease attack. The crRNAs had either a 19 base protospacer domain targeting *HPRT1* at the 5'-end (SEQ ID Nos. 1, 9 10, 14-16, 22-24, 163-173) or a 20 base protospacer domain targeting the same site (SEQ ID Nos. 48, 174-237) with a 16 base tracrRNA binding domain at the 3'-end. The crRNAs listed in Table 7 were complexed with unmodified truncated (67 base) tracrRNA SEQ ID No. 2 (Table 1) or chemically-modified truncated (67 base) tracrRNA SEQ ID No. 100 (Table 6). The use of two tracrRNAs enables determination if function of chemical modified crRNAs varies if paired with a modified tracrRNA. The paired crRNA:tracrRNA RNA oligonucleotides were transfected into the HEK-Cas9 cells and processed as described previously. Relative gene editing activities were assessed by comparing cleavage rates in the *HPRT1* gene using the T7EI mismatch endonuclease cleavage assay, with quantitative measurement of products done using the Fragment Analyzer.

Table 7: Optimization of crRNA oligonucleotide modification patterns in mammalian cells.

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
1	uuauauccaacacuucgugguuuuagagcuaugcu	63	61
9	uuauauccaacacuucgugguuuuagagcuaugcu	1	0
10	uuauauccaacacuucgugguuuuagagcuaugcu	0	1
22	uuauauccaacacuucgugguuuuagagcuaugcu	1	1
23	uu**a**u**a**ucc**aa**cacuuc**g**u**gg**uuuu**agag**cu**a**u**g**cu	5	ND
24	*uuauauccaacacuucgugguuuuagagcuaugcu*	3	5
14	uuauauccaacacuucgugguuuuagagcuaugcu	63	26
15	uuauauccaacacuucgugguuuuagagcuaugcu	5	3
16	uuauauccaacacuucgugguuuuagagcuaugcu	5	5
163	C3-uuauauccaacacuucgugguuuuagagcuaugcu-C3	65	49

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
164	u*u*a*uauccaacacuucgugguuuuagagcuau *g*c*u	65	65
165	uuauauccaacacuucgugguuuuagagcuaugcu	0	3
166	uuauauccaacacuucgugguuuuagagcuaugcu	54	42
167	uuauauccaacacuucgugguuuuagagcuaugcu	49	58
168	uuauauccaacacuucgugguuuuagagcuaugcu	64	60
169	uuauauccaacacuucgugguuuuagagcuaugcu	16	16
170	uuauauccaacacuucgugguuuuagagcuaugcu	3	3
171	uuauauccaacacuucgugguuuuagagcuaugcu	42	62
172	uuauauccaacacuucgugguuuuagagcuaugcu	4	13
173	uuauauccaacacuucgugguuuuagagcuaugcu	1	1
48	cuuauauccaacacuucgugguuuuagagcuaugc u	61	60
174	cuuauauccaacacuucgugguuuuagagcuaugc u	60	59
175	cuuauauccaacacuucgugguuuuagagcuaugc u	62	60
176	cuuauauccaacacuucgugguuuuagagcuaugc u	61	59
177	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	60	59
178	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	61	59
179	C3-cuuauauccaacacuucgugguuuuagagcua ugcu	61	58
180	cuuauauccaacacuucgugguuuuagagcuaugc u-C3	57	59

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
181	C3-cuuauauccaacacuucguggguuuuagagcua ugcu-C3	62	59
182	ZEN-cuuauauccaacacuucguggguuuuagagcu augcu	64	62
183	cuuauauccaacacuucguggguuuuagagcuaugc u-ZEN	62	60
184	ZEN-cuuauauccaacacuucguggguuuuagagcu augcu-ZEN	64	64
185	ZEN-cuuauauccaacacuucguggguuuuagagcu augcu-ZEN	60	63
186	u*u*a*uauccaacacuucguggguuuuagagcuau *g*c*u	64	62
187	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	65	65
188	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
189	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	63	64
190	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	64	62
191	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	64	63
192	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	64	64

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
193	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	64	65
194	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	60	63
195	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	63	62
196	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	62	63
197	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	61	64
198	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	61	64
199	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	63	68
200	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	59	67
201	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	63	67
202	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	60	69
203	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	53	67
204	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	54	67

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
205	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	59	62
206	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	58	61
207	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	50	60
208	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	7
209	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
210	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
211	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
212	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	56	68
213	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	41	64
214	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	53	67
215	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	2

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
216	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
217	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
218	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
219	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
220	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
221	+c*+t*uauauccaacacuucguggguuuuagagcu aug*+c*+t	58	61
222	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	31	54
223	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	6	60
224	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	27	57
225	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	2
226	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	2	25
227	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	3	31

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
228	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	4	35
229	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
230	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
231	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	1
232	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	0	0
233	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	33	67
234	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	24	66
235	C3-cuuauauccaacacuucguggguuuuagagcua ugcu-C3	56	65
236	C3-cuuauauccaacacuucguggguuuuagagcua ugcu-C3	11	55
237	C3-cuuauauccaacacuucguggguuuuagagcua ugcu-InvT	62	65
238	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	17	67
239	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	39	66

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
240	C3- cuuauauccaacacuucguggguuuuagagcuaugc u-C3	27	63
241	C3- cuuauauccaacacuucguggguuuuagagcuaugc u-C3	14	46
242	ZEN- cuuauauccaacacuucguggguuuuagagcuaugc u-ZEN	41	67
243	ZEN- cuuauauccaacacuucguggguuuuagagcuaugc u-ZEN	23	24
244	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	ND	60
245	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	ND	65
246	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	ND	64
247	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	ND	64
248	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	ND	63
249	c*u*u*auauccaacacuucguggguuuuagagcua u*g*c*u	ND	53
250	c*u*u*auauccaacacUUCGUGGUUUuagagcua u*g*c*u	0	2

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
251	c*u*u*auauccaacacUUCGUGguuuuagagcua u*g*c*u	0	0
252	c*u*u*auauccaacacuucgugGUUUuagagcua u*g*c*u	0	18
253	c*u*u*auauccaacac*uucgugguuu*uagagcua u*g*c*u	0	3
254	c*u*u*auauccaacac*uucgug*guuuuagagcua u*g*c*u	5	0
255	c*u*u*auauccaacacuucgug*guuu*uagagcua u*g*c*u	0	0
256	C3-cuuauauccaacacuucgugguuuuagagcua ugcu-C3	27	53
257	C3-cuuauauccaacacuucgugguuuuagagcua ugcu-C3	10	50
258	c*u*u*auauccaacacuucgugguuuuagagcua u*g*c*u	29	47
259	c*u*u*auauccaacacuucgugguuuuagagcua *u*g*c	7	45
260	c*u*u*auauccaacacuucgugguuuuagagcu* a*u*g	0	4
261	c*u*u*auauccaacacuucgugguuuuagagc*u *a*u	0	0
262	c*u*u*auauccaacacuucgugguuuuagag*c* u*a	0	0

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
263	c*u*u*auauccaacacuucguggguuuuaga*g*c*u	0	0
264	c*u*u*auauccaacacuucguggguuuuagagcuau*g*c*u	50	62
265	c*u*u*auauccaacacuucgugg*u*u*u*uagagcuau*g*c*u	45	59
266	c*u*u*auaucca*a*c*a*c*u*u*c*g*u*g*guuuuagagcuau*g*c*u	26	36
267	c*u*u*auaucca*a*c*a*c*u*u*c*g*u*g*g*u*u*u*uagagcuau*g*c*u	20	34
268	C3-cuuauauccaacacuucgugguuuuagagcuaugcu-C3	27	59
269	C3-cuuauauccaacacuucgugg*u*u*u*uagagcuaugcu-C3	45	60
270	C3-cuuauaucca*a*c*a*c*u*u*c*g*u*g*guuuuagagcuaugcu-C3	16	43
271	C3-cuuauaucca*a*c*a*c*u*u*c*g*u*g*g*u*u*u*uagagcuaugcu-C3	22	45
272	cuuauauccaacacuucgugguuuuagagcuaugcu	63	57
273	cuuauauccaacacuucgugguuuuagagcuaugcu	59	60
274	cuuauauccaacacuucgugguuuuagagcuaugcu	63	63

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
275	cuuauauccaacacuucguggguuuuagagcuaugc u	64	62
276	cuuauauccaacacuucguggguuuuagagcuaugc u	0	1
277	cuuauauccaacacuucguggguuuuagagcuaugc u	5	16
278	cuuauauccaacacuucguggguuuuagagcuaugc u	64	61
279	cuuauauccaacacuucguggguuuuagagcuaugc u	64	63
280	cuuauauccaacacuucguggguuuuagagcuaugc u	30	49
281	cuuauauccaacacuucguggguuuuagagcuaugc u	56	60
282	cuuauauccaacacuucguggguuuuagagcuaugc u	53	61
283	cuuauauccaacacuucguggguuuuagagcuaugc u	0	3
284	cuuauauccaacacuucguggguuuuagagcuaugc u	0	2
285	cuuauauccaacacuucguggguuuuagagcuaugc u	2	8
286	cuuauauccaacacuucguggguuuuagagcuaugc u	48	61
287	a*u*a*uccaacacuucguggguuuuagagcuau*g *c*u	0	0

SEQ ID No.	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No 2	Cleavage % tracrRNA SEQ ID No. 100
288	u*a*u*ccaacacuucgugguuuuagagcuau*g*c*u	0	0
289	+A*+T*a*uccaacacuucgugguuuuagagcuau*g*c*u	2	14
290	+T*+A*u*ccaacacuucgugguuuuagagcuau*g*c*u	0	0

Oligonucleotide sequences are shown 5'-3'. Uppercase = DNA; Lowercase = RNA; Underlined = 2'-O-methyl RNA; Italics = 2'-fluoro RNA; +a, +c, +t, +g = LNA; C3 = C3 spacer (propanediol modifier); * = phosphorothioate internucleotide linkage; ZEN = napthyl-azo modifier; InvdT = inverted-dT. The relative functional activity of each species is indicated by the % cleavage in a T7EI heteroduplex assay when the indicated crRNA is paired with the indicated tracrRNA. ND = not determined.

[0100] Some kind of chemical modification is usually necessary for synthetic nucleic acids to function well in an intracellular environment due to the presence of exonucleases and endonucleases that degrade unmodified oligonucleotides. A wide range of modifications have been described that confer nuclease resistance to oligonucleotides. The precise combination and order of modifications employed that works well for a given application can vary with sequence context and the nature of the protein interactions required for biological function. Extensive prior work has been done relating to chemical modification of antisense oligonucleotides (which interact with RNase H1) and siRNAs (which interact with DICER, AGO2, and other proteins). It is expected that chemical modification will improve function of the CRISPR crRNA:tracrRNA complex. However, it is not possible to predict what modifications and/or pattern of modifications will be compatible with association of the RNAs with Cas9 in a functional way. The present disclosure defines minimal, moderate, and extensive chemical modification patterns for the crRNA that retain high levels of function to direct Cas9 mediated gene editing in mammalian cells. The survey in Example 7 was performed targeting a single site in the human *HPRT1* gene. Note that modification patterns of the 20 base 5'-end protospacer guide domain of the crRNA that perform well may vary with sequence context. However, it is likely that modification patterns of the 3'-end tracrRNA binding domain that perform well as defined herein will be affected when the sequence of the adjacent protospacer domain changes when different sites are targeted, so the 3'-domain modification patterns shown here will be "universal".

[0101] The results in Table 7 demonstrate that extensive modification is tolerated throughout the 5' and 3' ends of the crRNA. Modification of certain select positions within internal domains of the

crRNA lead to reduced activity or totally blocks activity, likely due to altered structure of the folded RNA and/or blocking of protein contact points with the 2'-OH of key RNA residues by the 2'OMe modification. For example, compound SEQ ID No. 204 has 21/36 residues modified with 2'OMe RNA (58%) and retains full activity compared with the unmodified sequence. Compound SEQ ID No. 239 has 30/36 residues modified with 2'OMe RNA (83%) and retains full activity compared with the unmodified sequence. Both of these compounds also have 3 phosphorothioate (PS) modified internucleotide linkages at the 5'- and 3'-ends, which provides additional protection against exonuclease attack. In contrast, SEQ ID No. 165 has only 4/36 residues modified with 2'OMe RNA (11%) yet has totally lost activity.

[0102] Large blocks of sequence were tolerant to 2'OMe modification at the 5'-end and 3'-end of the crRNA, however modification of certain residues in the central portion of the molecule led to inactivation. To further investigate which individual residues can be modified using 2'OMe RNA within the central domain of the crRNA, a single base modification 2'OMe RNA 'walk' was done (SEQ ID Nos. 272-286). Specific residues (positions within the crRNA) were identified that led to large reductions or complete loss of activity. Using the 36 base crRNA SEQ ID No. 239 as model and numbering from the 5'-end of the sequence, substitution of 2'OMe RNA for the natural RNA of residues U15 and U16 lead to substantial loss of activity and residue U19 led to a moderate loss of activity (FIG. 7). These 3 sites lie within the target-specific protospacer guide domain, so sequence varies with target (residues 15, 16, and 19, FIG. 7). It is possible that in certain sequence contexts that these sites will be tolerant to modification. Within the universal tracrRNA-binding domain (residues 21-36), substitution of 2'OMe RNA for the natural RNA of residues U22, U23, and U24 led to substantial loss of activity. Given that this domain does not change with sequence context, it is likely that these sites will not vary in modification tolerance as target sequence changes. Sequence-specific effects of modification in the 20-base target-specific protospacer guide domain are studies in greater detail in Example 10.

[0103] Antisense oligonucleotide are often made with complete PS modification, where every internucleotide linkage is phosphorothioate modified. This extensive level of modification is possible because the protein effector molecule RNase HI tolerates the PS modification in the ASO when forming a functional substrate/enzyme complex. On the other hand, siRNAs do not tolerate full PS modification; extensive PS modification disrupts productive interaction with the effector protein AGO2. Limited PS end modification of the crRNA can be done with no loss of activity (SEQ ID Nos. 177, 178, 239, etc., have 3 PS linkages on each end). End-modification is desirable as this adds additional protection from exonuclease attack. PS modification of select internal sites may also be tolerated and may provide additional protection from endonuclease attack. Using SEQ ID No. 264 as a base modification pattern, internal linkages were PS modified in the tracrRNA-binding domain (SEQ ID No. 265), in the 3'-end of the protospacer guide domain (seed region) (SEQ ID No. 266), or both regions (SEQ ID No. 267). Increasing level of PS modification led to reduced functional activity, with SEQ ID No. 267 having ~50% the activity of the less modified SEQ ID No. 264 variant. SEQ ID No 267 has 21 out of 35 internucleotide linkages modified and will be stable to nuclease exposure. In cases where exposure to a high nuclease environment is needed (such as direct IV administration for research or therapeutic indications), this highly modified variant may actually show higher activity than the less modified variants, which will be degraded more quickly.

[0104] There are experimental settings where the PS modification contributes to chemical toxicity. In this case use of other methods to block exonuclease attack are desirable. The crRNA can have a C3 spacer (propanediol modifier) or a ZEN (napthyl-azo modifier) placed on either or both the 5'-end and 3'-end to block exonuclease attack, obviating the need the PS modification. This strategy can be employed to eliminate the PS-end block modification (See SEQ ID Nos. 179-186). This strategy can be used to reduce PS content of more highly modified crRNA variants. SEQ ID No. 271 has the internal protospacer domain and tracrRNA binding domain PS-modified in the same pattern as SEQ ID No. 267, yet employs only 15 PS internucleotide linkages (instead of 21) and shows improved activity. Therefore combination of non-base end-blocks with internal PS modification may be used to increase nuclease stability while maintaining high activity.

EXAMPLE 8

[0105] The following example demonstrates improved potency of the modified CRISPR crRNAs and tracrRNAs of the present disclosure. Examples 2-7 employed transfection of crRNA:tracrRNA complexes into human HEK-Cas9 cells at 30 nM concentration. Experimental testing had previously shown that this dose represented the upper shoulder of the dose response curve such that using higher doses of RNA did not improve gene editing efficiency but use of lower doses resulted lower gene editing efficiency. Those measurements were done using unmodified RNAs. The present example re-examines the dose response of new optimized chemically modified RNAs of the present disclosure compared with unmodified RNAs and demonstrates that chemical modification (i.e., nuclease stabilization) results in more potent compounds which can be used at lower dose.

[0106] Example 5 demonstrated that the truncated guide RNAs performed superior to WT RNAs at 12 sites in the human *HPRT1* gene. Four of these sites (38087, 38231, 38133, and 38285) were chosen for comparison of unmodified vs. modified RNA in the present example. Unmodified crRNAs were paired with the unmodified tracrRNA (SEQ ID No. 2) at a 1:1 molar ratio. Unmodified crRNAs were paired with the modified tracrRNA (SEQ ID No. 100) at a 1:1 molar ratio. Modified crRNAs were paired with the modified tracrRNA (SEQ ID No. 100) at a 1:1 molar ratio. Sequences are shown in Table 8. RNAs were transfected into HEK-Cas9 cells as described previously at 30 nM, 10 nM, and 3 nM concentrations. Cells were incubated for 48 hours at 37°C, then were processed for DNA and studied for evidence of gene editing activity comparing cleavage rates at the *HPRT1* locus in the T7EI mismatch endonuclease assay, with quantitative measurement of products done using the Fragment Analyzer as previously described. Results are shown in Table 8.

Table 8: Increased potency of modified vs. unmodified crRNA:tracrRNA complexes to direct Cas9-mediated gene editing in mammalian cells.

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	30 nM Cleavage %	10 nM Cleavage %	3 nM Cleavage %
38087 Un-cr Un-tr	56	aauuauggggauuacuaggaguuuuagagcuaug cu	80	76	35
	2	agcauagcaaguuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgagucggugcuuu			
38087 Un-cr Mod-tr	56	aauuauggggauuacuaggaguuuuagagcuaug cu	83	76	50
	100	a*g*cauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggugcu* u*u			
38087 Mod-cr	445	a*a*u*uauggggauuacuaggaguuuuagagcu au*g*c*u	77	77	54
	100	a*g*cauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggugcu* u*u			
38231 Un-cr Un-tr	69	uuuuguaauuaacagcuugcguuuuagagcuaug cu	31	4	0
	2	agcauagcaaguuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgagucggugcuuu			
38231 Un-cr Mod-tr	69	uuuuguaauuaacagcuugcguuuuagagcuaug cu	45	14	1
	100	a*g*cauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggugcu* u*u			
38231 Mod-cr Mod-tr	446	u*u*u*uguaauuaacagcuugcguuuuagagcu au*g*c*u	48	25	4
	100	a*g*cauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggugcu* u*u			

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	30 nM Cleavage %	10 nM Cleavage %	3 nM Cleavage %
38133 Un-cr Un-tr	78	ggucacuuuuaacacacccaguuuuagagcuaugcu	73	61	27
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu			
38133 Un-cr Mod-tr	78	ggucacuuuuaacacacccaguuuuagagcuaugcu	74	61	37
	100	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u			
38133 Mod-cr Mod-tr	447	g*g*u*cacuuuuaacacacccaguuuuagagcuau*g*c*u	75	66	55
	100	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u			
38285 Un-cr Un-tr	48	cuuauauccaacacuucgugguuuuagagcuaugcu	66	16	2
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu			
38285 Un-cr Mod-tr	48	cuuauauccaacacuucgugguuuuagagcuaugcu	67	16	5
	100	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u			
38285 Mod-cr	178	c*u*u*auauccaacacuucgugguuuuagagcuau*g*c*u	62	60	26

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	30 nM Cleavage %	10 nM Cleavage %	3 nM Cleavage %
Mod-tr	100	a*g*cauagcaaguuaaaauaaggcuaguccguu aucaacuugaaaaaguggcaccgagucggugcu* u*u*			

Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA; Underlined = 2'-O-methyl RNA; * = phosphorothioate internucleotide linkage. Unmodified crRNA = Un-cr. Unmodified tracrRNA = Un-tr. Modified crRNA = Mod-cr. Modified tracrRNA = Mod-tr. The relative functional activity of each species is indicated by the % cleavage in a T7EI heteroduplex assay for each dose studied.

[0107] In general, modification of the crRNA and tracrRNA had a small impact on gene editing efficiency when the RNAs were transfected at high dose where the RNAs are present in excess. At lower doses, the modified reagents showed improved potency and, in some cases, markedly improved potency. The degree of improvement varied with site. The very potent site 38087 showed highly efficiency gene editing at the 30 nM and 10 nM doses with all crRNA/tracrRNA variants tested, but at the 3 nM use of the modified tracrRNA (with either of the crRNAs) showed improved activity. A low potency site, such as 38231, showed improved gene editing efficiency even at the highest dose tested (30 nM) using the modified RNAs. Modification of the tracrRNA alone showed benefit, but the greatest benefit was realized when both the crRNA and tracrRNA were modified. FIG. 8 shows a schematic of one effective modified crRNA (SEQ ID No. 178) paired with modified tracrRNA (SEQ ID No. 100), specific for *HPRT1* site 38285. FIG. 9 shows a schematic of a more highly modified pair that is also highly functional, crRNA (SEQ ID No. 239) paired with modified tracrRNA (SEQ ID No. 134), also specific for *HPRT1* site 38285.

[0108] The present example employed transfection of the crRNA:tracrRNA complex into HEK-Cas9 cells, where Cas9 protein is constitutively expressed. Therefore transfected RNAs can bind Cas9 protein immediately, minimizing risk of degradation in the cytoplasm by nucleases. It is anticipated that the benefit of chemical modification of the crRNA and/or tracrRNA will be greater in cases where the transfected RNAs must survive exposure to cellular nucleases while Cas9 protein is being made, as occurs when using protocols where Cas9 mRNA or a Cas9 expression vector is co-transfected with the targeting RNAs, such that Cas9 is not already expressed in the cells. The benefits of using highly modified RNAs will be greatest for *in vivo* applications (such as medical therapeutics) where the RNAs may be exposed to both nucleases present in serum (following IV administration) and cellular cytoplasmic nucleases.

EXAMPLE 9

[0109] Examples 2-8 demonstrate activity of truncated and/or chemically-modified CRISPR crRNAs and/or tracrRNAs to trigger Cas9-mediated genome editing in mammalian cells that constitutively express Cas9. The present example demonstrates that the truncated, modified RNA compositions can bind Cas9 protein and this complex can be transfected into human cells

and further that transfection of the ribonuclear protein (RNP) complex is sufficient to trigger highly efficient genome editing.

[0110] Reagents specific for human *HPRT1* site 38285 were employed in the present example. Unmodified crRNA was paired with unmodified tracrRNA at a 1:1 molar ratio. Unmodified crRNA was paired with modified tracrRNA at a 1:1 molar ratio. Modified crRNA was paired with modified tracrRNA at a 1:1 molar ratio. Sequences are shown in Table 9. RNAs were transfected into unmodified HEK293 cells as described above except that a 1:1 complex of Cas9 protein (Caribou Biosciences) with crRNA:tracrRNA were employed at 10 nM concentration using increased amounts of RNAiMAX lipid transfection reagent (1.2 µL, increased over the 0.75 µL amount used per 100 µL transfection in 96 well format for the 30 nM RNA-alone transfections in HEK-Cas9 cells). Cells were incubated for 48 hours at 37°C, then were processed for DNA and studied for evidence of gene editing activity comparing cleavage rates at the *HPRT1* locus in the T7EI mismatch endonuclease assay, with quantitative measurement of products done using the Fragment Analyzer as previously described. Results are shown in Table 9.

Table 9: Increased potency of modified vs. **unmodified crRNA:tracrRNA complexes to direct Cas9-mediated gene editing in mammalian cells.**

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	10 nM Cleavage %
38285 Un-cr Un-tr	48	cuuauauccaacacuucgugguuuuagagcuaugcu	42
	2	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaa aaaguggcaccgagucggugcuuu	
38285 Un-cr Mod-tr	48	cuuauauccaacacuucgugguuuuagagcuaugcu	41
	100	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuug aaaaaguggcaccgagucggugcu*u*u	
38285 Mod-cr Mod-tr	178	c*u*u*auauccaacacuucgugguuuuagagcuau*g*c*u	54
	100	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuug aaaaaguggcaccgagucggugcu*u*u	

Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA; Underlined = 2'-O-methyl RNA; * = phosphorothioate internucleotide linkage. Unmodified crRNA = Un-cr. Unmodified tracrRNA = Un-tr. Modified crRNA = Mod-cr. Modified tracrRNA = Mod-tr. The relative functional activity of each complex is indicated by the % cleavage in a T7EI heteroduplex assay for each dose studied.

[0111] All 3 CRISPR RNA complexes performed well in the RNP-transfection protocol for mammalian genome editing. The unmodified crRNA + unmodified tracrRNA pair (SEQ ID Nos. 48 and 2) and the unmodified crRNA + modified tracrRNA pair (SEQ ID Nos. 48 and 100) performed 2.5x better at 10 nM dose in the RNP protocol than in the HEK-Cas9 protocol, consistent with the

less modified RNAs suffering degradation between transfection and eventual complexation with Cas9 protein in the cytoplasm or nucleus. Thus higher doses are needed for unmodified RNAs and in some settings it is likely that unmodified RNAs will fail to direct any genome editing activity. The modified crRNA + modified tracrRNA (SEQ ID NOs. 178 and 100), on the other hand, worked with high efficiency in both protocols.

[0112] The modified, truncated CRISPR RNAs work well with direct Cas9 RNP transfection methods.

EXAMPLE 10

[0113] The chemical modification optimization studies performed in Examples 6 and 7 studied the activity of crRNAs having various modification patterns paired with a tracrRNA having various modification patterns. The tracrRNA is universal and the same sequence is employed at all target sites. It is expected that the performance of various modification patterns for the tracrRNA will be similar between different target sites. The crRNA, however, varies sequence between different target sites. In the optimized version tested in Examples 7 and 8, the 5'-20 bases of the crRNA are target-specific (i.e., the "protospacer domain") and the 3'-16 bases are universal (i.e., "the tracrRNA binding domain"). Like the tracrRNA, it is expected that the performance of various modification patterns in the universal 16 base 3'-domain of the crRNA will be similar at all target sites. However, it is possible that performance of different modification patterns may be influenced by the sequence context present in the 5'-20 base target-specific domain.

[0114] It is well established that effective modification patterns for small interfering RNAs (siRNAs) are affected by sequence context (Behlke, Oligonucleotides 18:305-320, 2008). For siRNAs, certain "limited modification" patterns can be applied to all sites, whereas for "heavy modification" it is not possible to predict which patterns will be functional for a given sequence and empiric testing is necessary. The present example studies the effect that sequence context has on the crRNA, testing different modification patterns within the 5'-20 base target-specific domain at different sites.

[0115] The modification studies in Examples 6 and 7 employed a single crRNA PAM site in the human *HPRT1* gene. The present study examines 12 sites in the human *HPRT1* gene, including the site previously examined, comparing functional performance of different modification patterns and establishes a single modification pattern that can be employed with good results at all sites. See Example 5 for other studies relating to these 12 sites.

[0116] A series of crRNAs (Table 10) were synthesized having a protospacer domain lengths of 20 bases specific to 12 sites in the human *HPRT1* gene with a 16mer universal tracrRNA binding sequence at the 3'-end. The crRNAs were made using a variety of chemical modifications, including: the ribose modifications 2'OMe RNA, the end-modifying groups propanediol spacer and napthyl-azo modifier (N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine, or "ZEN"), an inverted-dT residue; and select internucleotide linkages with phosphorothioate modifications. A schematic representation of the different modification patterns employed is shown in FIG. 10.

[0117] The crRNAs were paired with a highly modified 67mer tracrRNA (SEQ ID No. 100). The paired crRNA:tracrRNA RNA oligonucleotides were transfected into the HEK-Cas9 cells and processed as described in Example 2. Relative gene editing activities were assessed by comparing cleavage rates in the *HPRT1* gene using the T7EI mismatch endonuclease cleavage assay with quantitative measurement of products done using the Fragment Analyzer. Results are shown in Table 10 and in FIG. 11.

Table 10: **Optimization of crRNA oligonucleotide modification patterns in mammalian cells across 12 target sites.**

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38094	64	1	uccauuucauagucuuuccuguuuuagagcuaugcu	62
38231	69	1	uuuuguaauuaacagcuugcguuuuagagcuaugcu	35
38371	71	1	cuuagagaauauuuguagagguuuuagagcuaugcu	66
38509	73	1	uugacuauaaugaauacuucguuuuagagcuaugcu	71
38574	75	1	caaaacacgcauaaaaauuuguuuuagagcuaugcu	52
38087	56	1	aauuauggggauuacuaggaguuuuagagcuaugcu	72
38133	78	1	ggucacuuuuaacacacccaguuuuagagcuaugcu	65
38285	48	1	cuuauauccaacacuucgugguuuuagagcuaugcu	62
38287	80	1	ggcuuauauccaacacuucgguuuuagagcuaugcu	47
38358	60	1	auuucacauaaaacucuuuuguuuuagagcuaugcu	59
38636	83	1	ucaaauuaugaggugcuggaguuuuagagcuaugcu	27
38673	85	1	uacagcuuuaugugacuaauguuuuagagcuaugcu	49
38094	291	2	u*c*c*auuucauagucuuuccuguuuuagagcuau *g*c*u	71
38231	292	2	u*u*u*uguaauuaacagcuugcguuuuagagcuau *g*c*u	54
38371	293	2	c*u*u*agagaauauuuguagagguuuuagagcuau *g*c*u	65
38509	294	2	u*u*g*acuauaaugaauacuucguuuuagagcuau *g*c*u	78
38574	295	2	c*a*a*aacacgcauaaaaauuuguuuuagagcuau *g*c*u	56

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38087	296	2	a*a*u*uaugggauuacuaggaguuuuagagcuau *g*c*u	76
38133	297	2	g*g*u*cacuuuuaacacacccaguuuuagagcuau *g*c*u	70
38285	178	2	c*u*u*auauccaacacuucguggguuuuagagcuau *g*c*u	65
38287	298	2	g*g*c*uuauauccaacacuucgguuuuagagcuau *g*c*u	59
38358	299	2	a*u*u*ucacauaaaacucuuuuguuuuagagcuau *g*c*u	73
38636	300	2	u*c*a*aauuaugaggugcuggaguuuuagagcuau *g*c*u	29
38673	301	2	u*a*c*agcuuuaugugacuaauguuuuagagcuau *g*c*u	60
38094	302	3	u*c*c*auuucauagucuuuccuguuuuagagcuau *g*c*u	67
38231	303	3	u*u*u*uguaauuaacagcuugcguuuuagagcuau *g*c*u	57
38371	304	3	c*u*u*agagaauauuuguagaggguuuuagagcuau *g*c*u	65
38509	305	3	u*u*g*acuauaaugaauacuucguuuuagagcuau *g*c*u	79
38574	306	3	c*a*a*aacacgcauaaaaauuuguuuuagagcuau *g*c*u	52

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38087	307	3	a*a*u*uaugggauuacuaggaguuuuagagcuau *g*c*u	76
38133	308	3	g*g*u*cacuuuuaacacacccaguuuuagagcuau *g*c*u	66
38285	309	3	c*u*u*auauccaacacuucgugguuuuagagcuau *g*c*u	60
38287	310	3	g*g*c*uuauauccaacacuucgguuuuagagcuau *g*c*u	56
38358	311	3	a*u*u*ucacauaaaacucuuuuguuuuagagcuau *g*c*u	66
38636	312	3	u*c*a*aauuaugaggugcuggaguuuuagagcuau *g*c*u	24
38673	313	3	u*a*c*agcuuuaugugacuaauguuuuagagcuau *g*c*u	51
38094	314	4	u*c*c*auuucauagucuuuccuguuuuagagcuau *g*c*u	68
38231	315	4	u*u*u*uguaauuaacagcuugcguuuuagagcuau *g*c*u	53
38371	316	4	c*u*u*agagaauauuuguagaggguuuuagagcuau *g*c*u	65
38509	317	4	u*u*g*acuauaaugaauacuucguuuuagagcuau *g*c*u	76

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38574	318	4	c*a*a*aacacgcauaaaaauuuguuuuagagcuau *g*c*u	51
38087	319	4	a*a*u*uaugggauuacuaggaguuuuagagcuau *g*c*u	76
38133	320	4	g*g*u*cacuuuuaacacacccaguuuuagagcuau *g*c*u	70
38285	321	4	c*u*u*auauccaacacuucgugguuuuagagcuau *g*c*u	65
38287	322	4	g*g*c*uuauauccaacacuucgguuuuagagcuau *g*c*u	56
38358	323	4	a*u*u*ucacauaaaacucuuuuguuuuagagcuau *g*c*u	64
38636	324	4	u*c*a*aauuaugaggugcuggaguuuuagagcuau *g*c*u	23
38673	325	4	u*a*c*agcuuuaugugacuaauguuuuagagcuau *g*c*u	48
38094	326	5	u*c*c*auuucauagucuuuccuguuuuagagcuau *g*c*u	71
38231	327	5	u*u*u*uguaauuaacagcuugcguuuuagagcuau *g*c*u	53
38371	328	5	c*u*u*agagaauauuuguagaggguuuuagagcuau *g*c*u	69
38509	329	5	u*u*g*acuauaaugaauacuucguuuuagagcuau *g*c*u	77

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38574	330	5	c*a*a*aacacgcauaaaaauuuguuuuagagcuau *g*c*u	51
38087	331	5	a*a*u*uaugggauuacuaggaguuuuagagcuau *g*c*u	80
38133	332	5	g*g*u*cacuuuuaacacacccaguuuuagagcuau *g*c*u	70
38285	333	5	c*u*u*auauccaacacuucgugguuuuagagcuau *g*c*u	64
38287	334	5	g*g*c*uuauauccaacacuucgguuuuagagcuau *g*c*u	59
38358	335	5	a*u*u*ucacauaaaacucuuuuguuuuagagcuau *g*c*u	64
38636	336	5	u*c*a*aauuaugaggugcuggaguuuuagagcuau *g*c*u	25
38673	337	5	u*a*c*agcuuuaugugacuaauguuuuagagcuau *g*c*u	56
38094	338	6	u*c*c*auuucauagucuuuccuguuuuagagcuau *g*c*u	70
38231	339	6	u*u*u*uguaauuaacagcuugcguuuuagagcuau *g*c*u	53
38371	340	6	c*u*u*agagaauauuuguagaggguuuuagagcuau *g*c*u	68
38509	341	6	u*u*g*acuauaaugaauacuucguuuuagagcuau *g*c*u	72

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38574	342	6	c*a*a*aacacgcauaaaaauuuguuuuagagcuau *g*c*u	51
38087	343	6	a*a*u*uaugggguuacuaggaguuuuagagcuau *g*c*u	81
38133	344	6	g*g*u*cacuuuuaacacacccaguuuuagagcuau *g*c*u	71
38285	345	6	c*u*u*auauccaacacuucgugguuuuagagcuau *g*c*u	64
38287	346	6	g*g*c*uuauauccaacacuucgguuuuagagcuau *g*c*u	55
38358	347	6	a*u*u*ucacauaaaacucuuuuguuuuagagcuau *g*c*u	65
38636	348	6	u*c*a*aauuaugaggugcuggaguuuuagagcuau *g*c*u	24
38673	349	6	u*a*c*agcuuuaugugacuaauguuuuagagcuau *g*c*u	55
38094	350	7	u*c*c*auuucauagucuuuccuguuuuagagcuau *g*c*u	73
38231	351	7	u*u*u*uguaauuaacagcuugcguuuuagagcuau *g*c*u	51
38371	352	7	c*u*u*agagaauauuuguagaggguuuuagagcuau *g*c*u	73
38509	353	7	u*u*g*acuauaaugaauacuucguuuuagagcuau *g*c*u	78

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38574	354	7	c*a*a*aacacgcauaaaaauuuguuuuagagcuau *g*c*u	50
38087	355	7	a*a*u*uaugggauuacuaggaguuuuagagcuau *g*c*u	83
38133	356	7	g*g*u*cacuuuuaacacacccaguuuuagagcuau *g*c*u	63
38285	357	7	c*u*u*auauccaacacuucgugguuuuagagcuau *g*c*u	63
38287	358	7	g*g*c*uuauauccaacacuucgguuuuagagcuau *g*c*u	43
38358	359	7	a*u*u*ucacauaaaacucuuuuguuuuagagcuau *g*c*u	66
38636	360	7	u*c*a*aauuaugaggugcuggaguuuuagagcuau *g*c*u	28
38673	361	7	u*a*c*agcuuuaugugacuaauguuuuagagcuau *g*c*u	61
38094	362	8	u*c*c*auuucauagucuuuccuguuuuagagcuau *g*c*u	63
38231	363	8	u*u*u*uguaauuaacagcuugcguuuuagagcuau *g*c*u	40
38371	364	8	c*u*u*agagaauauuuguagaggguuuuagagcuau *g*c*u	64
38509	365	8	u*u*g*acuauaaugaauacuucguuuuagagcuau *g*c*u	67

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38574	366	8	c*a*a*aacacgcauaaaaauuuguuuuagagcuau *g*c*u	18
38087	367	8	a*a*u*uaugggauuacuaggaguuuuagagcuau *g*c*u	75
38133	368	8	g*g*u*cacuuuuaacacacccaguuuuagagcuau *g*c*u	48
38285	369	8	c*u*u*auauccaacacuucguggguuuuagagcuau *g*c*u	53
38287	370	8	g*g*c*uuauauccaacacuucgguuuuagagcuau *g*c*u	24
38358	371	8	a*u*u*ucacauaaaacucuuuuguuuuagagcuau *g*c*u	56
38636	372	8	u*c*a*aauuaugaggugcuggaguuuuagagcuau *g*c*u	22
38673	373	8	u*a*c*agcuuuaugugacuaauguuuuagagcuau *g*c*u	50
38094	374	9	u*c*c*auuucauagucuuuccuguuuuagagcuau *g*c*u	65
38231	375	9	u*u*u*uguaauuaacagcuugcguuuuagagcuau *g*c*u	7
38371	376	9	c*u*u*agagaauauuuguagaggguuuuagagcuau *g*c*u	70
38509	377	9	u*u*g*acuauaaugaauacuucguuuuagagcuau *g*c*u	57

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38574	378	9	c*a*a*aacacgcauaaaaauuuguuuuagagcuau *g*c*u	8
38087	379	9	a*a*u*uauggggauuacuaggaguuuuagagcuau *g*c*u	74
38133	380	9	g*g*u*cacuuuuaacacacccaguuuuagagcuau *g*c*u	38
38285	222	9	c*u*u*auauccaacacuucgugguuuuagagcuau *g*c*u	54
38287	381	9	g*g*c*uuauauccaacacuucgguuuuagagcuau *g*c*u	32
38358	382	9	a*u*u*ucacauaaaacucuuuuguuuuagagcuau *g*c*u	58
38636	383	9	u*c*a*aauuaugaggugcuggaguuuuagagcuau *g*c*u	19
38673	384	9	u*a*c*agcuuuaugugacuaauguuuuagagcuau *g*c*u	55
38094	385	10	C3-uccauuucauagucuuuccuguuuuagagcuaugcu-C3	66
38231	386	10	C3-uuuuguaauuaacagcuugcguuuuagagcuaugcu-C3	54
38371	387	10	C3-cuuagagaauauuuguagaggguuuuagagcuaugcu-C3	57
			C3-	

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38509	388	10	uugacuauaaugaauacuucguuuuagagcuaugcu –C3	75
38574	389	10	C3– caaaacacgcauaaaaauuuguuuuagagcuaugcu –C3	50
38087	390	10	C3– aauuaugggggauuacuaggaguuuuagagcuaugcu –C3	71
38133	391	10	C3– ggucacuuuuaacacacccaguuuuagagcuaugcu –C3	68
38285	181	10	C3– cuuauauccaacacuucgugguuuuagagcuaugcu –C3	58
38287	392	10	C3– ggcuuauauccaacacuucgguuuuagagcuaugcu –C3	57
38358	393	10	C3– auuucacauaaaacucuuuuguuuuagagcuaugcu –C3	64
38636	394	10	C3– ucaaauuaugaggugcuggaguuuuagagcuaugcu –C3	22
38673	395	10	C3– uacagcuuuaugugacuaauguuuuagagcuaugcu –C3	50
38094	396	11	**ZEN–** uccauuucauagucuuuccuguuuuagagcuaugcu **–ZEN**	74
38231	397	11	**ZEN–** uuuuguaauuaacagcuugcguuuuagagcuaugcu **–ZEN**	44

HPRT1 Target site	SEQ ID No.	Mod Pattern	crRNA Sequence (5'-3')	Cleavage % tracrRNA SEQ ID No. 100
38371	398	11	ZEN- cuuagagaauauuuguagagguuuuagagcuaugcu -ZEN	72
38509	399	11	ZEN- uugacuauaaugaauacuucguuuuagagcuaugcu -ZEN	74
38574	400	11	ZEN- caaaacacgcauaaaaauuuguuuuagagcuaugcu -ZEN	57
38087	401	11	ZEN- aauuauggggauuacuaggaguuuuagagcuaugcu -ZEN	82
38133	402	11	ZEN- ggucacuuuuaacacacccaguuuuagagcuaugcu -ZEN	73
38285	184	11	ZEN- cuuauauccaacacuucgugguuuuagagcuaugcu -ZEN	60
38287	403	11	ZEN- ggcuuauauccaacacuucgguuuuagagcuaugcu -ZEN	62
38358	404	11	ZEN- auuucacauaaaacucuuuuguuuuagagcuaugcu -ZEN	69
38636	405	11	ZEN- ucaaauuaugaggugcuggaguuuuagagcuaugcu -ZEN	26
38673	406	11	ZEN- uacagcuuuaugugacuaauguuuuagagcuaugcu -ZEN	44

Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA; Underlined = 2'-O-methyl RNA; C3 = C3 spacer (propanediol modifier); * = phosphorothioate internucleotide linkage; ZEN = napthyl-azo modifier. The relative functional activity of

each species is indicated by the % cleavage in a T7EI heteroduplex assay when the indicated crRNA is paired with the indicated tracrRNA at each of 12 sites in human *HRPT1*.

[0118] The modified crRNAs employed a fixed modification pattern in the 16-base 3'-end domain which is universal and binds the tracrRNA. Different modification pattern were tested/compared in the 5'-end domain that is target specific (i.e., sequence varies with target site). The test set comprised variants having 0, 3, 4, 6, 8, 10, 12, 13, or 14 contiguous 2'OMe RNA residues starting at the 5'-end and walking towards the 3'-end. The modification patterns avoided positions previously demonstrated to reduce functional performance of the crRNA (Example 7). Use of only non-base modifier end groups (C3 spacer or ZEN) were also tested (without additional modification). When functional activity is compared across all 12 sites in the survey, all sites tested showed full activity when 0-10 RNA residues at the 5'-end were replaced with 2'OMe RNA residues. Only 1/12 sites showed a slight reduction in activity with 12 residues modified, however 3/12 sites showed a reduction in activity when 13 residues were modified and 4/12 sites showed a reduction in activity when 14 residues were modified. The end-modifiers (C3, ZEN) showed full activity at all sites.

[0119] The highest level of crRNA modification that showed full activity at all sites tested included Mod Patterns 6 and 7 (FIG. 10). This represents 61% and 67% of the bases in the crRNA modified with 2'OMe RNA, respectively.

n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u	Mod Pattern 6 (SEQ ID NO.:434)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u	Mod Pattern 7 (SEQ ID NO.:435)

[0120] The data in the present example also demonstrates that individual sites can be modified at higher levels and retain potency. For example, 8 of the 12 sites studied showed full activity using Mod Pattern 8, which has 72% of the residues modified. Further, example 7 demonstrates that the crRNA targeting site 38285 in HPRT1 (SEQ ID No. 239) has full activity and has 30/36 residues modified (83%, with only 6 unmodified RNA residues remaining). A base modification pattern such as Mod Pattern 6 or Mod Pattern 7 can be used as a starting point for studies to empirically ascertain the extent that a particular sequence can be modified before activity is lost. FIG. 12 shows a schematic where a Mod Pattern 6 crRNA is paired with a highly modified tracrRNA, SEQ ID No. 134.

EXAMPLE 11

[0121] The Examples herein employ the Cas9 endonuclease from *Streptococcus pyogenes.* The native amino acid sequence of *S.py.* Cas9 (SpyCas9) is shown below (SEQ ID No. 407).

[0122] The native Cas9 DNA sequence was codon optimized for expression in *E.coli* bacteria and

had elements added for mammalian nuclear localization (nuclease localization signals) and aid protein purification (His-tag). The final amino-acid sequence of the recombinant protein is shown (SEQ ID No 408). The DNA sequence employed to express the recombinant protein in *E.coli* is shown (SEQ ID No. 409).

[0123] The native Cas9 DNA sequence was codon optimized for expression in human cells and had elements added for antibody recognition (V5 epitope) and mammalian nuclear localization (nuclease localization signals, NLS) added. The final amino-acid sequence is shown (SEQ ID No. 410) and DNA sequence follows (SEQ ID No 411).

[0124] The native *S.py* Cas9 DNA sequence codon was optimized for expression in human cells and assembled as a T7 RNA polymerase expression cassette (SEQ ID No. 412). The sequence contains a T7 RNA polymerase promoter, a V5 epitope tag, a nuclear localization signal, the codon optimized Cas9 sequence, a second nuclear localization signal, and the BGH (bovine growth hormone) gene 3'-UTR element with a polyadenylation signal. Sequence of mRNA made from this expression cassette is shown (SEQ ID No. 413).

S.py. Cas9 amino acid sequence (SEQ ID No. 407).

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRR
YTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKL
VDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILS
ARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGD
QYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS
KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHILRRQE
DFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNF
DKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYF
KKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYA
HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKA
QVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK

RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSID
NKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVET
RQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGT
ALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETN
GETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSP
TVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELEN
GRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKR
VILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
SITGLYETRIDLSQLGGD

S.py Cas9 amino acid sequence expressed from DNA codon optimized for expression in E.coli containing 3 NLS sequences and a purification His-tag (SEQ ID No. 408).

MGSSAPKKKRKVGIHGVPAAMDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL
LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNI
VDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYN
QLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKL
QLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLL
KALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFD
NGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWN
FEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAI
VDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDI
VLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFAN
RNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVI
EMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINR
LSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA
ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYK

VREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNF
FKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGY
KEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLF
VEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDT
TIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDAAPKKKRKVDPKKKRKVAAALEHHHHHH

S.py Cas9 DNA sequence codon optimized for expression in E.coli containing 3 NLS sequences and a purification His-tag (SEQ ID No. 409).

ATGGGCAGCAGCGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCATGGACAAAAA
GTACTCTATTGGCCTGGATATCGGGACCAACAGCGTCGGGTGGGCTGTTATCACCGACGAGTATAAAGTAC
CTTCGAAAAAGTTCAAAGTGCTGGGCAACACCGATCGCCATTCAATCAAAAAGAACTTGATTGGTGCGCTG
TTGTTTGACTCCGGGGAAACCGCCGAGGCGACTCGCCTTAAACGTACAGCACGTCGCCGGTACACTCGGCG
TAAGAATCGCATTTGCTATTTGCAGGAAATCTTTAGCAACGAGATGGCAAAAGTCGATGACTCGTTTTTCC
ACCGCCTCGAGGAAAGCTTTCTGGTGGAGGAAGACAAAAAGCATGAGCGTCACCCGATCTTCGGCAACATT
GTCGATGAAGTAGCGTATCATGAAAAATACCCAACCATTTACCACTTACGCAAAAAGCTGGTGGACAGCAC
TGACAAAGCTGATTTGCGCCTTATCTATTTAGCCCTGGCACATATGATTAAGTTTCGTGGTCACTTCCTGA
TCGAAGGAGACTTAAATCCCGACAACAGTGATGTTGATAAATTGTTTATTCAGCTTGTCCAAACTTACAAT
CAACTGTTCGAGGAAAACCCGATCAATGCCTCCGGTGTGGATGCAAAAGCCATTTTAAGTGCACGCCTTAG

CAAGTCCCGTCGCTTAGAAAACCTTATCGCGCAGCTGCCCGGCGAGAAAAAGAATGGTTTGTTTGGGAACC
TTATTGCCTTGAGCTTAGGCCTCACCCCGAATTTCAAAAGTAATTTCGATCTTGCAGAAGACGCCAAATTA
CAACTGTCGAAGGATACTTATGATGACGATCTCGATAATCTGTTAGCGCAGATTGGTGACCAATACGCCGA
TCTTTTTCTGGCGGCTAAAAATCTGAGCGACGCCATCTTGCTTTCGGATATTCTCCGCGTTAACACCGAAA
TCACGAAAGCGCCTCTTAGTGCCAGCATGATTAAACGTTATGATGAACACCACCAGGACCTGACCTTACTC
AAAGCGTTGGTTCGCCAGCAACTGCCAGAGAAGTACAAAGAAATCTTCTTTGATCAGTCAAAGAATGGTTA
TGCCGGCTATATTGACGGGGGTGCAAGCCAAGAGGAATTCTACAAATTTATCAAGCCTATTCTGGAGAAAA
TGGATGGCACCGAAGAGTTATTGGTGAAGCTTAACCGTGAAGACCTCCTGCGGAAACAGCGCACATTCGAT
AATGGTTCGATCCCACACCAAATCCATTTGGGGGAGTTACACGCTATTTTGCGTCGCCAGGAAGACTTTTA
CCCTTTCCTGAAGGATAACCGGGAGAAAATTGAGAAGATCCTTACCTTTCGTATTCCGTATTACGTAGGCC
CCTTAGCACGGGGTAATAGCCGTTTCGCGTGGATGACACGGAAGTCGGAAGAGACGATCACCCCGTGGAAC

TTCGAAGAGGTAGTCGACAAGGGCGCATCAGCGCAGTCTTTTATTGAACGTATGACGAATTTCGATAAAAA
CTTGCCCAATGAGAAGGTGCTTCCGAAACATTCCTTGTTATATGAATATTTTACAGTTTACAACGAGCTGA
CCAAGGTTAAATACGTGACGGAAGGAATGCGCAAGCCCGCTTTTCTTAGCGGTGAGCAAAAAAAGGCGATC
GTCGACCTGTTATTCAAAACGAATCGTAAGGTGACTGTAAAGCAACTCAAAGAAGATTACTTCAAAAAGAT
TGAGTGCTTCGACAGCGTCGAAATCTCTGGGGTAGAGGATCGGTTTAACGCAAGTTTAGGTACCTACCATG
ACCTGCTTAAAATCATTAAGGATAAAGACTTCTTAGATAATGAAGAGAACGAAGATATTCTCGAGGACATC
GTCTTGACGTTAACCTTATTTGAGGATCGTGAAATGATTGAGGAACGCCTCAAAACTTATGCCCACCTGTT
CGACGATAAGGTGATGAAGCAGCTGAAACGTCGGCGCTACACAGGATGGGGCCGCTTGAGTCGCAAACTTA
TTAACGGAATCCGTGACAAGCAATCCGGCAAAACGATTCTGGATTTCTTGAAGTCGGACGGATTTGCTAAT
CGCAACTTCATGCAGTTGATCCATGATGACTCCCTGACTTTTAAAGAGGATATTCAAAAGGCGCAGGTTAG
TGGTCAAGGCGACAGCTTACACGAACACATCGCAAATTTGGCTGGTTCGCCGGCCATTAAAAAGGGGATCC
TCCAGACCGTGAAAGTTGTAGATGAGCTTGTTAAGGTCATGGGTCGTCATAAGCCCGAAAACATCGTGATT
GAAATGGCGCGGGAGAATCAAACGACCCAGAAAGGACAAAAGAATAGCCGTGAACGGATGAAGCGGATCGA
GGAAGGCATTAAAGAGCTGGGGTCTCAAATCTTGAAGGAACACCCTGTGGAGAACACTCAGCTCCAAAATG
AAAAACTTTACCTGTACTATTTGCAGAACGGACGCGATATGTACGTGGACCAAGAGTTGGATATTAATCGG
CTGAGTGACTACGACGTTGATCATATCGTCCCGCAGAGCTTCCTCAAAGACGATTCTATTGACAATAAGGT
ACTGACGCGCTCTGATAAAAACCGTGGTAAGTCGGACAACGTGCCCTCCGAAGAGGTTGTGAAAAAGATGA
AAAATTATTGGCGCCAGCTTTTAAACGCGAAGCTGATCACACAACGTAAATTCGATAATTTGACCAAGGCT
GAACGGGGTGGCCTGAGCGAGTTAGATAAGGCAGGATTTATTAAACGCCAGTTAGTGGAGACTCGTCAAAT

CACCAAACATGTCGCGCAGATTTTGGACAGCCGGATGAACACCAAGTACGATGAAAATGACAAACTGATCC
GTGAGGTGAAAGTCATTACTCTGAAGTCCAAATTAGTTAGTGATTTCCGGAAGGACTTTCAATTCTACAAA
GTCCGTGAAATTAATAACTATCATCACGCACATGACGCGTACCTGAATGCAGTGGTTGGGACCGCCCTTAT
CAAGAAATATCCTAAGCTGGAGTCGGAGTTTGTCTATGGCGACTATAAGGTATACGATGTTCGCAAAATGA
TTGCGAAATCTGAGCAGGAGATCGGTAAGGCAACCGCAAAATATTTCTTTTACTCAAACATTATGAATTTC
TTTAAGACAGAAATCACTCTGGCCAACGGGGAGATTCGCAAACGTCCGTTGATCGAAACAAACGGCGAGAC
TGGCGAAATTGTTTGGGACAAAGGGCGTGATTTCGCGACGGTGCGCAAGGTACTGAGCATGCCTCAAGTCA
ATATTGTTAAGAAAACCGAAGTGCAGACGGGCGGGTTTTCCAAGGAAAGCATCTTACCCAAACGTAATTCA
GATAAACTTATTGCACGCAAAAAGGACTGGGATCCGAAAAAGTATGGAGGCTTCGACAGTCCAACCGTAGC
CTACTCTGTTCTCGTTGTAGCGAAAGTAGAAAAGGGTAAATCCAAGAAACTGAAATCTGTCAAGGAGTTGC
TTGGAATCACCATTATGGAGCGTAGCTCCTTCGAGAAGAACCCGATTGACTTTCTGGAAGCCAAAGGATAT
AAAGAGGTCAAGAAAGATCTTATCATTAAGCTGCCTAAGTATTCACTCTTCGAGCTGGAAAATGGTCGTAA
ACGCATGCTCGCTTCTGCCGGCGAGTTGCAGAAGGGCAATGAATTAGCACTTCCATCAAAGTACGTTAACT

TCCTGTATTTGGCCAGCCATTACGAGAAACTGAAGGGGTCTCCAGAGGACAACGAACAGAAACAATTATTT
GTAGAGCAGCACAAGCATTATCTTGATGAAATCATTGAGCAAATTTCCGAATTCAGTAAACGCGTAATCCT
GGCCGATGCAAACCTCGACAAGGTGCTGAGCGCTTACAATAAGCATCGCGACAAACCTATCCGTGAGCAGG
CTGAAAATATCATTCACCTGTTCACATTAACGAACCTGGGCGCTCCGGCCGCTTTTAAATATTTCGACACG
ACAATCGACCGTAAGCGCTATACCAGTACGAAAGAAGTGTTGGATGCGACCCTTATTCACCAGTCAATTAC
AGGATTATATGAGACCCGTATCGACCTTAGCCAATTAGGTGGGGATGCGGCCCCGAAGAAAAAACGCAAAG
TGGATCCGAAGAAAAAACGCAAAGTGGCGGCCGCACTCGAGCACCACCACCACCACCACTGA

S.py Cas9 amino acid sequence expressed from DNA codon optimized for expression in human cells containing a V5 epitope tag and 2 NLS sequences (SEQ ID No. 410).

MGKPIPNPLLGLDSTAPKKKRKVGIHGVPAADKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVL
GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFR
GHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLP
GEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRR
QEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQS
FIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKT
NRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS
DGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYY
LQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMK
NYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDE
NDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWD
KGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVA
YSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEII
EQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR
YTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSRADPKKKRKVEFHHTGLVDPSSVPSLSLNR

S.py Cas9 DNA sequence codon optimized for expression in human cells containing a V5 epitope tag and 2 NLS sequences (SEQ ID No. 411).

ATGGGCAAGCCCATCCCTAACCCCCTGTTGGGGCTGGACAGCACCGCTCCCAAAAAGAAAAGGAAGGTGGG
CATTCACGGCGTGCCTGCGGCCGACAAAAAGTACAGCATCGGCCTTGATATCGGCACCAATAGCGTGGGCT
GGGCCGTTATCACAGACGAATACAAGGTACCCAGCAAGAAGTTCAAGGTGCTGGGGAATACAGACAGGCAC
TCTATCAAGAAAAACCTTATCGGGGCTCTGCTGTTTGACTCAGGCGAGACCGCCGAGGCCACCAGGTTGAA
GAGGACCGCAAGGCGAAGGTACACCCGGAGGAAGAACAGGATCTGCTATCTGCAGGAGATCTTCAGCAACG
AGATGGCCAAGGTGGACGACAGCTTCTTCCACAGGCTGGAGGAGAGCTTCCTTGTCGAGGAGGATAAGAAG
CACGAACGACACCCCATCTTCGGCAACATAGTCGACGAGGTCGCTTATCACGAGAAGTACCCCACCATCTA
CCACCTGCGAAAGAAATTGGTGGATAGCACCGATAAAGCCGACTTGCGACTTATCTACTTGGCTCTGGCGC
ACATGATTAAGTTCAGGGGCCACTTCCTGATCGAGGGCGACCTTAACCCCGACAACAGTGACGTAGACAAA
TTGTTCATCCAGCTTGTACAGACCTATAACCAGCTGTTCGAGGAAAACCCTATTAACGCCAGCGGGGTGGA
TGCGAAGGCCATACTTAGCGCCAGGCTGAGCAAAAGCAGGCGCTTGGAGAACCTGATAGCCCAGCTGCCCG
GTGAAAAGAAGAACGGCCTCTTCGGTAATCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGC
AACTTCGACCTGGCAGAAGATGCCAAGCTGCAGTTGAGTAAGGACACCTATGACGACGACTTGGACAATCT
GCTCGCCCAAATCGGCGACCAGTACGCTGACCTGTTCCTCGCCGCCAAGAACCTTTCTGACGCAATCCTGC
TTAGCGATATCCTTAGGGTGAACACAGAGATCACCAAGGCCCCCCTGAGCGCCAGCATGATCAAGAGGTAC
GACGAGCACCATCAGGACCTGACCCTTCTGAAGGCCCTGGTGAGGCAGCAACTGCCCGAGAAGTACAAGGA
GATCTTTTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGAGCCAGCCAAGAGGAGTTCT

ACAAGTTCATCAAGCCCATCCTGGAGAAGATGGATGGCACCGAGGAGCTGCTGGTGAAGCTGAACAGGGAA
GATTTGCTCCGGAAGCAGAGGACCTTTGACAACGGTAGCATCCCCCACCAGATCCACCTGGGCGAGCTGCA
CGCAATACTGAGGCGACAGGAGGATTTCTACCCCTTCCTCAAGGACAATAGGGAGAAAATCGAAAAGATTC
TGACCTTCAGGATCCCCTACTACGTGGGCCCTCTTGCCAGGGGCAACAGCCGATTCGCTTGGATGACAAGA
AAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAAGGAGCAAGCGCGCAGTCTTT
CATCGAACGGATGACCAATTTCGACAAAAACCTGCCTAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTTT
ACGAGTACTTCACCGTGTACAACGAGCTCACCAAGGTGAAATATGTGACCGAGGGCATGCGAAAACCCGCT
TTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTGTTCAAGACCAACAGGAAGGTGACCGTGAA

TTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTGTTCAAGACCAACAGGAAGGTGACCGTGAA
GCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTTGATAGCGTGGAAATAAGCGGCGTGGAGGACA
GGTTCAACGCCAGCCTGGGCACCTACCACGACTTGTTGAAGATAATCAAAGACAAGGATTTCCTGGATAAT
GAGGAGAACGAGGATATACTCGAGGACATCGTGCTGACTTTGACCCTGTTTGAGGACCGAGAGATGATTGA
AGAAAGGCTCAAAACCTACGCCCACCTGTTCGACGACAAAGTGATGAAACAACTGAAGAGACGAAGATACA
CCGGCTGGGGCAGACTGTCCAGGAAGCTCATCAACGGCATTAGGGACAAGCAGAGCGGCAAGACCATCCTG
GATTTCCTGAAGTCCGACGGCTTCGCCAACCGAAACTTCATGCAGCTGATTCACGATGACAGCTTGACCTT
CAAGGAGGACATCCAGAAGGCCCAGGTTAGCGGCCAGGGCGACTCCCTGCACGAACATATTGCAAACCTGG
CAGGCTCCCCTGCGATCAAGAAGGGCATACTGCAGACCGTTAAGGTTGTGGACGAATTGGTCAAGGTCATG
GGCAGGCACAAGCCCGAAAACATAGTTATAGAGATGGCCAGAGAGAACCAGACCACCCAAAAGGGCCAGAA
GAACAGCCGGGAGCGCATGAAAAGGATCGAGGAGGGTATCAAGGAACTCGGAAGCCAGATCCTCAAAGAGC
ACCCCGTGGAGAATACCCAGCTCCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCAGGGACATG
TACGTTGACCAGGAGTTGGACATCAACAGGCTTTCAGACTATGACGTGGATCACATAGTGCCCCAGAGCTT
TCTTAAAGACGATAGCATCGACAACAAGGTCCTGACCCGCTCCGACAAAAACAGGGGCAAAAGCGACAACG
TGCCAAGCGAAGAGGTGGTTAAAAAGATGAAGAACTACTGGAGGCAACTGCTCAACGCGAAATTGATCACC
CAGAGAAAGTTCGATAACCTGACCAAGGCCGAGAGGGGCGGACTCTCCGAACTTGACAAAGCGGGCTTCAT
AAAGAGGCAGCTGGTCGAGACCCGACAGATCACGAAGCACGTGGCCCAAATCCTCGACAGCAGAATGAATA
CCAAGTACGATGAGAATGACAAACTCATCAGGGAAGTGAAAGTGATTACCCTGAAGAGCAAGTTGGTGTCC
GACTTTCGCAAAGATTTCCAGTTCTACAAGGTGAGGGAGATCAACAACTACCACCATGCCCACGACGCATA
CCTGAACGCCGTGGTCGGCACCGCCCTGATTAAGAAGTATCCAAAGCTGGAGTCCGAATTTGTCTACGGCG
ACTACAAAGTTTACGATGTGAGGAAGATGATCGCTAAGAGCGAACAGGAGATCGGCAAGGCCACCGCTAAG
TATTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATCACACTTGCCAACGGCGAAATCAGGAA
GAGGCCGCTTATCGAGACCAACGGTGAGACCGGCGAGATCGTGTGGGACAAGGGCAGGGACTTCGCCACCG
TGAGGAAAGTCCTGAGCATGCCCCAGGTGAATATTGTGAAAAAAACTGAGGTGCAGACAGGCGGCTTTAGC
AAGGAATCCATCCTGCCCAAGAGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCTAAGAA
GTATGGAGGCTTCGACAGCCCCACCGTAGCCTACAGCGTGCTGGTGGTCGCGAAGGTAGAGAAGGGGAAGA
GCAAGAAACTGAAGAGCGTGAAGGAGCTGCTCGGCATAACCATCATGGAGAGGTCCAGCTTTGAGAAGAAC
CCCATTGACTTTTTGGAAGCCAAGGGCTACAAAGAGGTCAAAAAGGACCTGATCATCAAACTCCCCAAGTA
CTCCCTGTTTGAATTGGAGAACGGCAGAAAGAGGATGCTGGCGAGCGCTGGGGAACTGCAAAAGGGCAACG
AACTGGCGCTGCCCAGCAAGTACGTGAATTTTCTGTACCTGGCGTCCCACTACGAAAAGCTGAAAGGCAGC
CCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCATTACCTGGACGAGATAATCGAGCA
AATCAGCGAGTTCAGCAAGAGGGTGATTCTGGCCGACGCGAACCTGGATAAGGTCCTCAGCGCCTACAACA
AGCACCGAGACAAACCCATCAGGGAGCAGGCCGAGAATATCATACACCTGTTCACCCTGACAAATCTGGGC
GCACCTGCGGCATTCAAATACTTCGATACCACCATCGACAGGAAAAGGTACACTAGCACTAAGGAGGTGCT
GGATGCCACCTTGATCCACCAGTCCATTACCGGCCTGTATGAGACCAGGATCGACCTGAGCCAGCTTGGAG
GCGACTCTAGGGCGGACCCAAAAAAGAAAAGGAAGGTGGAATTCCACCACACTGGACTAGTGGATCCGAGC
TCGGTACCAAGCTTAAGTTTAAACCGCTGA

S.py Cas9 DNA sequence codon optimized for expression in human cells as a T7 RNA polymerase expression cassette (SEQ ID No. 412). The sequence contains a T7 RNA polymerase promoter, a V5 epitope tag, a nuclear localization signal, the codon optimized Cas9 sequence, a second nuclear localization signal, and the BGH (bovine growth hormone) gene 3'-UTR element with a polyadenylation signal.

TAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAAACGGGCCCTCTAGACTCGAGCGGCCGC
CACCATGGGCAAGCCCATCCCTAACCCCCTGTTGGGGCTGGACAGCACCGCTCCCAAAAAGAAAAGGAAGG
TGGGCATTCACGGCGTGCCTGCGGCCGACAAAAAGTACAGCATCGGCCTTGATATCGGCACCAATAGCGTG
GGCTGGGCCGTTATCACAGACGAATACAAGGTACCCAGCAAGAAGTTCAAGGTGCTGGGGAATACAGACAG
GCACTCTATCAAGAAAAACCTTATCGGGGCTCTGCTGTTTGACTCAGGCGAGACCGCCGAGGCCACCAGGT
TGAAGAGGACCGCAAGGCGAAGGTACACCCGGAGGAAGAACAGGATCTGCTATCTGCAGGAGATCTTCAGC
AACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGGCTGGAGGAGAGCTTCCTTGTCGAGGAGGATAA
GAAGCACGAACGACACCCCATCTTCGGCAACATAGTCGACGAGGTCGCTTATCACGAGAAGTACCCCACCA
TCTACCACCTGCGAAAGAAATTGGTGGATAGCACCGATAAAGCCGACTTGCGACTTATCTACTTGGCTCTG
GCGCACATGATTAAGTTCAGGGGCCACTTCCTGATCGAGGGCGACCTTAACCCCGACAACAGTGACGTAGA
CAAATTGTTCATCCAGCTTGTACAGACCTATAACCAGCTGTTCGAGGAAAACCCTATTAACGCCAGCGGGG
TGGATGCGAAGGCCATACTTAGCGCCAGGCTGAGCAAAAGCAGGCGCTTGGAGAACCTGATAGCCCAGCTG
CCCGGTGAAAAGAAGAACGGCCTCTTCGGTAATCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAA
GAGCAACTTCGACCTGGCAGAAGATGCCAAGCTGCAGTTGAGTAAGGACACCTATGACGACGACTTGGACA
ATCTGCTCGCCCAAATCGGCGACCAGTACGCTGACCTGTTCCTCGCCGCCAAGAACCTTTCTGACGCAATC
CTGCTTAGCGATATCCTTAGGGTGAACACAGAGATCACCAAGGCCCCCCTGAGCGCCAGCATGATCAAGAG
GTACGACGAGCACCATCAGGACCTGACCCTTCTGAAGGCCCTGGTGAGGCAGCAACTGCCCGAGAAGTACA
AGGAGATCTTTTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGAGCCAGCCAAGAGGAG
TTCTACAAGTTCATCAAGCCCATCCTGGAGAAGATGGATGGCACCGAGGAGCTGCTGGTGAAGCTGAACAG
GGAAGATTTGCTCCGGAAGCAGAGGACCTTTGACAACGGTAGCATCCCCCACCAGATCCACCTGGGCGAGC

GCAACATTTGCTCCGAACCAGACGACCTTTGACAACGGTAGCATCCCCCACCAGATCCACCTGGGCGACC
TGCACGCAATACTGAGGCGACAGGAGGATTTCTACCCCTTCCTCAAGGACAATAGGGAGAAAATCGAAAAG
ATTCTGACCTTCAGGATCCCCTACTACGTGGGCCCTCTTGCCAGGGGCAACAGCCGATTCGCTTGGATGAC
AAGAAAGAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAAGGAGCAAGCGCGCAGT
CTTTCATCGAACGGATGACCAATTTCGACAAAAACCTGCCTAACGAGAAGGTGCTGCCCAAGCACAGCCTG
CTTTACGAGTACTTCACCGTGTACAACGAGCTCACCAAGGTGAAATATGTGACCGAGGGCATGCGAAAACC
CGCTTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTGTTCAAGACCAACAGGAAGGTGACCG
TGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTTGATAGCGTGGAAATAAGCGGCGTGGAG
GACAGGTTCAACGCCAGCCTGGGCACCTACCACGACTTGTTGAAGATAATCAAAGACAAGGATTTCCTGGA
TAATGAGGAGAACGAGGATATACTCGAGGACATCGTGCTGACTTTGACCCTGTTTGAGGACCGAGAGATGA
TTGAAGAAAGGCTCAAAACCTACGCCCACCTGTTCGACGACAAAGTGATGAAACAACTGAAGAGACGAAGA
TACACCGGCTGGGGCAGACTGTCCAGGAAGCTCATCAACGGCATTAGGGACAAGCAGAGCGGCAAGACCAT
CCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACCGAAACTTCATGCAGCTGATTCACGATGACAGCTTGA
CCTTCAAGGAGGACATCCAGAAGGCCCAGGTTAGCGGCCAGGGCGACTCCCTGCACGAACATATTGCAAAC
CTGGCAGGCTCCCCTGCGATCAAGAAGGGCATACTGCAGACCGTTAAGGTTGTGGACGAATTGGTCAAGGT
CATGGGCAGGCACAAGCCCGAAAACATAGTTATAGAGATGGCCAGAGAGAACCAGACCACCCAAAAGGGCC
AGAAGAACAGCCGGGAGCGCATGAAAAGGATCGAGGAGGGTATCAAGGAACTCGGAAGCCAGATCCTCAAA
GAGCACCCCGTGGAGAATACCCAGCTCCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCAGGGA
CATGTACGTTGACCAGGAGTTGGACATCAACAGGCTTTCAGACTATGACGTGGATCACATAGTGCCCCAGA
GCTTTCTTAAAGACGATAGCATCGACAACAAGGTCCTGACCCGCTCCGACAAAAACAGGGGCAAAAGCGAC
AACGTGCCAAGCGAAGAGGTGGTTAAAAAGATGAAGAACTACTGGAGGCAACTGCTCAACGCGAAATTGAT
CACCCAGAGAAAGTTCGATAACCTGACCAAGGCCGAGAGGGGCGGACTCTCCGAACTTGACAAAGCGGGCT
TCATAAAGAGGCAGCTGGTCGAGACCCGACAGATCACGAAGCACGTGGCCCAAATCCTCGACAGCAGAATG
AATACCAAGTACGATGAGAATGACAAACTCATCAGGGAAGTGAAAGTGATTACCCTGAAGAGCAAGTTGGT
GTCCGACTTTCGCAAAGATTTCCAGTTCTACAAGGTGAGGGAGATCAACAACTACCACCATGCCCACGACG
CATACCTGAACGCCGTGGTCGGCACCGCCCTGATTAAGAAGTATCCAAAGCTGGAGTCCGAATTTGTCTAC
GGCGACTACAAAGTTTACGATGTGAGGAAGATGATCGCTAAGAGCGAACAGGAGATCGGCAAGGCCACCGC
TAAGTATTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATCACACTTGCCAACGGCGAAATCA
GGAAGAGGCCGCTTATCGAGACCAACGGTGAGACCGGCGAGATCGTGTGGGACAAGGGCAGGGACTTCGCC

ACCGTGAGGAAAGTCCTGAGCATGCCCCAGGTGAATATTGTGAAAAAAACTGAGGTGCAGACAGGCGGCTT
TAGCAAGGAATCCATCCTGCCCAAGAGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCTA
AGAAGTATGGAGGCTTCGACAGCCCCACCGTAGCCTACAGCGTGCTGGTGGTCGCGAAGGTAGAGAAGGGG
AAGAGCAAGAAACTGAAGAGCGTGAAGGAGCTGCTCGGCATAACCATCATGGAGAGGTCCAGCTTTGAGAA
GAACCCCATTGACTTTTTGGAAGCCAAGGGCTACAAAGAGGTCAAAAAGGACCTGATCATCAAACTCCCCA
AGTACTCCCTGTTTGAATTGGAGAACGGCAGAAAGAGGATGCTGGCGAGCGCTGGGGAACTGCAAAAGGGC
AACGAACTGGCGCTGCCCAGCAAGTACGTGAATTTTCTGTACCTGGCGTCCCACTACGAAAAGCTGAAAGG
CAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCATTACCTGGACGAGATAATCG
AGCAAATCAGCGAGTTCAGCAAGAGGGTGATTCTGGCCGACGCGAACCTGGATAAGGTCCTCAGCGCCTAC
AACAAGCACCGAGACAAACCCATCAGGGAGCAGGCCGAGAATATCATACACCTGTTCACCCTGACAAATCT
GGGCGCACCTGCGGCATTCAAATACTTCGATACCACCATCGACAGGAAAAGGTACACTAGCACTAAGGAGG
TGCTGGATGCCACCTTGATCCACCAGTCCATTACCGGCCTGTATGAGACCAGGATCGACCTGAGCCAGCTT
GGAGGCGACTCTAGGGCGGACCCAAAAAAGAAAAGGAAGGTGGAATTCCACCACACTGGACTAGTGGATCC
GAGCTCGGTACCAAGCTTAAGTTTAAACCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTG
TTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAAT
GAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAA
GGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGC

S.py Cas9 mRNA (SEQ ID No. 413) as made from the expression cassette (SEQ ID No. 412). The sequence contains a V5 epitope tag, a nuclear localization signal, the codon optimized Cas9 sequence, a second nuclear localization signal, and the BGH (bovine growth hormone) gene 3'-UTR element and poly-A tail.

GGGAGACCCAAGCUGGCUAGCGUUUAAACGGGCCCUCUAGACUCGAGCGGCCGCCACCAUGGGCAAGCCCA
UCCCUAACCCCCUGUUGGGGCUGGACAGCACCGCUCCCAAAAAGAAAAGGAAGGUGGGCAUUCACGGCGUG
CCUGCGGCCGACAAAAAGUACAGCAUCGGCCUUGAUAUCGGCACCAAUAGCGUGGGCUGGGCCGUUAUCAC
AGACGAAUACAAGGUACCCAGCAAGAAGUUCAAGGUGCUGGGGAAUACAGACAGGCACUCUAUCAAGAAAA
ACCUUAUCGGGGCUCUGCUGUUUGACUCAGGCGAGACCGCCGAGGCCACCAGGUUGAAGAGGACCGCAAGG
CGAAGGUACACCCGGAGGAAGAACAGGAUCUGCUAUCUGCAGGAGAUCUUCAGCAACGAGAUGGCCAAGGU
GGACGACAGCUUCUUCCACAGGCUGGAGGAGAGCUUCCUUGUCGAGGAGGAUAAGAAGCACGAACGACACC
CCAUCUUCGGCAACAUAGUCGACGAGGUCGCUUAUCACGAGAAGUACCCCACCAUCUACCACCUGCGAAAG
AAAUUGGUGGAUAGCACCGAUAAAGCCGACUUGCGACUUAUCUACUUGGCUCUGGCGCACAUGAUUAAGUU
CAGGGGCCACUUCCUGAUCGAGGGCGACCUUAACCCCGACAACAGUGACGUAGACAAAUUGUUCAUCCAGC
UUGUACAGACCUAUAACCAGCUGUUCGAGGAAAACCCUAUUAACGCCAGCGGGGUGGAUGCGAAGGCCAUA

CUUAGCGCCAGGCUGAGCAAAAGCAGGCGCUUGGAGAACCUGAUAGCCCAGCUGCCCGGUGAAAAGAAGAA
CGGCCUCUUCGGUAAUCUGAUUGCCCUGAGCCUGGGCCUGACCCCCAACUUCAAGAGCAACUUCGACCUGG
CAGAAGAUGCCAAGCUGCAGUUGAGUAAGGACACCUAUGACGACGACUUGGACAAUCUGCUCGCCCAAAUC
GGCGACCAGUACGCUGACCUGUUCCUCGCCGCCAAGAACCUUUCUGACGCAAUCCUGCUUAGCGAUAUCCU
UAGGGUGAACACAGAGAUCACCAAGGCCCCCCUGAGCGCCAGCAUGAUCAAGAGGUACGACGAGCACCAUC
AGGACCUGACCCUUCUGAAGGCCCUGGUGAGGCAGCAACUGCCCGAGAAGUACAAGGAGAUCUUUUUCGAC
CAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGCGGAGCCAGCCAAGAGGAGUUCUACAAGUUCAUCAA
GCCCAUCCUGGAGAAGAUGGAUGGCACCGAGGAGCUGCUGGUGAAGCUGAACAGGGAAGAUUUGCUCCGGA
AGCAGAGGACCUUUGACAACGGUAGCAUCCCCCACCAGAUCCACCUGGGCGAGCUGCACGCAAUACUGAGG
CGACAGGAGGAUUUCUACCCCUUCCUCAAGGACAAUAGGGAGAAAAUCGAAAAGAUUCUGACCUUCAGGAU
CCCCUACUACGUGGGCCCUCUUGCCAGGGGCAACAGCCGAUUCGCUUGGAUGACAAGAAAGAGCGAGGAGA
CCAUCACCCCCUGGAACUUCGAGGAAGUGGUGGACAAAGGAGCAAGCGCGCAGUCUUUCAUCGAACGGAUG
ACCAAUUUCGACAAAAACCUGCCUAACGAGAAGGUGCUGCCCAAGCACAGCCUGCUUUACGAGUACUUCAC
CGUGUACAACGAGCUCACCAAGGUGAAAUAUGUGACCGAGGGCAUGCGAAAACCCGCUUUCCUGAGCGGCG
AGCAGAAGAAGGCCAUCGUGGACCUGCUGUUCAAGACCAACAGGAAGGUGACCGUGAAGCAGCUGAAGGAG
GACUACUUCAAGAAGAUCGAGUGCUUUGAUAGCGUGGAAAUAAGCGGCGUGGAGGACAGGUUCAACGCCAG

CCUGGGCACCUACCACGACUUGUUGAAGAUAAUCAAAGACAAGGAUUUCCUGGAUAAUGAGGAGAACGAGG
AUAUACUCGAGGACAUCGUGCUGACUUUGACCCUGUUUGAGGACCGAGAGAUGAUUGAAGAAAGGCUCAAA
ACCUACGCCCACCUGUUCGACGACAAAGUGAUGAAACAACUGAAGAGACGAAGAUACACCGGCUGGGGCAG
ACUGUCCAGGAAGCUCAUCAACGGCAUUAGGGACAAGCAGAGCGGCAAGACCAUCCUGGAUUUCCUGAAGU
CCGACGGCUUCGCCAACCGAAACUUCAUGCAGCUGAUUCACGAUGACAGCUUGACCUUCAAGGAGGACAUC
CAGAAGGCCCAGGUUAGCGGCCAGGGCGACUCCCUGCACGAACAUAUUGCAAACCUGGCAGGCUCCCCUGC
GAUCAAGAAGGGCAUACUGCAGACCGUUAAGGUUGUGGACGAAUUGGUCAAGGUCAUGGGCAGGCACAAGC
CCGAAAACAUAGUUAUAGAGAUGGCCAGAGAGAACCAGACCACCCAAAAGGGCCAGAAGAACAGCCGGGAG
CGCAUGAAAAGGAUCGAGGAGGGUAUCAAGGAACUCGGAAGCCAGAUCCUCAAAGAGCACCCCGUGGAGAA
UACCCAGCUCCAGAACGAGAAGCUGUACCUGUACUACCUGCAGAACGGCAGGGACAUGUACGUUGACCAGG
AGUUGGACAUCAACAGGCUUUCAGACUAUGACGUGGAUCACAUAGUGCCCCAGAGCUUUCUUAAAGACGAU
AGCAUCGACAACAAGGUCCUGACCCGCUCCGACAAAAACAGGGGCAAAAGCGACAACGUGCCAAGCGAAGA
GGUGGUUAAAAAGAUGAAGAACUACUGGAGGCAACUGCUCAACGCGAAAUUGAUCACCCAGAGAAAGUUCG
AUAACCUGACCAAGGCCGAGAGGGGCGGACUCUCCGAACUUGACAAAGCGGGCUUCAUAAAGAGGCAGCUG
GUCGAGACCCGACAGAUCACGAAGCACGUGGCCCAAAUCCUCGACAGCAGAAUGAAUACCAAGUACGAUGA
GAAUGACAAACUCAUCAGGGAAGUGAAAGUGAUUACCCUGAAGAGCAAGUUGGUGUCCGACUUUCGCAAAG
AUUUCCAGUUCUACAAGGUGAGGGAGAUCAACAACUACCACCAUGCCCACGACGCAUACCUGAACGCCGUG
GUCGGCACCGCCCUGAUUAAGAAGUAUCCAAAGCUGGAGUCCGAAUUUGUCUACGGCGACUACAAAGUUUA
CGAUGUGAGGAAGAUGAUCGCUAAGAGCGAACAGGAGAUCGGCAAGGCCACCGCUAAGUAUUUCUUCUACA
GCAACAUCAUGAACUUUUUCAAGACCGAGAUCACACUUGCCAACGGCGAAAUCAGGAAGAGGCCGCUUAUC
GAGACCAACGGUGAGACCGGCGAGAUCGUGUGGGACAAGGGCAGGGACUUCGCCACCGUGAGGAAAGUCCU
GAGCAUGCCCCAGGUGAAUAUUGUGAAAAAAACUGAGGUGCAGACAGGCGGCUUUAGCAAGGAAUCCAUCC
UGCCCAAGAGGAACAGCGACAAGCUGAUCGCCCGGAAGAAGGACUGGGACCCUAAGAAGUAUGGAGGCUUC
GACAGCCCCACCGUAGCCUACAGCGUGCUGGUGGUCGCGAAGGUAGAGAAGGGGAAGAGCAAGAAACUGAA
GAGCGUGAAGGAGCUGCUCGGCAUAACCAUCAUGGAGAGGUCCAGCUUUGAGAAGAACCCCAUUGACUUUU
UGGAAGCCAAGGGCUACAAAGAGGUCAAAAAGGACCUGAUCAUCAAACUCCCCAAGUACUCCCUGUUUGAA
UUGGAGAACGGCAGAAAGAGGAUGCUGGCGAGCGCUGGGGAACUGCAAAAGGGCAACGAACUGGCGCUGCC
CAGCAAGUACGUGAAUUUUCUGUACCUGGCGUCCCACUACGAAAAGCUGAAAGGCAGCCCCGAGGACAACG
AGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCAUUACCUGGACGAGAUAAUCGAGCAAAUCAGCGAGUUC
AGCAAGAGGGUGAUUCUGGCCGACGCGAACCUGGAUAAGGUCCUCAGCGCCUACAACAAGCACCGAGACAA
ACCCAUCAGGGAGCAGGCCGAGAAUAUCAUACACCUGUUCACCCUGACAAAUCUGGGCGCACCUGCGGCAU
UCAAAUACUUCGAUACCACCAUCGACAGGAAAAGGUACACUAGCACUAAGGAGGUGCUGGAUGCCACCUUG
AUCCACCAGUCCAUUACCGGCCUGUAUGAGACCAGGAUCGACCUGAGCCAGCUUGGAGGCGACUCUAGGGC
GGACCCAAAAAAGAAAAGGAAGGUGGAAUUCCACCACACUGGACUAGUGGAUCCGAGCUCGGUACCAAGCU
UAAGUUUAAACCGCUGAUCAGCCUCGACUGUGCCUUCUAGUUGCCAGCCAUCUGUUGUUUGCCCCUCCCCC
GUGCCUUCCUUGACCCUGGAAGGUGCCACUCCCACUGUCCUUUCCUAAUAAAAUGAGGAAAUUGCAUCGCA
UUGUCUGAGUAGGUGUCAUUCUAUUCUGGGGGGUGGGGUGGGGCAGGACAGCAAGGGGGAGGAUUGGGAAG
ACAAUAGCAGGCAUGCUGGGGAUGCGGUGGGCUCUAUGGC – polyA

EXAMPLE 12

[0125] The following example demonstrates reduced stimulation of the innate immune system in mammalian cells by the truncated chemically modified crRNA:tracrRNA complexes of the present disclosure when compared with unmodified IVT sgRNAs.

[0126] Mammalian cells possess a variety of receptors intended to identify and respond to foreign RNAs as part of anti-viral immunity. This includes receptors such as TLR-3, TLR-7, TLR8, RIG-I, MDA5, OAS, PKR, and others. In broad terms, RNAs that are short or contain chemical modifications present in mammalian cells (such as 2'OMe RNA) evade detection or are less stimulatory than are long, unmodified RNAs. The present example compares the level of stimulation of 2 immune response associated genes (*IFIT1* and *IFITM1)* when mammalian HEK293 cells are transfected with truncated unmodified or truncated modified crRNA:tracrRNA complexes with a commercial IVT sgRNA (Thermo Fisher Scientific, Waltham, MA).

[0127] CRISPR guide RNAs specific to human *HPRT1* site 38285 were employed. Sequences are shown in Table 11 below. The unmodified crRNA:tracrRNA complexes (SEQ ID Nos. 48 and 2), the modified crRNA:tracrRNA complexes (SEQ ID Nos. 178 and 100) and the sgRNA (SEQ ID No. 414) were transfected into HEK-Cas9 cells at 30 nM concentration as outlined in Example 2 above. RNA was prepared 24 hours after transfection using the SV96 Total RNA Isolation Kit (Promega, Madison, WI). cDNA was synthesized using 150 ng total RNA with SuperScript™-II Reverse Transcriptase (Invitrogen, Carlsbad, CA) per the manufacturer's instructions using both random hexamer and oligo-dT priming. Transfection experiments were all performed a minimum of three times.

[0128] Quantitative real-time PCR was performed using 10 ng cDNA per 10 µL reaction with Immolase™ DNA Polymerase (Bioline, Randolph, MA), 200 nM primers, and 200 nM probe. Cycling conditions employed were: 95°C for 10 minutes followed by 40 cycles of 2-step PCR with 95°C for 15 seconds and 60°C for 1 minute. PCR and fluorescence measurements were done using an ABI Prism™ 7900 Sequence Detector (Applied Biosystems Inc., Foster City, CA). All reactions were performed in triplicate using 2-color multiplexing. Expression data were normalized against an average of two internal control genes. Copy number standards were linearized cloned amplicons for all assays. Unknowns were extrapolated against standards to establish absolute quantitative measurements. Housekeeping internal control normalization assays were *HPRT1* (primers and probe SEQ ID Nos. 415-417) and *SFRS9* (primers and probe SEQ ID Nos. 418-420). Immune activation pathway assays were *IFITM1* (primers and probe SEQ ID Nos. 421-423) and *IFIT1* (primers and probe SEQ ID Nos. 424-426). The results were normalized using non-transfected cells as baseline and are shown in FIG. 13.

Table 11. Nucleic acid reagents employed in immune activation experiments in Example 12.

SEQ ID No.	Reagent	Sequence
48	Unmodified crRNA	cuuauauccaacacuucgugguuuuagagcuaugcu
2	Unmodified tracrRNA	agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcuuu

SEQ ID No.	Reagent	Sequence
178	Modified crRNA	**c*u*u*auauccaacacuucgugguuuuagagcuau*g*c*u**
100	Modified tracrRNA	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu*u*u
414	IVT sgRNA	ppp-gcuuauauccaacacuucgugguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuuuuuu
415	Hs HPRT F517	GACTTTGCTTTCCTTGGTCAG
416	Hs HPRT R591	GGCTTATATCCAACACTTCGTGGG
I[1]	Hs HPRT P554	FAM-ATGGTCAAG(ZEN)GTCGCAAGCTTGCTGGT-ZEN
418	Hs SFRS9 F569	TGTGCAGAAGGATGGAGT
419	Hs SFRS9 R712	CTGGTGCTTCTCTCAGGATA
II[2]	Hs SFRS9 P644	HEX-TGGAATATG(ZEN)CCCTGCGTAAACTGGA-ZEN
421	Hs IFITM1 For	CTCTTCTTGAACTGGTGCTGTCTG
422	Hs IFITM1 Rev	CAGGATGAATCCAATGGTCATGAGG
III[3]	Hs IFITM1 Probe FAM	FAM-AAGTGCCTG(ZEN)AACATCTGGGCCCTGATT-ZEN
424	Hs IFIT1 For	CCATTGTCTGGATTTAAGCGG
425	Hs IFIT1 Rev	GCCACAAAAAATCACAAGCCA
IV[4]	Hs IFIT1 Probe HEX	HEX-TTTCTTTGC(ZEN)TTCCCCTAAGGCAGGCTG-ZEN

[1] Compound I is an oligonucleotide having the formula SEQ ID NO: 417-(ZEN)-SEQ ID NO: 441.

[2] Compound II is an oligonucleotide having the formula SEQ ID NO: 420-(ZEN)-SEQ ID NO: 442.

[3] Compound III is an oligonucleotide having the formula SEQ ID NO: 423-(ZEN)-SEQ ID NO: 443.

[4] Compound IV is an oligonucleotide having the formula SEQ ID NO: 426-(ZEN)-SEQ ID NO: 444. Oligonucleotide sequences are shown 5'-3'. Uppercase = DNA; Lowercase = RNA; Underlined = 2'-O-methyl RNA; * = phosphorothioate

internucleotide linkage; ppp = triphosphate; ZEN = napthyl-azo modifier, dark quencher; FAM = 6-carboxyfluorescein; HEX = hexachlorofluorescein.

[0129] Treatment with the unmodified or chemically modified truncated crRNA:tracrRNA complex did not lead to detectable increases in IFIT1 or IFITM1 expression over baseline. In contrast, treatment with the longer IVT sgRNA led to a 45-fold induction of IFITM1 and a 220-fold induction of IFIT1. Thus, significant stimulation of the innate immune system occurred using the sgRNA that was absent using the short crRNA:tracrRNA complexes of the present disclosure.

EXAMPLE 13

[0130] The following example combines modification patterns identified in Examples 6 and 7 as being particularly efficacious to demonstrate new highly modified crRNA and tracrRNA compositions that perform with high efficiency in mammalian CRISPR genome editing applications.

[0131] A series of crRNAs and tracrRNAs (Table 12) were synthesized having chemical modifications as indicated. The crRNAs employed a 20 base protospacer domain targeting the same site in the human *HPRT1* gene (38285) at the 5'- end with a 16 base tracrRNA binding domain at the 3'-end. The tracrRNAs were synthesized having chemical modifications as indicated, using the 67 nucleotide or 62 nucleotide truncated versions of the tracrRNA sequence. The crRNAs and tracrRNAs listed in Table 12 were paired as indicated and transfected into the HEK-Cas9 cells at 30 nM concentration and processed as described in previous Examples. Relative gene editing activities were assessed by comparing cleavage rates in the *HPRT1* gene using the T7EI mismatch endonuclease cleavage assay, with quantitative measurement of products done using the Fragment Analyzer.

Table 12: Activity of highly modified crRNA:tracrRNA complexes to direct Cas9-mediated gene editing in mammalian cells.

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Cleavage %
1	448	*c*u*u*auaucca***acacuucgugguuu***uagagcuau*g*c*u*	57
	2	**agcauagcaaguuaaaauaaggcuaguccguuaucaacuugaa aaaguggcaccgagucggugcuuu**	
2	448	*c*u*u*auaucca***acacuucgugguuu***uagagcuau*g*c*u*	58
	100	**a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuug aaaaaguggcaccgagucggugcu*u*u**	
	48	cuuauauccaacacuucgugguuuuagagcuaugcu	

cr/tracr RNA pair	SEQ ID No.	crRNA Sequence tracrRNA Sequence	Cleavage %
3	449	*a*g*cauagcaag***uuaaaauaaggcuaguccguu***aucaacuug* *aaaaaguggcaccgagucggugcu*u*u*	58
4	48	cuuauauccaacacuucgugguuuuagagcuaugcu	57
	450	*a*g*cauagca***aguuaaaauaaggcuaguccguuaucaacuug** **aaaaaguggcaccgag***ucggugcu*u*u*	
5	48	cuuauauccaacacuucgugguuuuagagcuaugcu	65
	451	a*g*cauagcaaguuaaaauaaggcuaguccguuaucaacuug aaaaaguggcaccgagucg*g*u	

[0132] Oligonucleotide sequences are shown 5'-3'. Lowercase = RNA; Underlined = 2'-O-methyl RNA; Lowercase italic = 2'F RNA; * = phosphorothioate internucleotide linkage. The relative functional activity of each complex is indicated by the % cleavage in a T7EI heteroduplex assay for each dose studied.

[0133] The crRNA:tracrRNA pairs #1 and #2 show that a highly 2'F RNA modified crRNA (SEQ ID No. 448, which has 22/36 residues modified, or 61%) is highly functional when paired with either an unmodified tracrRNA (SEQ ID No. 2) or a highly 2'OMe modified tracrRNA (SEQ ID No. 100). The crRNA:tracrRNA pairs #3 and #4 show that tracrRNA compositions having moderate (SEQ ID No. 450, with 19/67 residues modified, or 28%) or high (SEQ ID No. 449, with 46/67 residues modified, or 69%) levels of 2'F RNA modification are highly functional. Information derived from Example 6 (in particular, the 2'OMe "walk", SEQ ID Nos. 144-162) was used to identify specific residues that can be modified within the internal domain of the tracrRNA (see Fig. 6). The crRNA:tracrRNA pair #5 demonstrates that an extremely highly modified tracrRNA, which in this case was a truncated 62 nucleotide design (SEQ ID No. 451, having 51/62 residues modified with 2'OMe RNA, or 82%), has high potency in triggering CRISPR genome editing in mammalian cells. Therefore, the original 89 RNA nucleotide wild-type tracrRNA has been optimized herein to a form that has as little as 11 RNA residues remaining (11/62), thereby significantly reducing risk of RNA-based activation of the mammalian innate immune system and reducing the nuclease-susceptible RNA content of the tracrRNA to a minimal level.

[0134] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the subject matter of the present disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve

as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the claimed subject matter and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the claimed subject matter.

SEQUENCE LISTING

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<120> CRISPR-BASED COMPOSITIONS AND METHODS OF USE

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<140> EP 15828610.4
<141> 2015-12-18

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<210> 46
<211> 42
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 46
cuuauaucca acacuucgug guuuuagagc uaugcuguuu ug 42

<210> 47
<211> 39
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 47
cuuauaucca acacuucgug guuuuagagc uaugcuguu 39

<210> 48
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 48
cuuauaucca acacuucgug guuuuagagc uaugcu 36

<210> 49
<211> 34
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 49
cuuauaucca acacuucgug guuuuagagc uaug 34

<210> 50
<211> 74
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 50
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gagucggugc uuuu 74

<210> 51
<211> 70
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 51
aacagcauag caaguuaaaa uaaggcuagu ccguuaucaa cuugaaaaag uggcaccgag 60

ucggugcuuu 70

<210> 52
<211> 65
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 52
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gugcu 65

<210> 53
<211> 63
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 53
cauagcaagu uaaaauaagg cuaguccguu aucaacuuga aaaaguggca ccgagucggu 60

gcu 63

<210> 54
<211> 34
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 54
uauauccaac acuucguggu uuuagagcua ugcu 34

<210> 55
<211> 33
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 55
auauccaaca cuucgugguu uuagagcuau gcu 33

<210> 56
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 56
aauuaugggg auuacuagga guuuuagagc uaugcu 36

<210> 57
<211> 35
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 57
auuaugggga uuacuaggag uuuuagagcu augcu 35

<210> 58
<211> 34
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 58
uuauggggau uacuaggagu uuuagagcua ugcu 34

<210> 59
<211> 33
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 59
uauggggauu acuaggaguu uuagagcuau gcu 33

<210> 60
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 60
auuucacaua aaacucuuuu guuuuagagc uaugcu 36

<210> 61
<211> 35
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 61
uuucacauaa aacucuuuug uuuuagagcu augcu 35

<210> 62
<211> 34
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 62

uucacauaaa acucuuuugu uuuagagcua ugcu 34

<210> 63
<211> 33
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 63
ucacauaaaa cucuuuuguu uuagagcuau gcu 33

<210> 64
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 64
uccauuucau agucuuuccu guuuuagagc uaugcu 36

<210> 65
<211> 35
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 65
ccauuucaua gucuuuccug uuuuagagcu augcu 35

<210> 66
<211> 34
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 66
cauuucauag ucuuuccugu uuuagagcua ugcu 34

<210> 67
<211> 33
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 67
auuucauagu cuuuccuguu uuagagcuau gcu 33

<210> 68
<211> 42
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 68
uccauuucau agucuuuccu guuuuagagc uaugcuguuu ug 42

<210> 69
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 69
uuuuguaauu aacagcuugc guuuuagagc uaugcu 36

<210> 70
<211> 42
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<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 70
uuuuguaauu aacagcuugc guuuuagagc uaugcuguuu ug 42

<210> 71

<211> 36
<212> RNA
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<220>
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<400> 71
cuuagagaau auuuguagag guuuuagagc uaugcu 36

<210> 72
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<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 72
cuuagagaau auuuguagag guuuuagagc uaugcuguuu ug 42

<210> 73
<211> 36
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<213> Artificial Sequence

<220>
<221> source
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<400> 73
uugacuauaa ugaauacuuc guuuuagagc uaugcu 36

<210> 74
<211> 42
<212> RNA
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<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 74
uugacuauaa ugaauacuuc guuuuagagc uaugcuguuu ug 42

<210> 75
<211> 36

<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 75
caaaacacgc auaaaaauuu guuuuagagc uaugcu 36

<210> 76
<211> 42
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<220>
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<400> 76
caaaacacgc auaaaaauuu guuuuagagc uaugcuguuu ug 42

<210> 77
<211> 42
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<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 77
aauuaugggg auuacuagga guuuuagagc uaugcuguuu ug 42

<210> 78
<211> 36
<212> RNA
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<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 78
ggucacuuuu aacacaccca guuuuagagc uaugcu 36

<210> 79
<211> 42
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 79
ggucacuuuu aacacaccca guuuuagagc uaugcuguuu ug 42

<210> 80
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 80
ggcuuauauc caacacuucg guuuuagagc uaugcu 36

<210> 81
<211> 42
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<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 81
ggcuuauauc caacacuucg guuuuagagc uaugcuguuu ug 42

<210> 82
<211> 42
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 82
auuucacaua aaacucuuuu guuuuagagc uaugcuguuu ug 42

<210> 83
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
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<400> 83
ucaaauuaug aggugcugga guuuuagagc uaugcu 36

<210> 84
<211> 42
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<213> Artificial Sequence

<220>
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<400> 84
ucaaauuaug aggugcugga guuuuagagc uaugcuguuu ug 42

<210> 85
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 85
uacagcuuua ugugacuaau guuuuagagc uaugcu 36

<210> 86
<211> 42
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 86
uacagcuuua ugugacuaau guuuuagagc uaugcuguuu ug 42

<210> 87
<211> 67
<212> RNA
<213> Artificial Sequence

<220>

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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<222> (1) .. (5)
<223> 2'-O-methyl nucleotide

<400> 87
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60
gugcuuu 67

<210> 88
<211> 67
<212> RNA
<213> Artificial Sequence

<220>
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<220>
<221> modified_base
<222> (65) .. (67)
<223> 2'-O-methyl nucleotide

<400> 88
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60
gugcuuu 67

<210> 89
<211> 67
<212> RNA
<213> Artificial Sequence

<220>
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<220>
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<222> (1) .. (5)
<223> 2'-O-methyl nucleotide

<220>
<221> modified_base
<222> (65) .. (67)
<223> 2'-O-methyl nucleotide

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gugcuuu 67

<210> 90
<211> 65
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<222> (1) .. (7)
<223> 2'-O-methyl nucleotide

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gugcu 65

<210> 91
<211> 65
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<222> (59) .. (64)
<223> 2'-O-methyl nucleotide

<400> 91
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gugcu 65

<210> 92
<211> 65
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<222> (45) .. (54)
<223> 2'-O-methyl nucleotide

<400> 92
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gugcu 65

<210> 93
<211> 65
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<220>
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<220>
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<222> (38) .. (40)
<223> 2'-O-methyl nucleotide

<220>
<221> modified_base
<222> (45) .. (54)
<223> 2'-O-methyl nucleotide

<400> 93
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gugcu 65

<210> 94
<211> 65
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<220>
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<220>
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<222> (38) .. (40)
<223> 2'-O-methyl nucleotide

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<223> 2'-O-methyl nucleotide

<220>
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<222> (59) .. (64)
<223> 2'-O-methyl nucleotide

<400> 94
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gugcu 65

<210> 95
<211> 65
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<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<223> 2'-O-methyl nucleotide

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<222> (38) .. (40)
<223> 2'-O-methyl nucleotide

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<222> (45) .. (54)
<223> 2'-O-methyl nucleotide

<220>
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<222> (59) .. (64)
<223> 2'-O-methyl nucleotide

<400> 95
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gugcu 65

<210> 96
<211> 65
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<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<222> (41) .. (44)
<223> 2'-O-methyl nucleotide

<220>
<221> modified_base
<222> (55) .. (58)
<223> 2'-O-methyl nucleotide

<400> 96
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gugcu 65

<210> 97
<211> 67
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
<221> source
<223> /note="5' C3 spacer"

<220>
<221> source
<223> /note="3' C3 spacer"

<400> 97
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gugcuuu 67

<210> 98
<211> 67
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>

<221> misc_feature
<222> (1) .. (3)
<223> /note="Phosphorothioate linkage"

<220>
<221> misc_feature
<222> (65) .. (67)
<223> /note="Phosphorothioate linkage"

<400> 98
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gugcuuu 67

<210> 99
<211> 67
<212> RNA
<213> Artificial Sequence

<220>
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<220>
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<220>
<221> modified_base
<222> (1) .. (9)
<223> 2'-O-methyl nucleotide

<220>
<221> modified_base
<222> (38) .. (67)
<223> 2'-O-methyl nucleotide

<220>
<221> source
<223> /note="3' C3 spacer"

<400> 99
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gugcuuu 67

<210> 100
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<213> Artificial Sequence

<220>
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<220>
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<222> (1) .. (3)
<223> /note="Phosphorothioate linkage"

<220>
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<222> (1) .. (9)
<223> 2'-O-methyl nucleotide

<220>
<221> modified_base
<222> (38) .. (67)
<223> 2'-O-methyl nucleotide

<220>
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<222> (65) .. (67)
<223> /note="Phosphorothioate linkage"

<400> 100
agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gugcuuu 67

<210> 101
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<220>
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<220>
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<220>
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<222> (64) .. (67)
<223> /note="Phosphorothioate linkage"

<400> 101
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gugcuuu 67

<210> 102
<211> 67
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<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<223> 2'-O-methyl nucleotide

<220>
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<222> (65) .. (67)
<223> 2'-O-methyl nucleotide

<400> 102
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gugcuuu 67

<210> 103
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<223> /note="Phosphorothioate linkage"

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<223> 2'-O-methyl nucleotide

<220>
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<222> (64) .. (67)
<223> /note="Phosphorothioate linkage"

<220>
<221> modified_base
<222> (65) .. (67)
<223> 2'-O-methyl nucleotide

<400> 103
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gugcuuu 67

<210> 104
<211> 67
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<223> /note="3' ZEN modifier"

<400> 104
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gugcuuu 67

<210> 105
<211> 67
<212> RNA
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<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<223> /note="5' ZEN modifier"

<220>
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<400> 105
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gugcuuu 67

<210> 106
<211> 62

<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

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<220>
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<223> 2'-O-methyl nucleotide

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<222> (60) .. (62)
<223> /note="Phosphorothioate linkage"

<400> 106
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<222> (60) .. (62)
<223> /note="Phosphorothioate linkage"

<400> 107
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gugcuuu 67

<210> 108
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<220>
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<222> (38) .. (67)
<223> 2'-O-methyl nucleotide

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<222> (65) .. (67)
<223> /note="Phosphorothioate linkage"

<400> 108
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gugcuuu 67

<210> 109

<211> 67
<212> RNA
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<220>
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<223> 2'-O-methyl nucleotide

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<222> (65) .. (67)
<223> /note="Phosphorothioate linkage"

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gugcuuu 67

<210> 110
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<223> /note="Phosphorothioate linkage"

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<223> 2'-O-methyl nucleotide

<220>
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gugcuuu 67

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gugcuuu 67

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<213> Artificial Sequence

<220>
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<220>
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<220>
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<400> 393
auuucacaua aaacucuuuu guuuuagagc uaugcu 36

<210> 394
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<213> Artificial Sequence

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<400> 394
ucaaauuaug aggugcugga guuuuagagc uaugcu 36

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<220>
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<400> 395
uacagcuuua ugugacuaau guuuuagagc uaugcu 36

<210> 396
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<213> Artificial Sequence

<220>
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<220>
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<220>
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<400> 396
uccauuucau agucuuuccu guuuuagagc uaugcu 36

<210> 397
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<213> Artificial Sequence

<220>

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<400> 397
uuuuguaauu aacagcuugc guuuuagagc uaugcu 36

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<213> Artificial Sequence

<220>
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<220>
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<220>
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<400> 398
cuuagagaau auuuguagag guuuuagagc uaugcu 36

<210> 399
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<213> Artificial Sequence

<220>
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<220>
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<220>
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<400> 399
uugacuauaa ugaauacuuc guuuuagagc uaugcu 36

<210> 400
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<213> Artificial Sequence

<220>
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<400> 400
caaaacacgc auaaaaauuu guuuuagagc uaugcu 36

<210> 401
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<212> RNA
<213> Artificial Sequence

<220>
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<400> 401
aauuaugggg auuacuagga guuuuagagc uaugcu 36

<210> 402
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<212> RNA
<213> Artificial Sequence

<220>
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<400> 402
ggucacuuuu aacacaccca guuuuagagc uaugcu 36

<210> 403
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
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<400> 403
ggcuuauauc caacacuucg guuuuagagc uaugcu 36

<210> 404
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
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<220>
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<223> /note="5' ZEN modifier"

<220>

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<400> 404
auuucacaua aaacucuuuu guuuuagagc uaugcu 36

<210> 405
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<220>
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<400> 405
ucaaauuaug aggugcugga guuuuagagc uaugcu 36

<210> 406
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<223> /note="5' ZEN modifier"

<220>
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<223> /note="3' ZEN modifier"

<400> 406
uacagcuuua ugugacuaau guuuuagagc uaugcu 36

<210> 407
<211> 1367
<212> PRT

<213> Streptococcus pyogenes

<400> 407

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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 Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe Leu
            420                 425                 430

Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile Pro
        435                 440                 445

Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp Met
    450                 455                 460

Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val
465                 470                 475                 480

Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr Asn
                485                 490                 495

Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser Leu
            500                 505                 510

Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr
        515                 520                 525

Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys
    530                 535                 540

Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val
545                 550                 555                 560

Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser
                565                 570                 575

Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly Thr
            580                 585                 590

Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp Asn
        595                 600                 605
```

595 600 605

Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu
610 615 620

Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala His
625 630 635 640

Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr Thr
645 650 655

Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp Lys
660 665 670

Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala
675 680 685

Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys
690 695 700

Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His
705 710 715 720

Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile
725 730 735

Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg
740 745 750

His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr
755 760 765

Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu
770 775 780

Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro Val
785 790 795 800

Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln
805 810 815

Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg Leu
820 825 830

Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp
835 840 845

Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly
850 855 860

Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn
865 870 875 880

Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe
885 890 895

Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys
900 905 910

```
Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys
        915                 920                 925

His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp Glu
    930                 935                 940

Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys
945                 950                 955                 960

Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg Glu
                965                 970                 975

Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val Val
            980                 985                 990

Gly Thr Ala Leu Ile Lys Lys Tyr  Pro Lys Leu Glu Ser  Glu Phe Val
        995                 1000                1005

Tyr Gly  Asp Tyr Lys Val Tyr  Asp Val Arg Lys Met  Ile Ala Lys

    1010                1015                1020

Ser Glu  Gln Glu Ile Gly Lys  Ala Thr Ala Lys Tyr  Phe Phe Tyr
    1025                1030                1035

Ser Asn  Ile Met Asn Phe Phe  Lys Thr Glu Ile Thr  Leu Ala Asn
    1040                1045                1050

Gly Glu  Ile Arg Lys Arg Pro  Leu Ile Glu Thr Asn  Gly Glu Thr
    1055                1060                1065

Gly Glu  Ile Val Trp Asp Lys  Gly Arg Asp Phe Ala  Thr Val Arg
    1070                1075                1080

Lys Val  Leu Ser Met Pro Gln  Val Asn Ile Val Lys  Lys Thr Glu
    1085                1090                1095

Val Gln  Thr Gly Gly Phe Ser  Lys Glu Ser Ile Leu  Pro Lys Arg
    1100                1105                1110

Asn Ser  Asp Lys Leu Ile Ala  Arg Lys Lys Asp Trp  Asp Pro Lys
    1115                1120                1125

Lys Tyr  Gly Gly Phe Asp Ser  Pro Thr Val Ala Tyr  Ser Val Leu
    1130                1135                1140

Val Val  Ala Lys Val Glu Lys  Gly Lys Ser Lys Lys  Leu Lys Ser
    1145                1150                1155

Val Lys  Glu Leu Leu Gly Ile  Thr Ile Met Glu Arg  Ser Ser Phe
    1160                1165                1170

Glu Lys  Asn Pro Ile Asp Phe  Leu Glu Ala Lys Gly  Tyr Lys Glu
    1175                1180                1185

Val Lys  Lys Asp Leu Ile Ile  Lys Leu Pro Lys Tyr  Ser Leu Phe
    1190                1195                1200
```

```
Glu Leu  Glu Asn Gly Arg Lys  Arg Met Leu Ala Ser  Ala Gly Glu
    1205                 1210                 1215

Leu Gln  Lys Gly Asn Glu Leu  Ala Leu Pro Ser Lys  Tyr Val Asn
    1220                 1225                 1230

Phe Leu  Tyr Leu Ala Ser His  Tyr Glu Lys Leu Lys  Gly Ser Pro
    1235                 1240                 1245

Glu Asp  Asn Glu Gln Lys Gln  Leu Phe Val Glu Gln  His Lys His
    1250                 1255                 1260

Tyr Leu  Asp Glu Ile Ile Glu  Gln Ile Ser Glu Phe  Ser Lys Arg
    1265                 1270                 1275

Val Ile  Leu Ala Asp Ala Asn  Leu Asp Lys Val Leu  Ser Ala Tyr
    1280                 1285                 1290

Asn Lys  His Arg Asp Lys Pro  Ile Arg Glu Gln Ala  Glu Asn Ile
    1295                 1300                 1305

Ile His  Leu Phe Thr Leu Thr  Asn Leu Gly Ala Pro  Ala Ala Phe
    1310                 1315                 1320

Lys Tyr  Phe Asp Thr Thr Ile  Asp Arg Lys Arg Tyr  Thr Ser Thr
    1325                 1330                 1335

Lys Glu  Val Leu Asp Ala Thr  Leu Ile His Gln Ser  Ile Thr Gly
    1340                 1345                 1350

Leu Tyr  Glu Thr Arg Ile Asp  Leu Ser Gln Leu Gly  Gly Asp
    1355                 1360                 1365
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<210> 408

<211> 1416

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 408

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Met Gly Ser Ser Ala Pro Lys Lys Lys Arg Lys Val Gly Ile His Gly
1               5                  10                  15

Val Pro Ala Ala Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly
            20                  25                  30

Thr Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro
        35                  40                  45

Ser Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys
    50                  55                  60
```

```
Lys Asn Leu Ile Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu
65                  70                  75                  80

Ala Thr Arg Leu Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys
                85                  90                  95

Asn Arg Ile Cys Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys
            100                 105                 110

Val Asp Asp Ser Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu
        115                 120                 125

Glu Asp Lys Lys His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp
    130                 135                 140

Glu Val Ala Tyr His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys
145                 150                 155                 160

Lys Leu Val Asp Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu
                165                 170                 175

Ala Leu Ala His Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly
            180                 185                 190

Asp Leu Asn Pro Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu
        195                 200                 205

Val Gln Thr Tyr Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser
    210                 215                 220

Gly Val Asp Ala Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg
225                 230                 235                 240

Arg Leu Glu Asn Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly
                245                 250                 255

Leu Phe Gly Asn Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe
            260                 265                 270

Lys Ser Asn Phe Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys
        275                 280                 285

Asp Thr Tyr Asp Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp
    290                 295                 300

Gln Tyr Ala Asp Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile
305                 310                 315                 320

Leu Leu Ser Asp Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro
                325                 330                 335

Leu Ser Ala Ser Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu
            340                 345                 350

Thr Leu Leu Lys Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys
        355                 360                 365

Glu Ile Phe Phe Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp
    370                 375                 380
```

Gly Gly Ala Ser Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu
385 390 395 400

Glu Lys Met Asp Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu
405 410 415

Asp Leu Leu Arg Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His
420 425 430

Gln Ile His Leu Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp
435 440 445

Phe Tyr Pro Phe Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu
450 455 460

Thr Phe Arg Ile Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser
465 470 475 480

Arg Phe Ala Trp Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp
485 490 495

Asn Phe Glu Glu Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile
500 505 510

Glu Arg Met Thr Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu
515 520 525

Pro Lys His Ser Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu
530 535 540

Thr Lys Val Lys Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu
545 550 555 560

Ser Gly Glu Gln Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn
565 570 575

Arg Lys Val Thr Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile
580 585 590

Glu Cys Phe Asp Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn
595 600 605

Ala Ser Leu Gly Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys
610 615 620

Asp Phe Leu Asp Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val
625 630 635 640

Leu Thr Leu Thr Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu
645 650 655

Lys Thr Tyr Ala His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys
660 665 670

Arg Arg Arg Tyr Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn
675 680 685

```
Gly Ile Arg Asp Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys
    690                 695                 700

Ser Asp Gly Phe Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp
705                 710                 715                 720

Ser Leu Thr Phe Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln
                725                 730                 735

Gly Asp Ser Leu His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala
            740                 745                 750

Ile Lys Lys Gly Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val
        755                 760                 765

Lys Val Met Gly Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala
    770                 775                 780

Arg Glu Asn Gln Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg
785                 790                 795                 800

Met Lys Arg Ile Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu
                805                 810                 815

Lys Glu His Pro Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr
            820                 825                 830

Leu Tyr Tyr Leu Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu
        835                 840                 845

Asp Ile Asn Arg Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln
    850                 855                 860

Ser Phe Leu Lys Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser
865                 870                 875                 880

Asp Lys Asn Arg Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val
                885                 890                 895

Lys Lys Met Lys Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile
            900                 905                 910

Thr Gln Arg Lys Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu
        915                 920                 925

Ser Glu Leu Asp Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr
    930                 935                 940

Arg Gln Ile Thr Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn
945                 950                 955                 960

Thr Lys Tyr Asp Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile
                965                 970                 975

Thr Leu Lys Ser Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe
            980                 985                 990
```

```
Tyr Lys Val Arg Glu Ile Asn Asn  Tyr His His Ala His  Asp Ala Tyr
        995                 1000                1005

Leu Asn  Ala Val Val Gly Thr  Ala Leu Ile Lys Lys  Tyr Pro Lys
    1010                1015                1020

Leu Glu  Ser Glu Phe Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val
    1025                1030                1035

Arg Lys  Met Ile Ala Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr
    1040                1045                1050

Ala Lys  Tyr Phe Phe Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr
    1055                1060                1065

Glu Ile  Thr Leu Ala Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile
    1070                1075                1080

Glu Thr  Asn Gly Glu Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg
    1085                1090                1095

Asp Phe  Ala Thr Val Arg Lys  Val Leu Ser Met Pro  Gln Val Asn
    1100                1105                1110

Ile Val  Lys Lys Thr Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu
    1115                1120                1125

Ser Ile  Leu Pro Lys Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys
    1130                1135                1140

Lys Asp  Trp Asp Pro Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr
    1145                1150                1155

Val Ala  Tyr Ser Val Leu Val  Val Ala Lys Val Glu  Lys Gly Lys
    1160                1165                1170

Ser Lys  Lys Leu Lys Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile
    1175                1180                1185

Met Glu  Arg Ser Ser Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu
    1190                1195                1200

Ala Lys  Gly Tyr Lys Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu
    1205                1210                1215

Pro Lys  Tyr Ser Leu Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met
    1220                1225                1230

Leu Ala  Ser Ala Gly Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu
    1235                1240                1245

Pro Ser  Lys Tyr Val Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu
    1250                1255                1260

Lys Leu  Lys Gly Ser Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe
    1265                1270                1275

Val Glu  Gln His Lys His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile
```

```
    1280                1285                1290

Ser Glu  Phe Ser Lys Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp
    1295                1300                1305

Lys Val  Leu Ser Ala Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg
    1310                1315                1320

Glu Gln  Ala Glu Asn Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu
    1325                1330                 1335

Gly Ala  Pro Ala Ala Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg
    1340                1345                1350

Lys Arg  Tyr Thr Ser Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile
    1355                1360                1365

His Gln  Ser Ile Thr Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser
    1370                1375                1380

Gln Leu  Gly Gly Asp Ala Ala  Pro Lys Lys Lys Arg  Lys Val Asp
    1385                1390                1395

Pro Lys  Lys Lys Arg Lys Val  Ala Ala Ala Leu Glu  His His His
    1400                1405                1410

His His  His
    1415
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<210> 409
<211> 4251
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 409

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atggacaaaa agtactctat tggcctggat atcgggacca acagcgtcgg gtgggctgtt     120

atcaccgacg agtataaagt accttcgaaa aagttcaaag tgctgggcaa caccgatcgc     180

cattcaatca aaaagaactt gattggtgcg ctgttgtttg actccgggga aaccgccgag     240

gcgactcgcc ttaaacgtac agcacgtcgc cggtacactc ggcgtaagaa tcgcatttgc     300

tatttgcagg aaatctttag caacgagatg gcaaaagtcg atgactcgtt tttccaccgc     360

ctcgaggaaa gctttctggt ggaggaagac aaaaagcatg agcgtcaccc gatcttcggc     420

aacattgtcg atgaagtagc gtatcatgaa aaatacccaa ccatttacca cttacgcaaa     480

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<211> 1429
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 410

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Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val Gly Trp
35 40 45

Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val
50 55 60

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Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile Gly Ala
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Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg
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Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu
            100                 105                 110

Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser Phe Phe
        115                 120                 125

His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys His Glu
    130                 135                 140

Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr His Glu
145                 150                 155                 160

Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp Ser Thr
                165                 170                 175

Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His Met Ile
            180                 185                 190

Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn
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Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln
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Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala Lys Ala
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Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn Leu Ile
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Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile
            260                 265                 270

Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu
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Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp
    290                 295                 300

Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe
305                 310                 315                 320

Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu
                325                 330                 335

Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile
            340                 345                 350

Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu
        355                 360                 365
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Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe Asp Gln
    370                 375                 380

Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser Gln Glu
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Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp Gly Thr
                405                 410                 415

Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg Lys Gln
            420                 425                 430

Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu Gly Glu
        435                 440                 445

Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys
    450                 455                 460

Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr
465                 470                 475                 480

Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp Met Thr

                485                 490                 495

Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val Val
            500                 505                 510

Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr Asn Phe
        515                 520                 525

Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser Leu Leu
    530                 535                 540

Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val
545                 550                 555                 560

Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys
                565                 570                 575

Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val Lys
            580                 585                 590

Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser Val
        595                 600                 605

Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly Thr Tyr
    610                 615                 620

His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp Asn Glu
625                 630                 635                 640

Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu Phe
                645                 650                 655

Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala His Leu
            660                 665                 670
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Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr Thr Gly
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Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp Lys Gln
    690                 695                 700

Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn
705                 710                 715                 720

Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys Glu
                725                 730                 735

Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His Glu
            740                 745                 750

His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu
        755                 760                 765

Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg His
    770                 775                 780

Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr Thr
785                 790                 795                 800

Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu Glu
                805                 810                 815

Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro Val Glu
            820                 825                 830

Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn
        835                 840                 845

Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg Leu Ser
    850                 855                 860

Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp
865                 870                 875                 880

Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys
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Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn Tyr
            900                 905                 910

Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp
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Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala
    930                 935                 940

Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys His
945                 950                 955                 960

Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp Glu Asn
                965                 970                 975

Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys Leu
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Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg Glu Ile
995 1000 1005

Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val Val
1010 1015 1020

Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
1025 1030 1035

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
1040 1045 1050

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
1055 1060 1065

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
1100 1105 1110

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
1115 1120 1125

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
1130 1135 1140

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1145 1150 1155

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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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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Ser Arg  Ala Asp Pro Lys Lys  Lys Arg Lys Val Glu  Phe His His
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<210> 411
<211> 4290
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 411

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<210> 412

<211> 4601

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 412

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aggaccgcaa ggcgaaggta cacccggagg aagaacagga tctgctatct gcaggagatc 420

ttcagcaacg agatggccaa ggtggacgac agcttcttcc acaggctgga ggagagcttc 480

cttgtcgagg aggataagaa gcacgaacga caccccatct tcggcaacat agtcgacgag 540

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ccttctagtt gccagccatc tgttgtttgc ccctcccccg tgccttcctt gaccctggaa 4440

ggtgccactc ccactgtcct ttcctaataa aatgaggaaa ttgcatcgca ttgtctgagt 4500

aggtgtcatt ctattctggg gggtggggtg gggcaggaca gcaaggggga ggattgggaa 4560

gacaatagca ggcatgctgg ggatgcggtg ggctctatgg c 4601

<210> 413

<211> 4584

<212> RNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 413

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gggcaagccc aucccuaacc cccuguuggg gcuggacagc accgcuccca aaaagaaaag 120

gaaggugggc auucacggcg ugccugcggc cgacaaaaag uacagcaucg gccuugauau 180

cggcaccaau agcgugggcu gggccguuau cacagacgaa uacaagguac ccagcaagaa 240

guucaaggug cuggggaaua cagacaggca cucuaucaag aaaaaccuua ucggggcucu 300

gcuguuugac ucaggcgaga ccgccgaggc caccagguug aagaggaccg caaggcgaag 360

guacacccgg aggaagaaca ggaucugcua ucugcaggag aucuucagca acgagauggc 420

caagguggac gacagcuucu uccacaggcu ggaggagagc uuccuugucg aggaggauaa 480

gaagcacgaa cgacacccca ucuucggcaa cauagucgac gaggucgcuu aucacgagaa 540

guaccccacc aucuaccacc ugcgaaagaa auugguggau agcaccgaua aagccgacuu 600

gcgacuuauc uacuuggcuc uggcgcacau gauuaaguuc aggggccacu uccugaucga 660

gggcgaccuu aaccccgaca acagugacgu agacaaauug uucauccagc uuguacagac 720

cuauaaccag cuguucgagg aaaacccuau uaacgccagc gggguggaug cgaaggccau 780

acuuagcgcc aggcugagca aaagcaggcg cuuggagaac cugauagccc agcugcccgg 840

ugaaaagaag aacggccucu ucgguaaucu gauugcccug agccugggcc ugacccccaa 900

cuucaagagc aacuucgacc uggcagaaga ugccaagcug caguugagua aggacaccua 960

ugacgacgac uuggacaauc ugcucgccca aaucggcgac caguacgcug accuguuccu 1020

cgccgccaag aaccuuucug acgcaauccu gcuuagcgau auccuuaggg ugaacacaga 1080

gaucaccaag gccccccuga gcgccagcau gaucaagagg uacgacgagc accaucagga 1140

ccugacccuu cugaaggccc uggugaggca gcaacugccc gagaaguaca aggagaucuu 1200

uuucgaccag agcaagaacg gcuacgccgg cuacaucgac ggcggagcca gccaagagga 1260

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ccaccagauc caccugggcg agcugcacgc aauacugagg cgacaggagg auuucuaccc 1440

cuuccucaag gacaauaggg agaaaaucga aaagauucug accuucagga ucccccuacua 1500

cgugggcccu cuugccaggg gcaacagccg auucgcuugg augacaagaa agagcgagga 1560

gaccaucacc cccuggaacu ucgaggaagu gguggacaaa ggagcaagcg cgcagucuuu 1620

caucgaacgg augaccaauu ucgacaaaaa ccugccuaac gagaaggugc ugcccaagca 1680

cagccugcuu uacgaguacu ucaccgugua caacgagcuc accaagguga aauaugugac 1740

cgagggcaug cgaaaacccg cuuuccugag cggcgagcag aagaaggcca ucguggaccu 1800

gcuguucaag accaacagga aggugaccgu gaagcagcug aaggaggacu acuucaagaa 1860

gaucgagugc uuugauagcg uggaaauaag cggcguggag gacagguuca acgccagccu 1920

gggcaccuac cacgacuugu ugaagauaau caaagacaag gauuuccugg auaaugagga 1980

gaacgaggau auacucgagg acaucgugcu gacuuugacc cuguuugagg accgagagau 2040

gauugaagaa aggcucaaaa ccuacgccca ccuguucgac gacaaaguga ugaaacaacu 2100

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ggcccaaauc cucgacagca gaaugaauac caaguacgau gagaaugaca aacucaucag 3000

ggaagugaaa gugauuaccc ugaagagcaa guuggugucc gacuuucgca aagauuucca 3060

guucuacaag gugagggaga ucaacaacua ccaccaugcc cacgacgcau accugaacgc 3120

cguggucggc accgcccuga uuaagaagua uccaaagcug gaguccgaau uugucuacgg 3180

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acuugccaac ggcgaaauca ggaagaggcc gcuuaucgag accaacggug agaccggcga 3360

gaucgugugg gacaagggca gggacuucgc caccgugagg aaaguccuga gcaugcccca 3420

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uggggaugcg gugggcucua uggc 4584

<210> 414
<211> 104
<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<220>
<221> source
<223> /note="5' Triphosphate"

<400> 414
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ccguuaucaa cuugaaaaag uggcaccgag ucggugcuuu uuuu 104

<210> 415
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 415
gactttgctt tccttggtca g 21

<210> 416
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 416
ggcttatatc caacacttcg tggg 24

<210> 417
<211> 9

<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
<221> source
<223> /note="5' FAM"

<220>
<221> source
<223> /note="3' ZEN modifier"

<400> 417
atggtcaag 9

<210> 418
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 418
tgtgcagaag gatggagt 18

<210> 419
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 419
ctggtgcttc tctcaggata 20

<210> 420
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
<221> source
<223> /note="5' HEX"

<220>
<221> source
<223> /note="3' ZEN modifier"

<400> 420
tggaatatg 9

<210> 421
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 421
ctcttcttga actggtgctg tctg 24

<210> 422
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic primer "

<400> 422
caggatgaat ccaatggtca tgagg 25

<210> 423
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic probe"

<220>
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<223> /note="5' FAM"

<220>
<221> source
<223> /note="3' ZEN modifier"

<400> 423
aagtgcctg 9

<210> 424
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 424
ccattgtctg gatttaagcg g 21

<210> 425
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic primer "

<400> 425
gccacaaaaa atcacaagcc a 21

<210> 426
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic probe"

<220>
<221> source
<223> /note="5' HEX modifier"

<220>
<221> source
<223> /note="3' ZEN modifier"

<400> 426

tttctttgc 9

<210> 427
<211> 64
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 427
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cuuu 64

<210> 428
<211> 99
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 428
cuuauaucca acacuucgug guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuu 99

<210> 429
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (1)..(20)
<223> a, c, u, g, unknown or other

<400> 429
nnnnnnnnnn nnnnnnnnnn guuuuagagc uaugcu 36

<210> 430
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<222> (1)..(4)
<223> /note="Phosphorothioate linkage"

<220>
<221> modified_base
<222> (1)..(3)
<223> 2'-O-methyl nucleotide

<220>
<221> modified_base
<222> (1)..(20)
<223> a, c, u, g, unknown or other

<220>
<221> misc_feature
<222> (33)..(36)
<223> /note="Phosphorothioate linkage"

<220>
<221> modified_base
<222> (34)..(36)
<223> 2'-O-methyl nucleotide

<400> 430
nnnnnnnnnn nnnnnnnnnn guuuuagagc uaugcu 36

<210> 431
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
<221> source
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<220>
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<222> (1)..(4)
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<222> (1)..(20)
<223> a, c, u, g, unknown or other

<220>
<221> modified_base
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<223> 2'-O-methyl nucleotide

<220>
<221> misc_feature
<222> (33)..(36)
<223> /note="Phosphorothioate linkage"

<400> 431
nnnnnnnnnn nnnnnnnnnn guuuuagagc uaugcu 36

<210> 432
<211> 36
<212> RNA
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<220>
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<223> /note="Phosphorothioate linkage"

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<222> (1)..(6)
<223> 2'-O-methyl nucleotide

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<222> (1)..(20)
<223> a, c, u, g, unknown or other

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<221> modified_base
<222> (25)..(36)

<223> 2'-O-methyl nucleotide

<220>
<221> misc_feature
<222> (33)..(36)
<223> /note="Phosphorothioate linkage"

<400> 432
nnnnnnnnnn nnnnnnnnnn guuuuagagc uaugcu 36

<210> 433
<211> 36
<212> RNA
<213> Artificial Sequence

<220>
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<223> 2'-O-methyl nucleotide

<220>
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<222> (1)..(20)
<223> a, c, u, g, unknown or other

<220>
<221> modified_base
<222> (25)..(36)
<223> 2'-O-methyl nucleotide

<220>
<221> misc_feature
<222> (33)..(36)
<223> /note="Phosphorothioate linkage"

<400> 433
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<210> 434
<211> 36

<212> RNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<220>
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<222> (1)..(4)
<223> /note="Phosphorothioate linkage"

<220>
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<222> (1)..(20)
<223> a, c, u, g, unknown or other

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<222> (1)..(10)
<223> 2'-O-methyl nucleotide

<220>
<221> modified_base
<222> (25)..(36)
<223> 2'-O-methyl nucleotide

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nnnnnnnnnn nnnnnnnnnn guuuuagagc uaugcu 36

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<400> 439
nnnnnnnnnn nnnnnnnnnn guuuuagagc uaugcu 36

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gugcuuu 67

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gugcuuu 67

<210> 451
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agcauagcaa guuaaaauaa ggcuaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gu 62

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US20142732 32A1 [0010]
- WO2014144592A2 [0011]
- WO2014065596A1 [0012]
- WO2014124226A1 [0013]
- US5672695A [0027]
- US6248878B [0027]
- EP15828610 [0135]
- US2015066942W [0135]
- WO62239546A [0135]
- WO62093588A [0135]

Non-patent literature cited in the description

- **XU, T. et al.**Appl Environ Microbiol, 2014, vol. 80, 51544-52 [0004] [0056]
- Science, 2012, vol. 337, 6096816-21 [0006]
- Science, 2014, vol. 343, 1215- [0007]
- Cell, 2014, vol. 156, 5935-49 [0008]
- **BRINER et al.**Mol Cell, 2014, vol. 56, 2333-9 [0009]
- **REGINA CENCIC et al.**Protospacer Adjacent Motif (PAM)-Distal Sequences Engage CRISPR Cas9 DNA Target CleavagePLOS ONE, 2014, vol. 9, 10 [0014]
- **EDER et al.**Antisense Research and Development, 1991, vol. 1, 141-151 [0017]
- **KURRECK**Eur. J. Biochem., 2003, vol. 270, 1628-1644 [0017]
- **BEHLKE**Oligonucleotides, 2008, vol. 18, 305-320 [0017] [0114]
- **LENNOX et al.**Gene Therapy, 2011, vol. 18, 1111-1120 [0017]
- **BEHLKE, M.A.**Oligonucleotides, 2008, vol. 18, 4305-19 [0028]
- **SAHIN, U. et al.**Nat Rev Drug Discov, 2014, vol. 13, 10759-80 [0028]
- **O'CONNELL, M.R. et al.**Nature, 2014, vol. 516, 263- [0056]
- **FU et al.**Nature Biotechnol., 2014, vol. 32, 279- [0085]
- **LENNOX et al.**Molecular Therapy Nucleic Acids, 2013, vol. 2, e117- [0091] [0099]

Patentkrav

1. Isoleret tracrRNA omfattende en kemisk modificeret form af en af SEQ ID NOs.:2, 18, 30-33 og 36-39, hvor det isolerede tracrRNA udviser aktivitet i et CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-CRISPR-associeret (Cas) (CRISPR-Cas)-endonuklease-system, hvor den kemisk modificerede form af en af SEQ ID NOs.:2, 18, 30-33 og 36-39 omfatter et kemisk modificeret nukleotid med en ende-modificerende gruppe udvalgt fra en gruppe bestående af en propandiol (C3)-spacer, N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamin ("ZEN") og en inverted-dT-rest.

2. Isoleret tracrRNA ifølge krav 1, hvor den kemisk modificerede form af en af SEQ ID NOs.:2, 18, 30-33 og 36-39 endvidere omfatter et kemisk modificeret nukleotid, som har en yderligere modifikation udvalgt fra en gruppe bestående af en ribose-modifikation og en internukleotid-modificerende binding.

3. Isoleret tracrRNA ifølge krav 2, hvor det kemisk modificerede nukleotid, som har en yderligere modifikation, består af en ribose-modifikation udvalgt fra gruppen bestående af 2'OMe, 2'F, en bicyklisk nukleinsyre og en låst nukleinsyre (LNA).

4. Isoleret tracrRNA ifølge krav 2, hvor det kemisk modificerede nukleotid, som har en yderligere modifikation, består af en internukleotid-modificerende binding bestående af phosphorothioat-modifikation.

5. Isoleret tracrRNA ifølge krav 2, hvor det isolerede tracrRNA er udvalgt fra gruppen bestående af SEQ ID NOs.: 97, 99, 104, 105, 130, 131 og 132.

6. Isoleret crRNA omfattende en kemisk modificeret form af formel (I):

$$5'\text{-}X\text{-}Z\text{-}3' \qquad (I),$$

hvor X repræsenterer sekvenser omfattende et målspecifikt protospacer-domæne omfattende fra 17 nukleotider til 20 nukleotider, og Z repræsenter sekvenser omfattende et tracrRNA-bindingsdomæne omfattende fra 12 nukleotider til 19 nukleotider,

hvor det isolerede crRNA udviser aktivitet i et CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-CRISPR-associeret (Cas) (CRISPR-Cas)-endonuklease-system,
hvor den kemisk modificerede form af formel (I) omfatter et kemisk modificeret nukleotid, som har en ende-modificerende gruppe udvalgt fra gruppen bestående af en propandiol (C3)-spacer, N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamin ("ZEN") og en inverted-dT-rest.

7. Isoleret crRNA ifølge krav 6, hvor den kemisk modificerede form af formel (I) endvidere omfatter et kemisk modificeret nukleotid, som har en yderligere modifikation udvalgt fra en gruppe bestående af en ribose-modifikation og en internukleotid-modificerende binding.

8. Isoleret crRNA ifølge krav 7, hvor det kemisk modificerede nukleotid, som har den yderligere modifikation, består af en ribose-modifikation udvalgt fra en gruppe bestående af 2'OMe, 2'F, en bicyklisk nukleinsyre og låst nukleinsyre (LNA).

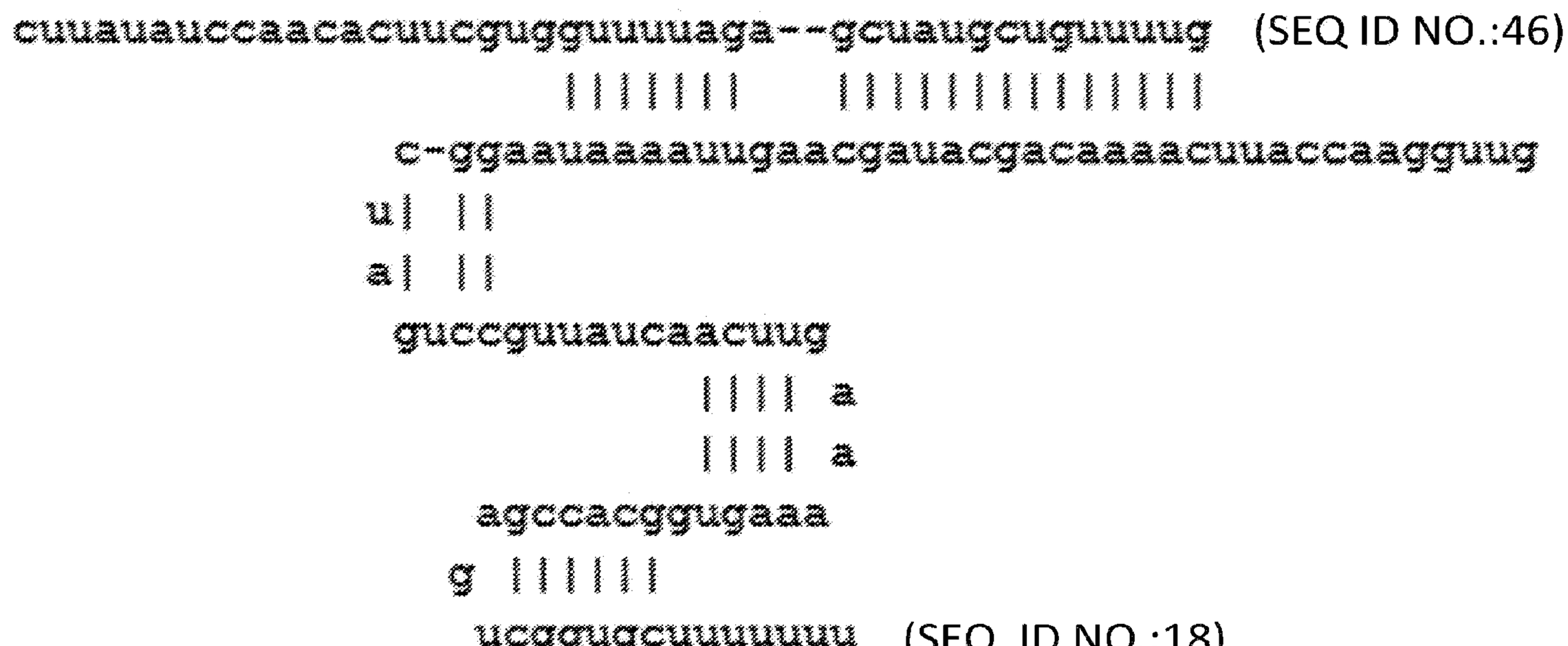
9. Isoleret crRNA ifølge krav 7, hvor det kemisk modificerede nukleotid, som har den yderligere modifikation, består af en internukleotid-modificerende binding bestående af phosphorothioat-modifikation.

10. Isoleret crRNA ifølge krav 7, hvor den kemisk modificerede form af formel (I) er udvalgt blandt SEQ ID NOs.: 163, 179-181, 235-237, 240-243, 256, 257, 268-271, 385-406, 438 og 439.

11. Isoleret tracrRNA udvalgt fra gruppen bestående af SEQ ID NOs.:11-13, 17, 87-106, 110-114, 116, 117, 126, 129, 130, 131, 132, 134, 136, 144, 145 og 147-156, 159, 160, 449 og 451, hvor det isolerede tracrRNA er aktivt i et CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/CRISPR-associeret protein-endonuklease-system.

12. Isoleret crRNA udvalgt fra gruppen bestående af SEQ ID NOs.: 14, 164, 166-169, 171, 174-178, 186, 187, 189-207, 212-214, 221-224, 226-228, 233-249, 252, 256-259, 264-271, 291-374, 376, 377, 379-384, 430-437 og 445-

448, hvor det isolerede crRNA er aktivt i et CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/CRISPR-associeret protein-endonuklease-system.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c-ggaauaaaauugaacgauacgacaaaacuuaccaagguug
u
a
guccguuaucaacuug
a
a
agccacggugaaa
g
ucggugcuuuuuuu (SEQ ID NO.:18)

FIG. 1

```
cuuauauccaacacuucgugguuuuaga--gcuag
                    |||||||    ||||  a
                    |||||||    ||||  a
  c-ggaauaaaauugaacgaua
u|  ||
a|  ||
 guccguuaucaacuug
                      ||||  a
                      ||||  a
   agccacggugaaa
g  ||||||
  ucggugcuuu
```

(SEQ ID NO.:428)

FIG. 2

SEQ ID No.	tracrRNA Sequence (5'-3')	Cleavage (%)
18	GUUGGAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU	38
30	CAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	26
31	AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU	32
2	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU	57
32	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU	47
33	CAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU	27
34	AGCAUAGCAAGUUAAAAUA GUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU	0
35	AGCAUAGCAAGUUAAAAUA AACUUGAAAAAGUGGCACCGAGUCGGUGCU	0
36	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC	53
37	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUG	56
38	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGU	56
39	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGG	53
40	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG CCGAGUCGG	5
41	AGCAUAGCAAGUUAAAAUAAGGCUAGUCC AACUUGAAAAAGUGGCACCGAGUCGGUGCU	0
42	AGCAUAGCAAGUUAAAAUAAGGCUAGUCC AACUUGAAAAAGUGGCACCGAGUCGG	0
43	AGCAUAGCAAGUUAAAAUAAGGCUAGUCC AACUUGAAAAAGUG CCGAGUCGG	0
44	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG	0
45	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA GCACCGAGUCGGUGCU	0
427	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGU CAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU	4

FIG. 3

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SEQ ID No.	crRNA and tracrRNA Sequences 5'-3'	Length
46	CUUAUAUCCAACACUUCGUGGUUUUAGAGCUAUGCUGUUUUG	42 base crRNA
47	CUUAUAUCCAACACUUCGUGGUUUUAGAGCUAUGCUGUU	39 base crRNA
48	CUUAUAUCCAACACUUCGUGGUUUUAGAGCUAUGCU	36 base crRNA
49	CUUAUAUCCAACACUUCGUGGUUUUAGAGCUAUG	34 base crRNA
18	GUUGGAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU	89 base tracrRNA
50	CAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	74 base tracrRNA
51	AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU	70 base tracrRNA
2	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU	67 base tracrRNA
52	AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU	65 base tracrRNA
53	CAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU	63 base tracrRNA

FIG. 4

```
cuuauauccaacacuucgugguuuuaga--gcuaugcu  (SEQ ID NO.:48 )
                        |||||||    ||||||||
                 c-ggaauaaaauugaacgauacga
                u|  ||
                a|  ||
                 guccguuaucaacuug
                              |||| a
                              |||| a
                   agccacggugaaa
                  g ||||||
                   ucggugcuuu   (SEQ ID NO.: 2)
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FIG. 5

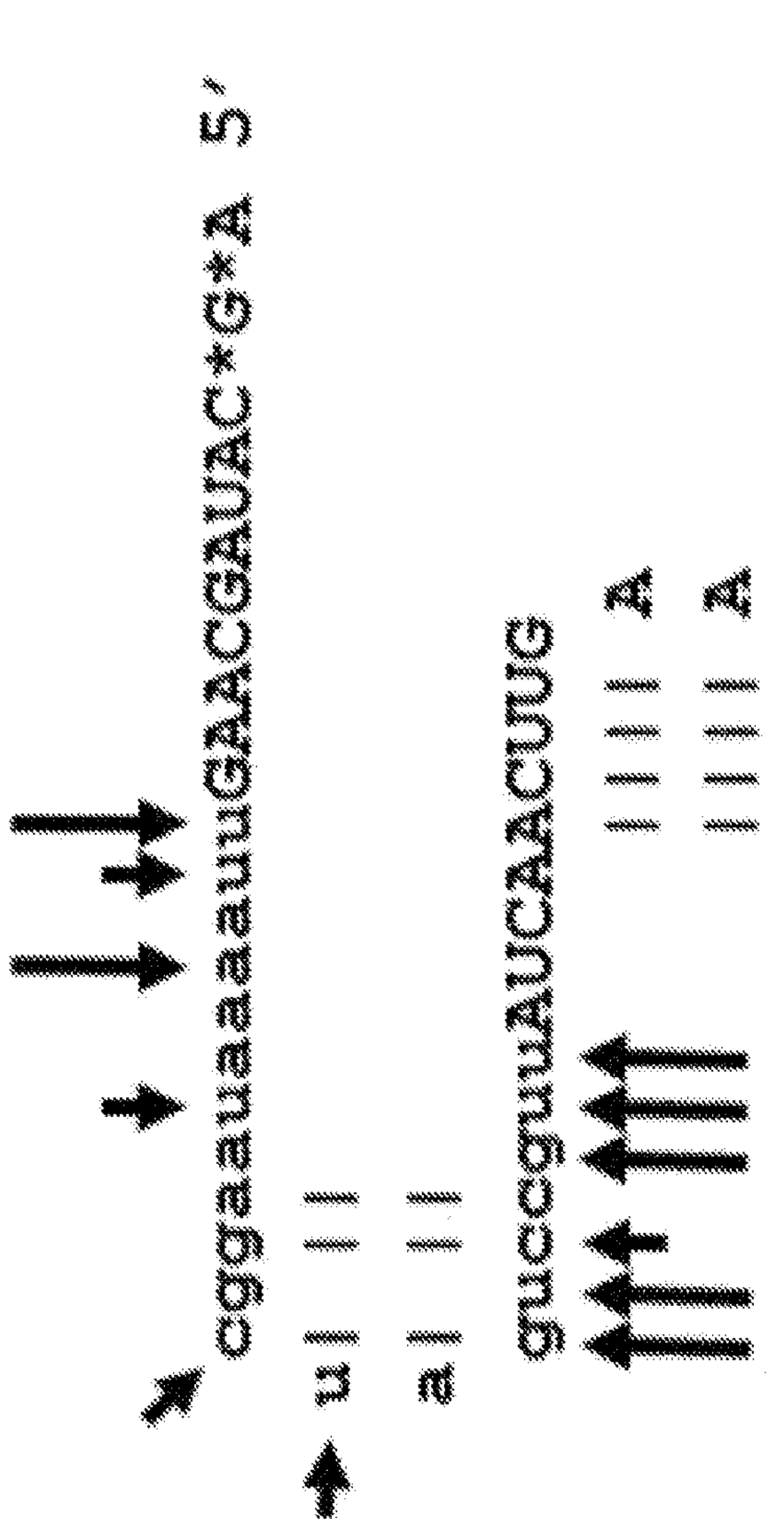
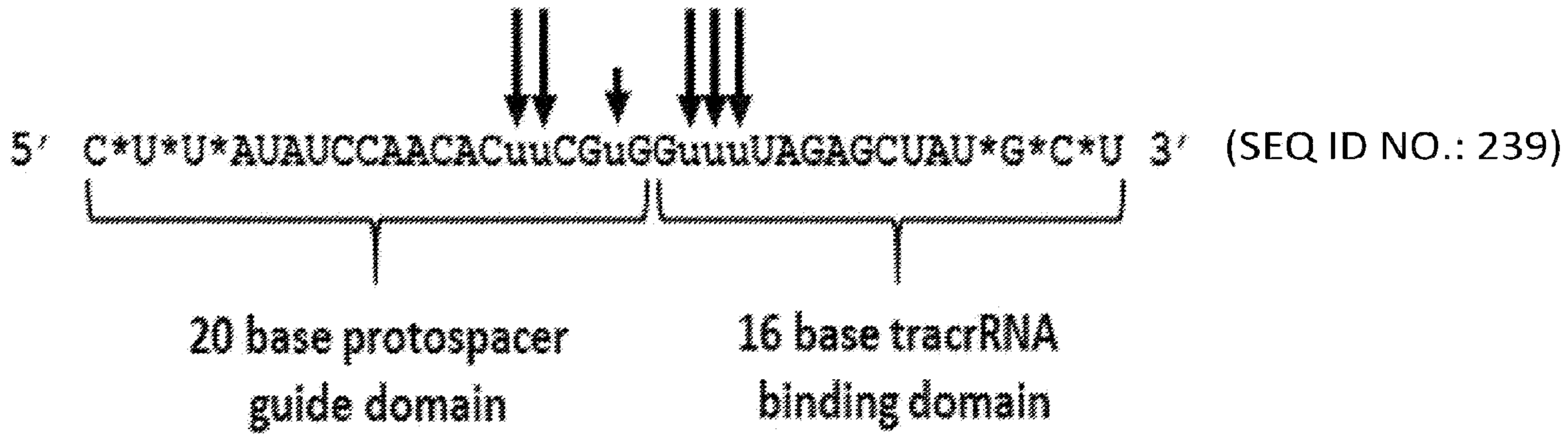

cggaauaaaauGAAGAUGAU 5'
guccguAUCACACUG
AGCACGGUGAA
G
UCGUGCU*U*U 3'
(SEQ ID NO.: 134)

FIG. 6



(SEQ ID NO.: 239)

FIG. 7

DK/EP 3234133 T3

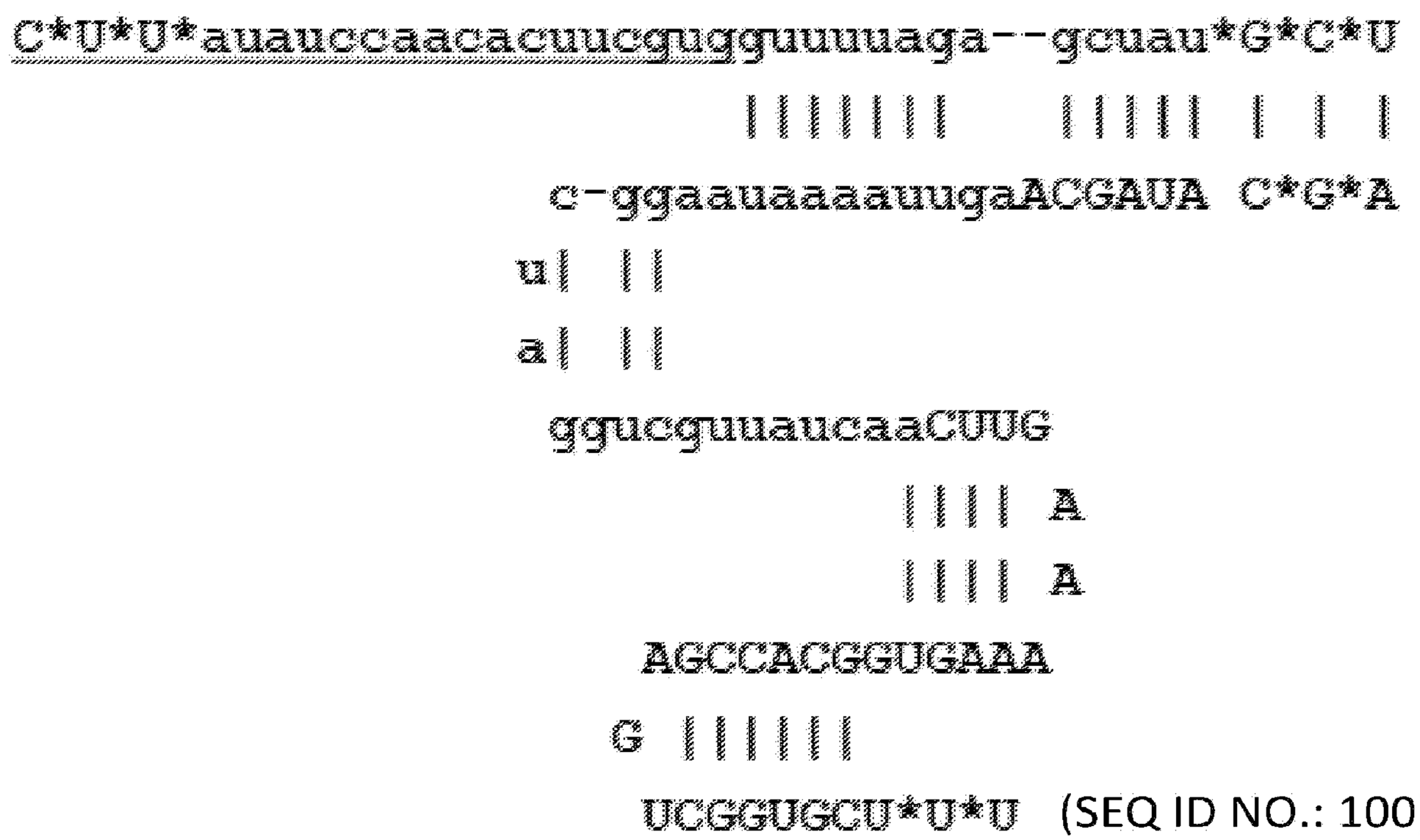


FIG. 8

DK/EP 3234133 T3

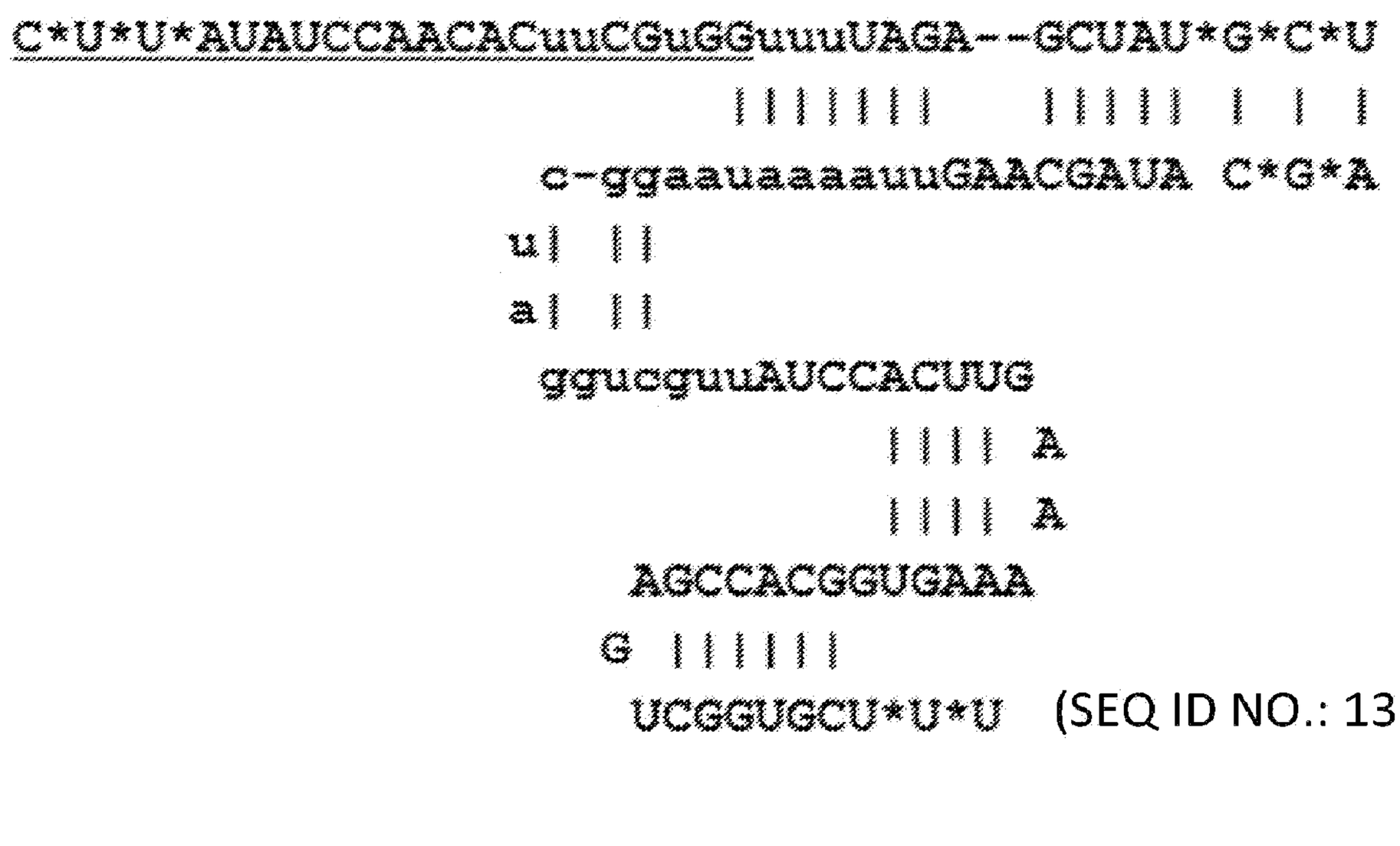


FIG. 9

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20 base protospacer guide Target-specific domain	16 base universal tracrRNA-binding domain	Modification Pattern #
n n n nnnnnnnnnnnnnnnnnguuuuagagcuau g c u		1 (SEQ ID NO.:429)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u		2 (SEQ ID NO.:430)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u		3 (SEQ ID NO.:431)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u		4 (SEQ ID NO.:432)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u		5 (SEQ ID NO.:433)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u		6 (SEQ ID NO.:434)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u		7 (SEQ ID NO.:435)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u		8 (SEQ ID NO.:436)
n*n*n*nnnnnnnnnnnnnnnnnguuuuagagcuau*g*c*u		9 (SEQ ID NO.:437)
C3-n n n nnnnnnnnnnnnnnnnnguuuuagagcuau g c u-C3		10 (SEQ ID NO.:438)
ZEN-n n n nnnnnnnnnnnnnnnnnguuuuagagcuau g c u-ZEN		11 (SEQ ID NO.:439)

FIG. 10

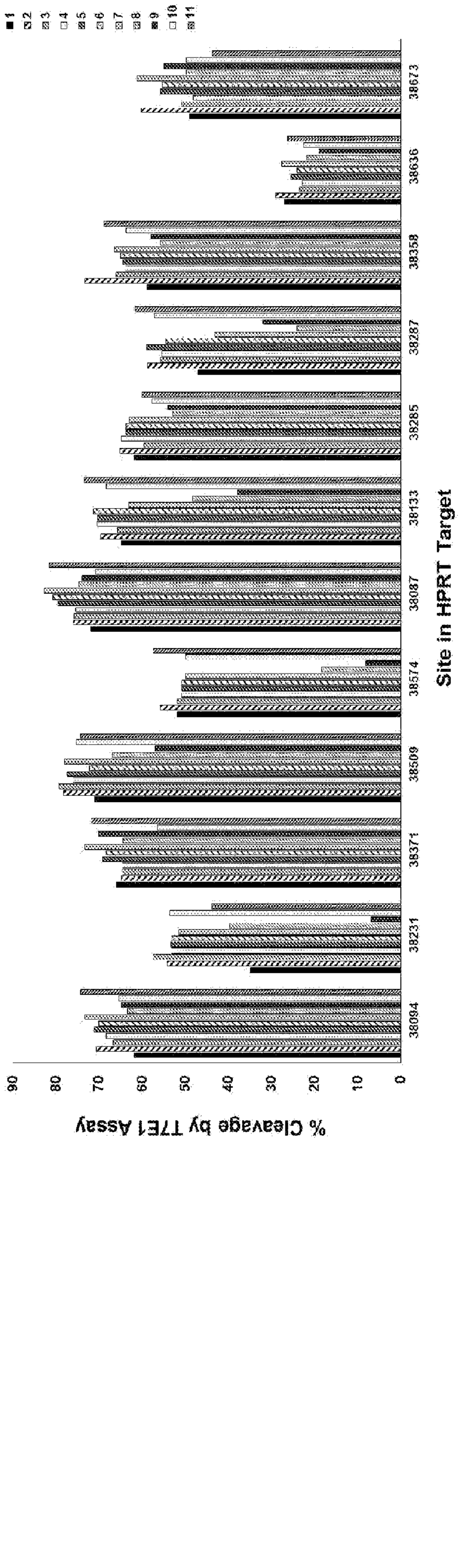
1
2
3
4
5
6
7
8
9
10
11
38673
38636
38358
38287
38285
38133
38087
38574
38509
38371
38231
38094
Site in HPRT Target
% Cleavage by T7E1 Assay
0
10
20
30
40
50
60
70
80
90

FIG. 11

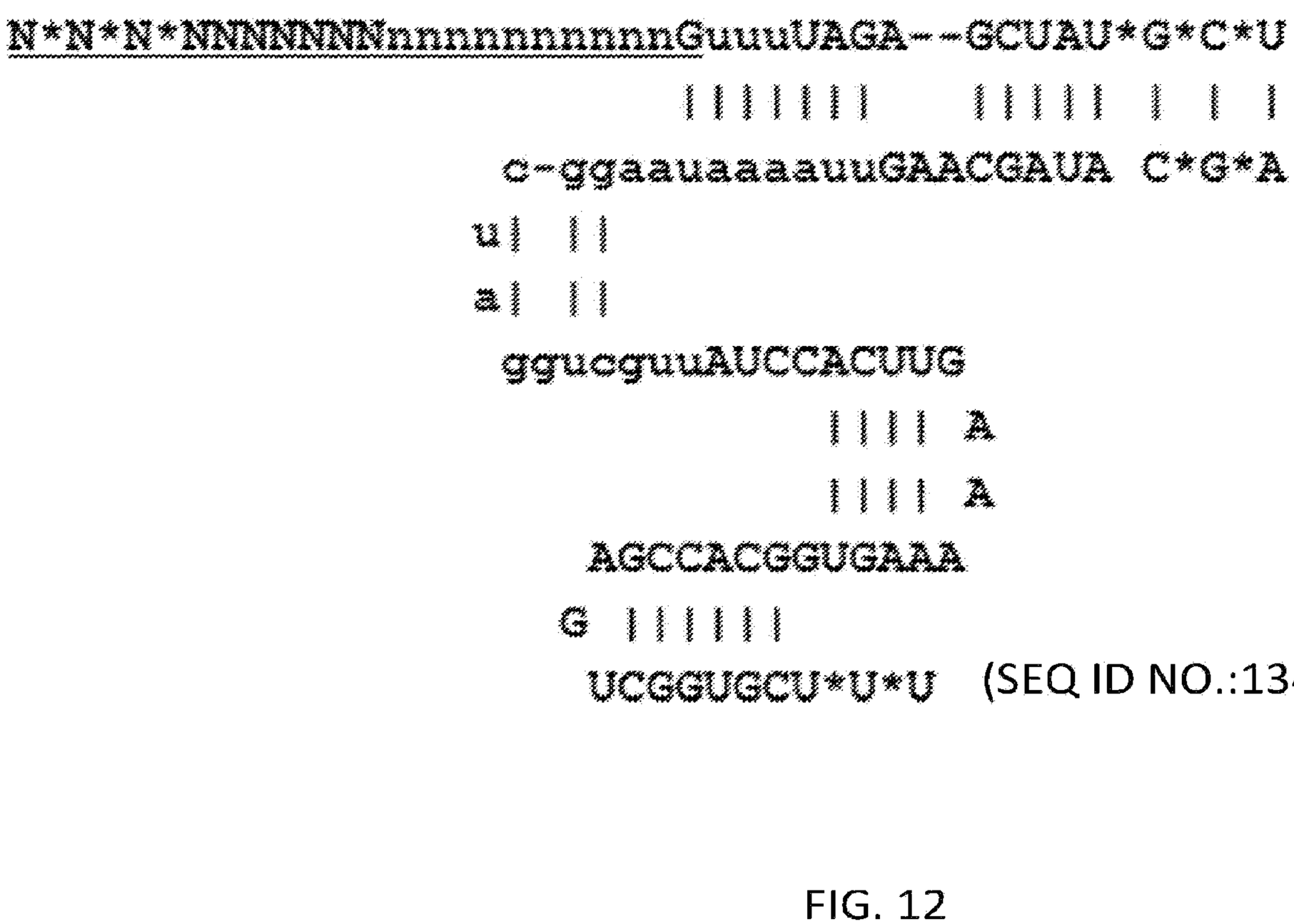
N*N*N*NNNNNNNnnnnnnnnnnnGuuuUAGA--GCUAU*G*C*U (SEQ ID NO.:434)
c-ggaauaaaauuGAACGAUA C*G*A
u
a
ggucguuAUCCACUUG
A
A
AGCCACGGUGAAA
G
UCGGUGCU*U*U (SEQ ID NO.:134)

FIG. 12

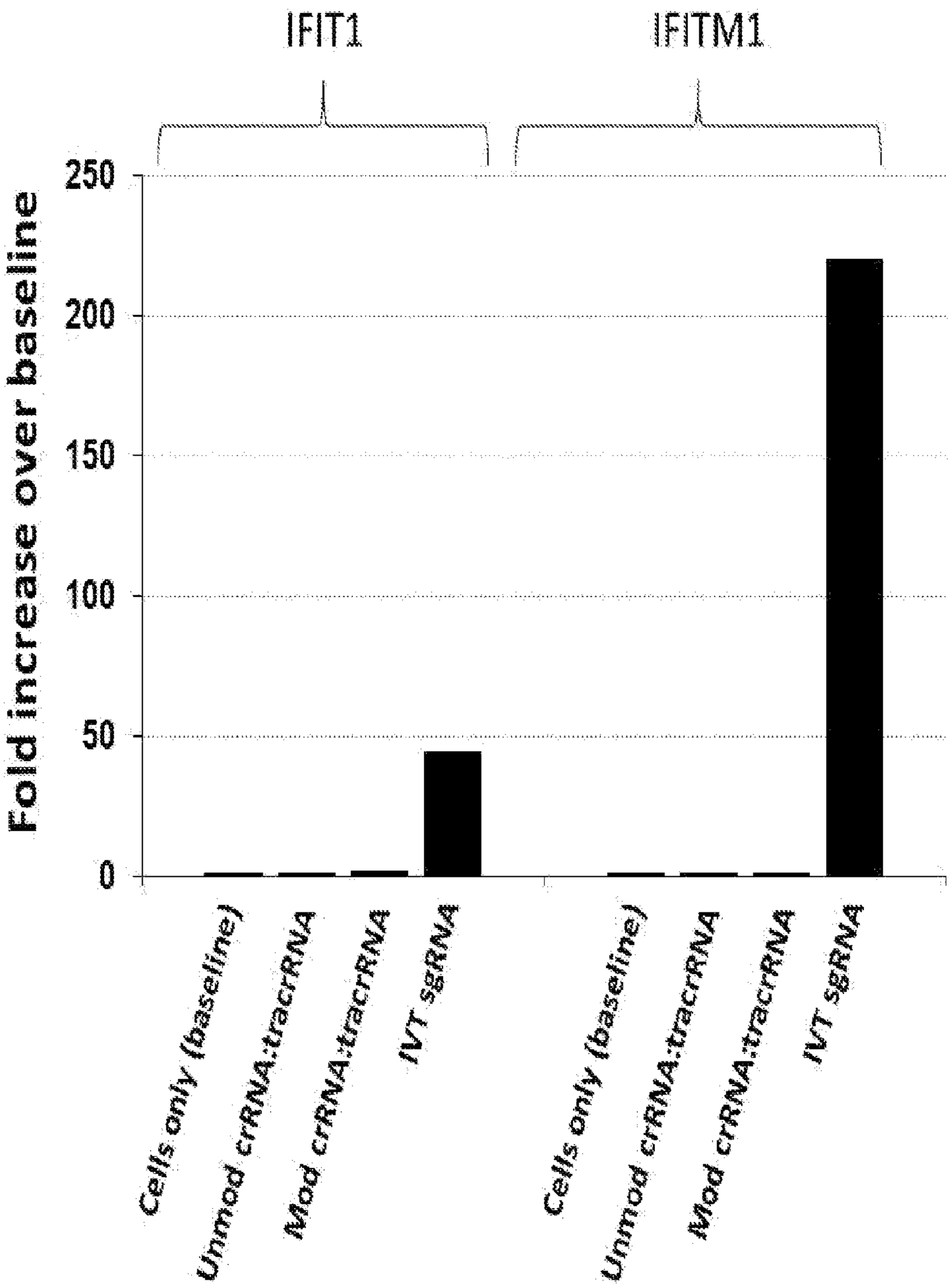
IFIT1
IFITM1
250
200
150
100
50
0
Fold increase over baseline
Cells only (baseline)
Unmod crRNA:tracrRNA
Mod crRNA:tracrRNA
IVT sgRNA
Cells only (baseline)
Unmod crRNA:tracrRNA
Mod crRNA:tracrRNA
IVT sgRNA

FIG. 13