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(54) **Title:** NOVEL CRISPR NUCLEASES

(57) **Abstract:** The present invention provides a method of modifying a nucleotide sequence at a target site in the genome of a mammalian cell comprising introducing into the cell (i) a composition comprising a CRISPR nuclease having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-9 or a nucleic acid molecule comprising a sequence encoding a CRISPR nuclease which sequence has at least 95% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs:23-27 and (ii) a DNA-targeting RNA molecule, or a DNA polynucleotide encoding a DNA-targeting RNA molecule, comprising a nucleotide sequence that is complementary to a sequence in the target DNA.

NOVEL CRISPR NUCLEASES

[0001] This application claims the benefit of U.S. Provisional Application No. 62/810,878, filed February 26, 2019, U.S. Provisional Application No. 62/810,835, filed February 26, 2019, U.S. Provisional Application No. 62/736,976, filed September 26, 2018, and U.S. Provisional
5 Application No. 62/737,733, filed September 27, 2018 the contents of each of which are hereby incorporated by reference.

[0002] Throughout this application, various publications are referenced, including referenced in parenthesis. The disclosures of all publications mentioned in this application in their entireties are hereby incorporated by reference into this application in order to provide additional description of
10 the art to which this invention pertains and of the features in the art which can be employed with this invention.

REFERENCE TO SEQUENCE LISTING

[0003] This application incorporates-by-reference nucleotide sequences which are present in the file named “190925_90617-A-PCT_Sequence_Listing_DH.txt”, which is 636 kilobytes in size,
15 and which was created on September 24, 2019 in the IBM-PC machine format, having an operating system compatibility with MS-Windows, which is contained in the text file filed September 25, 2019 as part of this application.

FIELD OF THE INVENTION

[0004] The present invention is directed to, *inter alia*, composition and methods for genome
20 editing.

BACKGROUND OF THE INVENTION

[0005] The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) systems of bacterial and archaeal adaptive immunity show extreme diversity of protein composition and genomic loci architecture. The CRISPRs systems have become important tools for research and
25 genome engineering. Nevertheless, many details of CRISPR systems remain to be learned and the applicability of CRISPR nucleases may be limited by the sequence specificity requirements, expression, or delivery challenges. Different CRISPR nucleases have diverse characteristics such

as: size, PAM site, on target activity, specificity, cleavage pattern (e.g. blunt, staggered ends), and prominent pattern of indel formation following cleavage. Different sets of characteristics may be useful for different applications. For example, some CRISPR nucleases may be able to target particular genomic loci that other CRISPR nucleases cannot due to limitations of the PAM site. In 5 addition, some CRISPR nucleases currently in use exhibit pre-immunity, which may limit *in vivo* applicability. *See* Charlesworth, C. T., et al. (2019). Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nature medicine, 25(2), 249, and Wagner, D. L., et al. (2019). High prevalence of Streptococcus pyogenes Cas9-reactive T cells within the adult human population. Nature medicine, 25(2), 242.

10 [0006] Discovery, engineering and improvement of novel CRISPR nucleases is of importance.

SUMMARY OF THE INVENTION

[0007] Disclosed herein are compositions and methods that may be utilized for genomic engineering, epigenomic engineering, genome targeting, genome editing of cells, and/or in vitro diagnostics.

15 [0008] The disclosed compositions may be utilized for modifying genomic DNA sequences. As used herein, genomic DNA refers to linear and/or chromosomal DNA and/or to plasmid or other extrachromosomal DNA sequences present in the cell or cells of interest. In some embodiments, the cell of interest is a eukaryotic cell. In some embodiments, the cell of interest is a prokaryotic cell. In some embodiments, the methods produce double-stranded breaks (DSBs) at pre-20 determined target sites in a genomic DNA sequence, resulting in mutation, insertion, and/or deletion of DNA sequences at the target site(s) in a genome.

[0009] Accordingly, in some embodiments, the compositions comprise a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) nucleases. In some embodiments, the CRISPR nuclease is CRISPR-associated protein.

25 [0010] In some embodiments, the compositions comprise a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) nucleases having 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90% identity to CRISPR nucleases derived from Clostridium cocleatum (e.g., Clostridium cocleatum-loc2), Acetitomaculum ruminis, Alloscardovia macacae, Enterococcus devriesei, Enterococcus thailandicus, Fructobacillus ficulneus, Aquimarina sp. (e.g., Aquimarina

sp. w0), Leuconostoc lactis (e.g., Leuconostoc lactis CCK940), Dolosicoccus paucivorans (e.g., Dolosicoccus paucivorans UMB0860), Lactobacillus kefiri (Lactobacillus kefiri OG2). Each possibility represents a separate embodiment.

OMNI Nucleases

5 [0011] Embodiments of the present invention provide for CRISPR nucleases designated "OMNI" nucleases as provided in Table 1 hereinbelow. Column 1 of Table 1 indicates each OMNI designation; column 2 indicates the SEQ ID NO for OMNI; column 3 indicates the DNA sequence encoding each OMNI; column 4 indicates the DNA sequence encoding each OMNI codon optimized for E. coli or bacterial cultures; column 5 indicates the DNA sequence encoding each
10 OMNI codon optimized for mammalian cells.

Table 1: "OMNI" Nucleases and encoding sequences

"OMNI" name	SEQ ID NO of Amino Acid Sequence	Source Organism	SEQ ID NO of DNA sequence encoding OMNI	SEQ ID NO of DNA sequence codon optimized for encoding OMNI in E. Coli	SEQ ID NO of DNA sequence codon optimized for encoding OMNI in mammalian cells
OMNI 4	2	Dolosicoccus paucivorans	30	52	24
OMNI 6	1	Clostridium cocleatum	29	51	23
OMNI 7	10	Acetitomaculum ruminis	38	60	--
OMNI 8	11	Alloscardovia macacae	39	61	28
OMNI 10	12	Aquimarina sp. w01	40	62	--
OMNI 11	13	Leuconostoc lactis	41	63	--
OMNI 13	4	Anaeromassilibacillus sp. An172	32	54	26

OMNI 14	14	Campylobacter blaseri	42	64	--
OMNI 16	15	Dehalobacterium formicoaceticum	43	65	--
OMNI 17	5	Drancourtella sp. An177	33	55	--
OMNI 18	6	Lachnoclostridium sp. SNUG30099	34	56	--
OMNI 19	7	Lactomassilus timonensis	35	57	27
OMNI 20	3	Monoglobus pectinilyticus	31	53	--
OMNI 21	16	Raoultibacter timonensis	44	66	--
OMNI 22	17	Elioraea	45	67	--
OMNI 23	8	Azospirillum brasilense	36	58	--
OMNI 24	9	Gramella	37	59	--
OMNI 26	18	Macrococcus bohemicus	46	68	--
OMNI 27	19	Mordavella sp. Marseille-P3756	47	69	--
OMNI 29	20	Polaribacter sp. ALD11	48	70	319
OMNI 30	21	Robinsoniella sp	49	71	--
OMNI 31	22	Aequorivita antarctica	50	72	--

[0012] This invention provides a method of modifying a nucleotide sequence at a target site in the genome of a mammalian cell comprising introducing into the cell (i) a composition comprising a CRISPR nuclease having at least 95% identity to an amino acid sequence selected from the group
5 consisting of SEQ ID NOs: 1-9 or a nucleic acid molecule comprising a sequence encoding a

CRISPR nuclease which sequence has at least 95% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs:23-27 and (ii) a DNA-targeting RNA molecule, or a DNA polynucleotide encoding a DNA-targeting RNA molecule, comprising a nucleotide sequence that is complementary to a sequence in the target DNA.

5 [0013] This invention also provides a non-naturally occurring composition comprising a CRISPR nuclease comprising a sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding the CRISPR nuclease.

[0014] This invention also provides a non-naturally occurring composition comprising a 10 CRISPR associated system comprising:

a) one or more RNA molecules comprising a guide sequence portion linked to a direct repeat sequence, wherein the guide sequence is capable of hybridizing with a target sequence, or one or more nucleotide sequences encoding the one or more RNA molecules; and

b) an CRISPR nuclease comprising an amino acid sequence having at least 95% identity to 15 the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding the CRISPR nuclease; and

wherein the one or more RNA molecules hybridize to the target sequence, wherein the target sequence is 3' of a Protospacer Adjacent Motif (PAM), and the one or more RNA molecules 20 form a complex with the RNA-guided nuclease.

[0015] This invention also provides a non-naturally occurring composition comprising:

a) a CRISPR nuclease comprising a sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding 25 the CRISPR nuclease; and

b) one or more RNA molecules, or one or more DNA polynucleotide encoding the one or more RNA molecules, comprising at least one of:

i) a nuclease-binding RNA nucleotide sequence capable of interacting with/binding to the CRISPR nuclease; and

ii) a DNA-targeting RNA nucleotide sequence comprising a sequence complementary to a sequence in a target DNA sequence,

wherein the CRISPR nuclease is capable of complexing with the one or more RNA molecules to form a complex capable of hybridizing with the target DNA sequence.

5

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **Fig. 1A-B**: **Fig. 1A**, An example of the predicted secondary structures of the full duplex RNA elements (crRNA:tracrRNA chimera) used for identification of possible “Nexus” and “hairpins” in the design of sgRNAs for each nuclease; **Fig. 1B**, an example of variations in the
5 sequence and predicted structure between regions of two different sgRNAs, V1 and V2, designed for use with a single nuclease. By shortening the duplex at the upper stem at different locations, the crRNA and tracrRNA were connected with tetra-loop ‘gaaa’, generating different sgRNA scaffolds.

[0017] **Figs. 2A-B**: **Fig. 2A**, a condensed 4N window library of all possible PAM locations along
10 an 8bp sequence for OMNI 4 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 2B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 4 sgRNA v2.

[0018] **Figs. 3A-B**: **Fig. 3A**, a condensed 4N window library of all possible PAM locations along
15 an 8bp sequence for OMNI 6 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 3B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 6 sgRNA v2.

[0019] **Figs**. **4A-B: Fig. 4A**, a condensed 4N window library of all possible PAM locations along
20 an 8bp sequence for OMNI 8 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 4B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 8 sgRNA v2.

[0020] **Figs. 5A-B**: **Fig. 5A**, a condensed 4N window library of all possible PAM locations along
25 an 8bp sequence for OMNI 10 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 5B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 10 sgRNA v2.

[0021] **Figs. 6A-B**: **Fig. 6A**, a condensed 4N window library of all possible PAM locations along an 8bp sequence for OMNI 11 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 6B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 11 sgRNA v2.

[0022] **Figs. 7A-B**: **Fig. 7A**, a condensed 4N window library of all possible PAM locations along an 8bp sequence for OMNI 13 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 7B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 13 sgRNA v2.

[0023] **Figs. 8A-B**: **Fig. 8A**, a condensed 4N window library of all possible PAM locations along an 8bp sequence for OMNI 17 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 8B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 17 sgRNA v2.

[0024] **Figs. 9A-B**: **Fig. 9A**, a condensed 4N window library of all possible PAM locations along an 8bp sequence for OMNI 18 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 9B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 18 sgRNA v2.

[0025] **Figs. 10A-B**: **Fig. 10A**, a condensed 4N window library of all possible PAM locations along an 8bp sequence for OMNI 19 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 10B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 19 sgRNA v2.

[0026] **Figs. 11A-B**: **Fig. 11A**, a condensed 4N window library of all possible PAM locations along an 8bp sequence for OMNI 20 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 11B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 20 sgRNA v2.

[0027] **Figs. 12A-B**: **Fig. 12A**, a condensed 4N window library of all possible PAM locations along an 8bp sequence for OMNI 23 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 12B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 23 sgRNA v2.

[0028] **Figs. 13A-B**: **Fig. 13A**, a condensed 4N window library of all possible PAM locations along an 8bp sequence for OMNI 24 sgRNA v1 in *E. coli* and sequence motifs generated. Activity estimated based on the average of the two most depleted sequences and was calculated as 1 – Depletion score; **Fig. 13B** discloses the sequence motifs generated for all possible PAM locations along an 8bp sequence for the OMNI 24 sgRNA v2.

[0029] **Figure 14**: Depletions of PAM sites along positions 1-4 of an 8bp sequence for spCas9 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0030] **Figure 15:** Depletions of PAM sites along positions 3-6 of an 8bp sequence for OMNI 4 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0031] **Figure 16**: Depletions of PAM sites along positions 1-4 of an 8bp sequence for OMNI 6 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0032] **Figure 17**: Depletions of PAM sites along positions 1-4 of an 8bp sequence for OMNI 8 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0033] **Figure 18**: Depletions of PAM sites along positions 1-4 (top panel) and positions 5-8 (middle panel) of an 8bp sequence for OMNI 10 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0034] **Figure 19**: Depletions of PAM sites along positions 3-7 of an 8bp sequence for OMNI 13 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0035] **Figure 20**: Depletions of PAM sites along positions 3-6 of an 8bp sequence for OMNI 17 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0036] **Figure 21:** Depletions of PAM sites along positions 1-4 of an 8bp sequence for OMNI 18 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0037] **Figure 22**: Depletions of PAM sites along positions 1-4 of an 8bp sequence for OMNI 19 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0038] **Figure 23**: Depletions of PAM sites along positions 1-6 of an 8bp sequence for OMNI 20 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0039] **Figure 24**: Depletions of PAM sites along positions 2-6 of an 8bp sequence for OMNI 23 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0040] **Figure 25**: Depletions of PAM sites along positions 2-5 of an 8bp sequence for OMNI 24 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0041] **Fig. 26A-B**: **Fig. 26A**, depletions of PAM sites along positions 1-6 of an 8bp sequence for OMNI 16 using sgRNA v1 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results; **Fig. 26B**, depletions of PAM sites along positions 1-6 of an 8bp sequence for OMNI 16 using sgRNA v2 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0042] **Fig. 27A-B**: **Fig. 27A**, depletions of PAM sites along positions 2-5 of an 8bp sequence for OMNI 21 using sgRNA v1 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results; **Fig. 27B**, depletions of PAM sites along positions 2-5 of an 8bp sequence for OMNI 21 using sgRNA v2 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0043] **Fig. 28A-B**: **Fig. 28A**, depletions of PAM sites along positions 4-7 of an 8bp sequence for OMNI 27 using sgRNA v1 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results; **Fig. 28B**, depletions of PAM sites along positions 4-7 of an 8bp sequence for OMNI 27 using sgRNA v2 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0044] **Fig. 29A-B**: **Fig. 29A**, depletions of PAM sites along positions 3-6 of an 8bp sequence for OMNI 30 using sgRNA v1 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results; **Fig. 29B**, depletions of PAM sites along positions 3-6 of an 8bp sequence for OMNI 30 using sgRNA v2 in a cell-free *in vitro* TXTL system. Sequence motifs generated for PAM sites based on depletion assay results.

[0045] **Fig. 30A-C**: **Fig. 30A**, growth selective plates with Arabinose for nuclease complexes for OMNI 4, OMNI 6, OMNI 8, and OMNI 10 along identified PAM sites. OMNI complexes that cleave the positive plasmid targeted survive in the presence of Arabinose, whereas OMNI complexes that do not cleave the positive plasmid cannot grow on selective plates; **Fig. 30B**, growth selective plates for nucleases complexes for OMNI 11, OMNI 13, OMNI 17, and OMNI 18; **Fig. 30C**, growth selective plates for nucleases complexes for OMNI 19, OMNI 20, OMNI 23, and OMNI 24.

[0046] **Fig. 31A-C**: **Fig. 31A**, SpCas9 as a control and OMNI 4, OMNI 6, OMNI 8, OMNI 22, OMNI 20 nucleases tested for *in vitro* cleavage of different PAM sites in. E. coli strain BW25141 (λDE3) co-expressing the respective OMNI nucleases and sgRNA were lysed using BugBuster lysis solution. The lysate was reacted in the recommended cleavage buffer with linear DNA substrates containing the PAM sequences flanked by a unique protospacer targeted by the sgRNA (T1) or a non-targeted protospacer (T2) as a control with cleavage results shown; **Fig. 31B**, OMNI 20 PAM cleavage results for PAM sites with an sgRNA T1 spacer for PAM sites shown in Table 17, specifically PAM-spacer 0-T1 (column 3), 2D4-T1 (column 4), 3D2-T1 (column 5), 5-T1 (column 6), 6D1-T1 (column 7), 9D1-T1 (column 8) 10-T1 (column 9) and a negative control utilizing a T2 spacer 0-T2 (column 10) and an uncut template (column 11); **Fig. 31C**, *(left panel gel)* OMNI 8 PAM cleavage results for PAM sites with an sgRNA T1 spacer for PAM sites shown in Table 17, specifically PAM-spacer 0-T1 (column 2), 6D1-T1 (column 3), 8D-1 (column 4) and

a negative control utilizing a T2 spacer 0T2 (column 4); *(right panel gel)* each of 0-T1, 6D1-T1, 8D1-T1, and 0-T2 uncut without OMNI nuclease.

[0047] **Fig. 32A-C**: **Fig. 32A**, OMNI 4 and OMNI 6 open-reading frames were cloned into bacterial expression plasmids and expressed in G10 cells. Purity of OMNI proteins expressed was measured by SDS-PAGE analysis; **Fig. 32B**, Synthetic sgRNA of OMNI 4 and OMNI 6 ribonucleoproteins (RNPs) were formed. The RNPs were reacted with a cleavage buffer with 100ng of linear DNA substrates containing the protospacer targeted by the sgRNA flanking each OMNI PAM sequence; **Fig. 32C**, *in vitro* DNA cleavage activity by RNP of purified OMNI 6 nucleases with corresponding synthetic sgRNA. Full cleavage of OMNI 6 with "On-target" protospacers was observed. OMNI 6 was further tested for specificity. A remarkable level of discrimination for protospacer with single nucleotide mismatch was demonstrated.

[0048] **Fig. 33A-H**: Activity of OMNI nucleases in Mammalian cells was assayed using a GFP Fluorescent gain-based reporter system in HEK 293T cells. Negative control cells were transfected with the reporter vector with only the OMNI nuclease or only the guide; **Fig. 33A**, OMNI 4 GFP signal for the nuclease + guide and guide only at PAM 4D2 with sgRNA v1; **Fig. 33B**, OMNI 13 GFP signal for the nuclease + guide, guide only, and nuclease only at PAM 4D1 with sgRNA v2; **Fig. 33C**, OMNI 17 GFP signal for the nuclease + guide, guide only, and nuclease only at PAM 17D2 with sgRNA v2; **Fig. 33D**, OMNI 18 GFP signal for the nuclease + guide, guide only, and nuclease only at PAM 1 with sgRNA v4; **Fig. 33E**, OMNI 19 GFP signal for the nuclease + guide, guide only, and nuclease only at PAM 4D2 with sgRNA v2; **Fig. 33F**, OMNI 20 GFP signal for the nuclease + guide, guide only, and nuclease only at PAM 20D2 with sgRNA v3; **Fig. 33G**, OMNI 23 GFP signal for the nuclease + guide, guide only, and nuclease only at PAM 6D2 with sgRNA v2; **Fig. 33H**, OMNI 24 GFP signal for the nuclease + guide, guide only, and nuclease only at PAM 8D2 with sgRNA v2.

[0049] **Fig. 34A-C**: Activity of OMNI nucleases with optimized expression vectors for *E. coli* and Human cells were assayed using a GFP Fluorescent gain-based reporter system. Negative control cells were transfected with the reporter vector with no guide. **Fig. 34A**, OMNI 6 activity with optimized expression vectors for *E. coli* and Human cells and sgRNAs sgRNA v2, sgRNA v2, sgRNA v4 and sgRNA v5; **Fig. 34B**, Activity of OMNI 18 encoded by a human optimized expression vector was compared to spCas9 activity. OMNI 18 was assayed with sgRNA v2,

sgRNA v3, and sgRNA v4. Negative controls for OMNI 18 and spCas9 were run with either no guide or no nuclease; **Fig. 34C**, Activity of OMNI 20 encoded by a human optimized expression vector was compared to spCas9 activity. OMNI 20 was assayed utilizing sgRNA-PAM pairings V2-0, V2-20D2,V3-0, and V3-20D2. SpCas9 activity was assayed at Pam site 1. Negative controls
5 for OMNI 30 and spCas9 were run with no nuclease.

[0050] **Fig. 35A-D**: In order to overcome potential transcriptional and structural constrains and to assess the plasticity of the sgRNA scaffold in the human cellular environmental context, several versions of sgRNA were tested. In each case the modifications represent small variations in the nucleotide sequence within the predicted duplex and/or hairpins that were introduced to several
10 synthetic sgRNA; **Fig. 35A**, the predicted secondary structure of the full duplex RNA elements of OMNI 6 sgRNA v2 (SEQ ID NO:88); **Fig. 35B**, the predicted secondary structure of the full duplex RNA elements of OMNI 6 sgRNA v3 (SEQ ID NO:89); **Fig. 35C**, the predicted secondary structure of the full duplex RNA elements of OMNI 6 sgRNA v4 (SEQ ID NO:90); **Fig. 35D**, the predicted secondary structure of the full duplex RNA elements of OMNI 6 sgRNA v5 (SEQ ID
15 NO:91).

[0051] **Fig. 36A-B**: The intrinsic fidelity of OMNI6 was measured by conducting an activity assay as described; **Fig. 36A**, an intrinsic fidelity assay of OMNI 6 at site ELANE g35 in HeLa cells as compared to SpCas9. The on target (SEQ ID NO:383) and off target (SEQ ID NO:384) editing efficiency ratio obtained by OMNI 6 was 2.43:1 while spCas9 on/off ratio was 1:1. **Fig.
20 36B**, an intrinsic fidelity assay of OMNI 6 at site ELANE g58 in HEK293 FRT cells as compared to SpCas9. The on target (SEQ ID NO: 101) and off target (SEQ ID NO:385) editing efficiency ratio obtained by OMNI 6 was 17.64:1 while spCas9 on/off ratio was 1.58:1.

[0052] **Figure 37**: synthetic sgRNAs for OMNI 4 and OMNI 6 were synthesized and expressed in U2OS cells. RNPs were formed. Cells were lysed and their genomic DNA content was used in PCR reaction, amplifying the corresponding putative genomic targets. Amplicons were subjected to NGS and the resulting sequences were then used calculate the percentage of editing events. **Figure 37**, *left panel*, OMNI 4 % editing at genomic site EMX1 on target (SEQ ID NO:386) was 50.4% as compared to 0.1% for the negative control with no sgRNA. **Figure 37**, *right panel*, OMNI 6 % editing at genomic site EMX1 on target (SEQ ID NO:101) was 48.1% as compared to 1.6% for the negative control with no sgRNA.

DETAILED DESCRIPTION

[0053] According to some aspects, the disclosed compositions comprise a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) nuclease and/or a nucleotide sequence encoding the same.

[0054] In some embodiments, the CRISPR nuclease comprises an amino acid sequence having at least 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, or 82% amino acid sequence identity to a CRISPR nuclease as set forth in any of SEQ ID NOs:1-22.

[0055] In some embodiments, the CRISPR nuclease comprises an amino acid sequence having at least 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 75% amino acid sequence identity to a CRISPR nucleases derived from Acetobacterium sp. KB-1, Alistipes sp.An54, Bartonella apis, Blastopirellula marina, Bryobacter aggregatus MPL3, Algoriphagus marinus, Butyrivibrio sp. AC2005, bacterium LF-3, Aliiarcobacter faecis, Caviibacter abscessus, Arcobacter sp.SM1702, Arcobacter mytili, Arcobacter thereius, carnobacterium funditum, Peptoniphilus obesi ph1, Carnobacterium iners, Lactobacillus allii, Ezakiella peruensis strain M6.X2, Bacteroides coagulans, Butyrivibrio sp. NC3005, Clostridium sp. AF02-29, or Algoriphagus antarcticus as set forth in any SEQ ID NOs:1-22.

[0056] In an embodiment, the CRISPR nuclease of the invention exhibits increased specificity to a target site compared to a spCas9 nuclease when complexed with the one or more RNA molecules.

[0057] In an embodiment, the complex of the CRISPR nuclease of the invention and one or more RNA molecules exhibits at least maintained on-target editing activity of the target site and reduced off-target activity compared to spCas9 nuclease.

[0058] In embodiments of the invention, the CRISPR nuclease is engineered or non-naturally occurring. The CRISPR nuclease may also be recombinant. Such CRISPR nucleases are produced using laboratory methods (molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms.

Compositions

[0059] This invention provides a non-naturally occurring composition comprising a CRISPR nuclease comprising a sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding the CRISPR nuclease.

[0060] In an embodiment, the CRISPR nuclease further comprises an RNA-binding portion capable of interacting with a DNA-targeting RNA molecule (gRNA) and an activity portion that exhibits site-directed enzymatic activity.

[0061] In an embodiment, the composition further comprises a DNA-targeting RNA molecule or a DNA polynucleotide encoding a DNA-targeting RNA molecule, wherein the DNA-targeting RNA molecule comprises a nucleotide sequence that is complementary to a sequence in a target region, wherein the DNA-targeting RNA molecule and the CRISPR nuclease do not naturally occur together.

[0062] In an embodiment, the DNA-targeting RNA molecule further comprises a nucleotide sequence that can form a complex with a CRISPR nuclease.

[0063] In an embodiment, the composition further comprises an RNA molecule comprising a nucleotide sequence that can form a complex with a CRISPR nuclease (tracrRNA) or a DNA polynucleotide comprising a sequence encoding an RNA molecule that can form a complex with the CRISPR nuclease.

[0064] In an embodiment, the composition further comprises a donor template for homology directed repair (HDR).

[0065] In an embodiment, the composition is capable of editing the target region in the genome of a cell.

[0066] In an embodiment of the composition:

a. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:1 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO: 73;

b. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:2 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:105.

c. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:3 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO: 127.

d. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:10 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:229;

e. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:11 the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:238;

f. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:12 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:248; or

g. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:13 the nucleotide sequence that can form a

complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:258.

[0067] This invention also provides a non-naturally occurring composition comprising a CRISPR associated system comprising:

5 a) one or more RNA molecules comprising a guide sequence portion linked to a direct repeat sequence, wherein the guide sequence is capable of hybridizing with a target sequence, or one or more nucleotide sequences encoding the one or more RNA molecules; and

b) an CRISPR nuclease comprising an amino acid sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID 10 NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding the CRISPR nuclease; and

wherein the one or more RNA molecules hybridize to the target sequence, wherein the target sequence is 3' of a Protospacer Adjacent Motif (PAM), and the one or more RNA molecules form a complex with the RNA-guided nuclease.

15 [0068] In an embodiment, the composition further comprises an RNA molecule comprising a nucleotide molecule that can form a complex with the RNA nuclease (tracrRNA) or a DNA polynucleotide encoding an RNA molecule comprising a nucleotide sequence that can form a complex with the CRISPR nuclease.

[0069] In an embodiment, the composition further comprises a donor template for homology 20 directed repair (HDR).

[0070] This invention also provides a non-naturally occurring composition comprising:

a) a CRISPR nuclease comprising a sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding 25 the CRISPR nuclease; and

b) one or more RNA molecules, or one or more DNA polynucleotide encoding the one or more RNA molecules, comprising at least one of:

i) a nuclease-binding RNA nucleotide sequence capable of interacting with/binding to the CRISPR nuclease; and

ii) a DNA-targeting RNA nucleotide sequence comprising a sequence complementary to a sequence in a target DNA sequence,

5 wherein the CRISPR nuclease is capable of complexing with the one or more RNA molecules to form a complex capable of hybridizing with the target DNA sequence.

[0071] In an embodiment, the CRISPR nuclease and the one or more RNA molecules form a CRISPR complex that is capable of binding to the target DNA sequence to effect cleavage of the target DNA sequence.

10 [0072] In an embodiment, the CRISPR nuclease and at least one of the one or more RNA molecules do not naturally occur together.

[0073] In an embodiment:

a) the CRISPR nuclease comprises an RNA-binding portion and an activity portion that exhibits site-directed enzymatic activity;

15 b) the DNA-targeting RNA nucleotide sequence comprises a nucleotide sequence that is complementary to a sequence in a target DNA sequence; and

c) the nuclease-binding RNA nucleotide sequence comprises a sequence that interacts with the RNA-binding portion of the CRISPR nuclease.

[0074] In an embodiment, the nuclease-binding RNA nucleotide sequence and the DNA-20 targeting RNA nucleotide sequence are on a single guide RNA molecule (sgRNA), wherein the sgRNA molecule can form a complex with the CRISPR nuclease and serve as the DNA targeting module.

[0075] In an embodiment, the sgRNA has a length of up to 1000 bases, 900 bases, 800 bases, 700 bases, 600 bases, 500 bases, 400 bases, 300 bases, 200 bases, 100 bases, 50 bases.

25 [0076] In an embodiment, the nuclease-binding RNA nucleotide sequence is on a first RNA molecule and the DNA-targeting RNA nucleotide sequence is on a single guiide RNA molecule, and wherein the first and second RNA sequence interact by base-paring or are fused together to

form one or more RNA molecules or sgRNA that complex with the CRISPR nuclease and serve as the targeting module.

[0077] In an embodiment, the composition further comprises a donor template for homology directed repair (HDR).

5 [0078] In an embodiment, the CRISPR nuclease comprises 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130,130-140, or 140-150 amino acid substitutions, deletions, and/or insertions compared to the amino acid sequence of the wild-type of the CRISPR nuclease.

[0079] In an embodiment, the CRISPR nuclease exhibits at least 2%, 5%, 7% 10%, 15%, 20%, 10 25%, 30, or 35% increased specificity compared the wild-type of the CRISPR nuclease.

[0080] In an embodiment, the CRISPR nuclease exhibits at least 2%, 5%, 7% 10%, 15%, 20%, 25%, 30, or 35% increased activity compared the wild-type of the CRISPR nuclease.

[0081] In an embodiment, the CRISPR nuclease has altered PAM specificity compared to the wild-type of the CRISPR nuclease.

15 [0082] In an embodiment, the CRISPR nuclease is non-naturally occurring.

[0083] In an embodiment, the CRISPR nuclease is engineered and comprises unnatural or synthetic amino acids.

[0084] In an embodiment, the CRISPR nuclease is engineered and comprises one or more of a nuclear localization sequences (NLS), cell penetrating peptide sequences, and/or affinity tags.

20 [0085] In an embodiment, the CRISPR nuclease comprises one or more nuclear localization sequences of sufficient strength to drive accumulation of a CRISPR complex comprising the CRISPR nuclease in a detectable amount in the nucleus of a eukaryotic cell.

[0086] In an embodiment, the CRISPR nuclease comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the amino-terminus, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near carboxy-25 terminus, or a combination of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the amino-terminus and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near carboxy-terminus. In an embodiment 1-4 NLSs are fused with the CRISPR nuclease. In an embodiment, an NLS is located within the open-reading frame (ORF) of the CRISPR nuclease.

[0087] Methods of fusing NLSs at or near the amino-terminus, at or near carboxy-terminus, or within the ORF of an expressed protein are well known in the art. As an example, to fuse an NLS to the amino-terminus of the CRISPR nuclease, the nucleic acid sequence of the NLS is placed immediately after the start codon of the CRISPR nuclease on the nucleic acid encoding the NLS-fused CRISPR nuclease. Conversely, to fuse an NLS to the carboxy-terminus of the CRISPR nuclease the nucleic acid sequence of the NLS is placed after the codon encoding the last amino acid of the CRISPR nuclease and before the stop codon.

[0088] Any combination of NLSs, cell penetrating peptide sequences, and/or affinity tags at any position along the ORF of the CRISPR nuclease is contemplated in this invention.

[0089] The amino acid sequences and nucleic acid sequences of the CRISPR nucleases provided herein may include NLS and/or TAGs inserted so as to interrupt the contiguous amino acid or nucleic acid sequences of the CRISPR nucleases.

[0090] In an embodiment, the one or more NLSs are in tandem repeats.

[0091] In an embodiment, the one or more NLSs are considered in proximity to the N- or C-terminus when the nearest amino acid of the NLS is within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, or more amino acids along the polypeptide chain from the N- or C-terminus.

[0092] As we discussed the CRISPR nuclease may be engineered to comprise one or more of a nuclear localization sequences (NLS), cell penetrating peptide sequences, and/or affinity tags.

[0093] In an embodiment, the CRISPR nuclease exhibits increased specificity to a target site compared to the wild-type of the CRISPR nuclease when complexed with the one or more RNA molecules.

[0094] In an embodiment, the complex of the CRISPR nuclease and one or more RNA molecules exhibits at least maintained on-target editing activity of the target site and reduced off-target activity compared to the wild-type of the CRISPR nuclease.

[0095] In an embodiment, the composition further comprises a recombinant nucleic acid molecule comprising a heterologous promoter operably linked to the nucleotide acid molecule comprising the sequence encoding the CRISPR nuclease.

[0096] In an embodiment, the CRISPR nuclease or nucleic acid molecule comprising a sequence encoding the CRISPR nuclease is non-naturally occurring or engineered.

[0097] This invention also provides a non-naturally occurring or engineered composition comprising a vector system comprising the nucleic acid molecule comprising a sequence encoding any of the CRISPR nucleases of the invention.

[0098] This invention also provides a method of modifying a nucleotide sequence at a target site in a cell-free system or the genome of a cell comprising introducing into the cell any of the compositions of the invention.

[0099] In an embodiment, the cell is a eukaryotic cell.

[00100] This invention also provides use of any of the compositions of the invention for the treatment of a subject afflicted with a disease associated with a genomic mutation comprising modifying a nucleotide sequence at a target site in the genome of the subject.

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[00101] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:1 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO:29 or SEQ ID NO:23.

[00102] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:1 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO: 51.

[00103] In an embodiment of the composition, the composition comprises an RNA molecule comprising a nuclease-binding RNA nucleotide sequence wherein the nucleotide binding RNA sequence is selected from the group consisting of SEQ ID NOs:73-79 and is suitable to form an active complex with the CRISPR nuclease.

[00104] In an embodiment of the composition, the composition comprises an RNA molecule comprising a nuclease-binding RNA nucleotide sequence wherein the nucleotide binding RNA sequence is selected from the group consisting of SEQ ID NOs: 88-91 and is suitable to form an active complex with the CRISPR nuclease.

[00105] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant selected from the group consisting of AGGGNNNN, CGGGNNNN, TGGGNNNN, GGGTNNNN, NGGNNNNN, CGGTCGAA, TGGTCCGC, and AGGACCTC.

[00106] In an embodiment, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 80 suitable to form an active complex with the CRISPR nuclease.

[00107] In an embodiment, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:81 suitable to form an active complex with the CRISPR nuclease.

[00108] In an embodiment, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:82 suitable to form an active complex with the CRISPR nuclease.

[00109] In an embodiment, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:83 suitable to form an active complex with the CRISPR nuclease.

[00110] In an embodiment, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86 and is suitable to form an active complex with the CRISPR nuclease.

[00111] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 87-91 suitable to form an active complex with the CRISPR nuclease.

[00112] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:88.

[00113] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of AGGGNNNN, CGGGNNNN, TGGGNNNN, GGGTNNNN, NGGNNNNN, CGGTCGAA, TGGTCCGC, AGGACCTC, NGGNN, NGGNM, and NGG.

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[00114] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:2 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO: 30 or SEQ ID NO: 24.

[00115] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:2 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO: 52.

[00116] In an embodiment of the composition, the composition comprises an RNA molecule
5 comprising a nuclease-binding RNA nucleotide sequence wherein the nucleotide binding RNA sequence is selected from the group consisting of SEQ ID NOs:105-107 and is suitable to form an active complex with the CRISPR nuclease.

[00117] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant selected from the group consisting of NGCACNNN, NATACNNN, NGTACNNN,
10 CGTANNNN, NRTAHNNN, TGTACTAA, TATACGAA, TGCACTAA.

[00118] In an embodiment, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:108 suitable to form an active complex with the CRISPR nuclease.

[00119] In an embodiment, the composition further comprises a tracrRNA molecule comprising
15 the nucleotide sequence as set forth in SEQ ID NO:109 suitable to form an active complex with the CRISPR nuclease.

[00120] In an embodiment, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:110 suitable to form an active complex with the CRISPR nuclease.

20 [00121] In an embodiment, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 111 suitable to form an active complex with the CRISPR nuclease.

[00122] In an embodiment, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO: 112, SEQ
25 ID NO:113, and SEQ ID NO: 114 and is suitable to form an active complex with the CRISPR nuclease.

[00123] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:115-116 suitable to form an active complex with the CRISPR nuclease.

[00124] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 115.

[00125] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NGCACNNN, NATACNNN, NGTACNNN, CGTANNNN, NRTAHNNN, TGTACTAA, TATACGAA, TGCACTAA, NVYAH, and YGTAM.

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[00126] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:3 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO:31 or SEQ ID NO:25.

[00127] In an embodiment of the composition, wherein the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:3 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO:53.

[00128] In an embodiment of the composition, the composition comprises an RNA molecule comprising a nuclease-binding RNA nucleotide sequence wherein the nucleotide binding RNA sequence is selected from the group consisting of SEQ ID NOs:127-128 and is suitable to form an active complex with the CRISPR nuclease.

[00129] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant selected from the group consisting of NNAAACNN, NCAAANNN, CGGANNNN, NNGAAGNN, NRRARNNN, TGGAAGCT, and AAAAAGCT.

[00130] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:129 suitable to form an active complex with the CRISPR nuclease.

[00131] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:130 suitable to form an active complex with the CRISPR nuclease.

[00132] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:131 suitable to form an active complex with the CRISPR nuclease.

[00133] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:132 suitable to form an active complex with the CRISPR nuclease.

[00134] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 133-134 and is suitable to form an active complex with the CRISPR nuclease.

[00135] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:135-137 suitable to form an active complex with the CRISPR nuclease.

[00136] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:136.

[00137] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NNAAACNN, NCAAANNN, CGGANNNN, NNGAAGNN, NRRARNNN, TGGAAGCT, AAAAAGCT, NVVRR, NRRRR, NVVRV, NRRAV, CGGGAGAG, TAAGGTCC, TGGTCCGC and GGATGAT.

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[00138] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:4 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 26, and SEQ ID NO: 54.

[00139] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:143 suitable to form an active complex with the CRISPR nuclease.

[00140] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:144 suitable to form an active complex with the CRISPR nuclease.

[00141] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 145 suitable to form an active complex with the CRISPR nuclease.

[00142] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:146 suitable to form an active complex with the CRISPR nuclease.

[00143] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:147, GGCUUCGCC, and SEQ ID NO: 148 and is suitable to form an active complex with the CRISPR nuclease.

[00144] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:149-150 suitable to form an active complex with the CRISPR nuclease.

[00145] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:150.

[00146] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of TGCACTAA, AGGACCTC, NVNVMY, NRNACY, NVDNMY, NRKACY.

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[00147] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:5 or the nucleic acid molecule comprising a sequence encoding the CRISPR nuclease has at least a 95% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO: 33 and SEQ ID NO:55.

[00148] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:157 suitable to form an active complex with the CRISPR nuclease.

[00149] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:158 suitable to form an active complex with the CRISPR nuclease.

[00150] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:159 suitable to form an active complex with the CRISPR nuclease.

[00151] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 160 suitable to form an active complex with the CRISPR nuclease.

[00152] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO: 161, AUUAUUAU, and SEQ ID NO:162 and is suitable to form an active complex with the CRISPR nuclease.

[00153] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:163-164 suitable to form an active complex with the CRISPR nuclease.

[00154] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:164.

[00155] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NNYVVH, NNYAAH, GGTAATAG, and GGCAAAAG.

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[00156] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:6 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO: 34 and SEQ ID NO: 56.

[00157] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:170 suitable to form an active complex with the CRISPR nuclease.

[00158] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:171 suitable to form an active complex with the CRISPR nuclease.

[00159] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:172 suitable to form an active complex with the CRISPR nuclease.

[00160] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:173 suitable to form an active complex with the CRISPR nuclease.

[00161] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:174, AGCUUAUGC, and SEQ ID NO:175 and is suitable to form an active complex with the CRISPR nuclease.

[00162] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 176-179 suitable to form an active complex with the CRISPR nuclease.

[00163] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:177.

[00164] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NGGNN, NGGNM, TGGAAGCT, TGGTCCGC, TGGTTGAT, and CGGTCGAA .

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[00165] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO: 7 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:27, and SEQ ID NO:57.

[00166] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:186 suitable to form an active complex with the CRISPR nuclease.

[00167] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 187 suitable to form an active complex with the CRISPR nuclease.

[00168] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 188 suitable to form an active complex with the CRISPR nuclease.

[00169] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 189 suitable to form an active complex with the CRISPR nuclease.

[00170] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:190, SEQ ID NO: 191, and SEQ ID NO: 192 and is suitable to form an active complex with the CRISPR nuclease.

[00171] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 193-194 suitable to form an active complex with the CRISPR nuclease.

[00172] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:194.

[00173] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NRTAN, TGCACTAA, TATACGAA.

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[00174] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:8 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO: 36 and SEQ ID NO:58.

[00175] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:199 suitable to form an active complex with the CRISPR nuclease.

[00176] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:200 suitable to form an active complex with the CRISPR nuclease.

[00177] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:201 suitable to form an active complex with the CRISPR nuclease.

[00178] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:202 suitable to form an active complex with the CRISPR nuclease.

[00179] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 203-205 and is suitable to form an active complex with the CRISPR nuclease.

[00180] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 206-207 suitable to form an active complex with the CRISPR nuclease.

[00181] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:207.

[00182] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NGGNR, NGGNG, AGGACCTC, TGGCGTTG.

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[00183] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:9 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO: 37 and SEQ ID NO:59.

[00184] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 214 suitable to form an active complex with the CRISPR nuclease.

[00185] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 215 suitable to form an active complex with the CRISPR nuclease.

[00186] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 216 suitable to form an active complex with the CRISPR nuclease.

[00187] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 217 suitable to form an active complex with the CRISPR nuclease.

[00188] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 218, 219, and 220 and is suitable to form an active complex with the CRISPR nuclease.

[00189] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 221-222 suitable to form an active complex with the CRISPR nuclease.

[00190] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:222 .

[00191] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NVRNH, NRRNC, NVRNC, AGGACCTC, TGGCGTTG, and TAGGCTCT.

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[00192] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:10 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:38 and SEQ ID NO:60.

[00193] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:229 suitable to form an active complex with the CRISPR nuclease.

[00194] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:230 suitable to form an active complex with the CRISPR nuclease.

[00195] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:231 suitable to form an active complex with the CRISPR nuclease.

[00196] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:232 suitable to form an active complex with the CRISPR nuclease.

[00197] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:233 suitable to form an active complex with the CRISPR nuclease.

[00198] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:234, AGUUUACU, and SEQ ID NO: 235, and is suitable to form an active complex with the CRISPR nuclease.

[00199] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:236-237 suitable to form an active complex with the CRISPR nuclease.

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[00200] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:11 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:39, SEQ ID NO:28, and SEQ ID NO:61.

[00201] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:238 suitable to form an active complex with the CRISPR nuclease.

[00202] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:239 suitable to form an active complex with the CRISPR nuclease.

[00203] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:240 suitable to form an active complex with the CRISPR nuclease.

[00204] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:241 suitable to form an active complex with the CRISPR nuclease.

[00205] In an embodiment of the composition, the composition further comprises a tracrRNA 5 molecule comprising the nucleotide sequence as set forth in SEQ ID NO:242 suitable to form an active complex with the CRISPR nuclease.

[00206] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:243, 244, and 245 and is suitable to form an active complex with the CRISPR nuclease.

10 [00207] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:246-247 suitable to form an active complex with the CRISPR nuclease.

[00208] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence SEQ ID NO:247 suitable to form an active complex with the CRISPR 15 nuclease.

[00209] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NGGNN, NGGYK, NGGN, NGGY, and TGGTTGAT.

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20 [00210] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:12 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:40 and SEQ ID NO:62.

[00211] In an embodiment of the composition, the composition further comprises a tracrRNA 25 molecule comprising the nucleotide sequence as set forth in SEQ ID NO:248 suitable to form an active complex with the CRISPR nuclease.

[00212] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:249 suitable to form an active complex with the CRISPR nuclease.

[00213] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:250 suitable to form an active complex with the CRISPR nuclease.

[00214] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:251 suitable to form an active complex with the CRISPR nuclease.

[00215] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:252 suitable to form an active complex with the CRISPR nuclease.

[00216] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:253, 254, and 255 and is suitable to form an active complex with the CRISPR nuclease.

[00217] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 256-257 suitable to form an active complex with the CRISPR nuclease.

[00218] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence SEQ ID NO:257 suitable to form an active complex with the CRISPR nuclease.

[00219] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NVVHHY, NRRTTT, CAGTTTAA, and CAATTTAA.

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[00220] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:13 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:41 and SEQ ID NO:63.

[00221] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:258 suitable to form an active complex with the CRISPR nuclease.

[00222] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:259 suitable to form an active complex with the CRISPR nuclease.

[00223] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:260 suitable to form an active complex with the CRISPR nuclease.

[00224] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:261 suitable to form an active complex with the CRISPR nuclease.

[00225] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:262 suitable to form an active complex with the CRISPR nuclease.

[00226] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:263, 264, and 265 and is suitable to form an active complex with the CRISPR nuclease.

[00227] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:266-267 suitable to form an active complex with the CRISPR nuclease.

[00228] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence SEQ ID NO:267 suitable to form an active complex with the CRISPR nuclease.

[00229] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NNGWWB, NHGWWY, GTGTACTC, and GTGTTCTC.

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[00230] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:14 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:64.

[00231] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:268 suitable to form an active complex with the CRISPR nuclease.

[00232] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:269 suitable to form an active complex with the CRISPR nuclease.

[00233] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:270 suitable to form an active complex with the CRISPR nuclease.

[00234] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:271 suitable to form an active complex with the CRISPR nuclease.

[00235] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 272, 273 and 274 and is suitable to form an active complex with the CRISPR nuclease.

[00236] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 275-276 suitable to form an active complex with the CRISPR nuclease.

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[00237] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:15 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:43 and SEQ ID NO:65.

[00238] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:277 suitable to form an active complex with the CRISPR nuclease.

[00239] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:278 suitable to form an active complex with the CRISPR nuclease.

[00240] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:279 suitable to form an active complex with the CRISPR nuclease.

[00241] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:280 suitable to form an active complex with the CRISPR nuclease.

[00242] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:281, 282, and 283 and is suitable to form an active complex with the CRISPR nuclease.

[00243] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:284-285 suitable to form an active complex with the CRISPR nuclease.

[00244] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence SEQ ID NO:285 suitable to form an active complex with the CRISPR nuclease.

[00245] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NRAVR and NRHAAC.

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[00246] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:16 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:44 and SEQ ID NO:66.

[00247] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:286 to form an active complex with the CRISPR nuclease.

[00248] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:287 suitable to form an active complex with the CRISPR nuclease.

[00249] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:288 suitable to form an active complex with the CRISPR nuclease.

[00250] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:289 suitable to form an active complex with the CRISPR nuclease.

[00251] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 290, 291, and 292 and is suitable to form an active complex with the CRISPR nuclease.

[00252] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 293-294 suitable to form an active complex with the CRISPR nuclease.

[00253] In an embodiment of the composition, the single guide RNA molecule comprises the nucleotide sequence SEQ ID NO:293 suitable to form an active complex with the CRISPR nuclease.

[00254] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NRAVR and NAARG.

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[00255] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:17 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:45 and SEQ ID NO:67.

[00256] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:295 suitable to form an active complex with the CRISPR nuclease.

[00257] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:296 to form an active complex with the CRISPR nuclease.

[00258] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:297 suitable to form an active complex with the CRISPR nuclease.

[00259] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:298 suitable to form an active complex with the CRISPR nuclease.

[00260] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence SEQ ID NO:299 and is suitable to form an active complex with the CRISPR nuclease.

[00261] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:300-301 suitable to form an active complex with the CRISPR nuclease.

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[00262] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:18 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:46 and SEQ ID NO:68.

[00263] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:302 suitable to form an active complex with the CRISPR nuclease.

[00264] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:303 suitable to form an active complex with the CRISPR nuclease.

[00265] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:304 suitable to form an active complex with the CRISPR nuclease.

[00266] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:305 suitable to form an active complex with the CRISPR nuclease.

[00267] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO: 306, GCUUAAAGC, and SEQ ID NO:307, and is suitable to form an active complex with the CRISPR nuclease.

5 [00268] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:308-309 suitable to form an active complex with the CRISPR nuclease.

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[00269] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to 10 the amino acid sequence as set forth in SEQ ID NO:19 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:69.

[00270] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:310 suitable to form an 15 active complex with the CRISPR nuclease.

[00271] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:311 suitable to form an active complex with the CRISPR nuclease.

[00272] In an embodiment of the composition, the composition further comprises a crRNA 20 molecule comprising the nucleotide sequence as set forth in SEQ ID NO:312 suitable to form an active complex with the CRISPR nuclease.

[00273] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:313 suitable to form an active complex with the CRISPR nuclease.

25 [00274] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 314, 315 and 316 and is suitable to form an active complex with the CRISPR nuclease.

[00275] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:317-318 suitable to form an active complex with the CRISPR nuclease.

[00276] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NNDVYY and NNDAYT.

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[00277] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:20 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:70 and SEQ ID NO: 319.

[00278] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:320 suitable to form an active complex with the CRISPR nuclease.

[00279] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:321 suitable to form an active complex with the CRISPR nuclease.

[00280] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:322 suitable to form an active complex with the CRISPR nuclease.

[00281] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:323 suitable to form an active complex with the CRISPR nuclease.

[00282] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:324, 325, and 326 and is suitable to form an active complex with the CRISPR nuclease.

[00283] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 327-328 suitable to form an active complex with the CRISPR nuclease.

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[00284] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:21 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:49 and SEQ ID NO:71.

[00285] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:329 suitable to form an active complex with the CRISPR nuclease.

[00286] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:330 suitable to form an active complex with the CRISPR nuclease.

[00287] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:331 suitable to form an active complex with the CRISPR nuclease.

[00288] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:332 suitable to form an active complex with the CRISPR nuclease.

[00289] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:333, 334, and 335 and is suitable to form an active complex with the CRISPR nuclease.

[00290] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:336-337 suitable to form an active complex with the CRISPR nuclease.

[00291] In an embodiment of the composition, the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NNNVYT and NNNACT.

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[00292] In an embodiment of the composition, the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:22 or the sequence encoding the CRISPR

nuclease has at least a 95% sequence identity to nucleotide sequence selected from the group consisting of SEQ ID NO:50 and SEQ ID NO:72.

[00293] In an embodiment of the composition, the composition further comprises a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 338 suitable to form an
5 active complex with the CRISPR nuclease.

[00294] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 339 suitable to form an active complex with the CRISPR nuclease.

[00295] In an embodiment of the composition, the composition further comprises a crRNA
10 molecule comprising the nucleotide sequence as set forth in SEQ ID NO:340 suitable to form an active complex with the CRISPR nuclease.

[00296] In an embodiment of the composition, the composition further comprises a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:341 suitable to form an active complex with the CRISPR nuclease.

15 [00297] In an embodiment of the composition, the composition further comprises a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 342, 343, and 344 and is suitable to form an active complex with the CRISPR nuclease.

[00298] In an embodiment of the composition, the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:345-346 suitable to form
20 an active complex with the CRISPR nuclease.

Methods

[00299] This invention provides a method of modifying a nucleotide sequence at a target site in the genome of a mammalian cell comprising introducing into the cell (i) a composition comprising a CRISPR nuclease having at least 95% identity to an amino acid sequence selected from the group
25 consisting of SEQ ID NOs: 1-9 or a nucleic acid molecule comprising a sequence encoding a CRISPR nuclease which sequence has at least 95% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs:23-27 and (ii) a DNA-targeting RNA molecule, or a DNA polynucleotide encoding a DNA-targeting RNA molecule, comprising a nucleotide sequence that is complementary to a sequence in the target DNA.

[00300] In an embodiment, the method further comprises introducing into the cell: (iii) an RNA molecule comprising a nuclease-binding RNA sequence or a DNA polynucleotide encoding an RNA molecule comprising a nuclease-binding RNA that interacts with the CRISPR nuclease.

[00301] In an embodiment, the DNA targeting RNA molecule is a crRNA molecule suitable to form an active complex with the CRISPR nuclease.

[00302] In an embodiment, the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule suitable to form an active complex with the CRISPR nuclease.

[00303] In an embodiment, the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule.

[00304] In an embodiment, the method further comprises introducing into the cell: (iv) an RNA molecule comprising a sequence complementary to a protospacer sequence.

[00305] In an embodiment, the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a Protospacer Adjacent Motif (PAM).

[00306] In an embodiment of any of the methods described herein, the method is for treating a subject afflicted with a disease associated with a genomic mutation comprising modifying a nucleotide sequence at a target site in the genome of the subject.

[00307] In an embodiment, the method comprises first selecting a subject afflicted with a disease associated with a genomic mutation, and obtaining the cell from the subject.

[00308] This invention also provides a modified cell or cells obtained by any of the methods described herein. In an embodiment these modified cell or cells are capable of giving rise to progeny cells. In an embodiment these modified cell or cells are capable of giving rise to progeny cells after engraftment.

[00309] This invention also provides a composition comprising these modified cells and a pharmaceutically acceptable carrier. Also provided is an *in vitro* or *ex vivo* method of preparing this, comprising mixing the cells with the pharmaceutically acceptable carrier.

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[00310] In an embodiment, the CRISPR nuclease has at least 95% identity to an amino acid sequence as set forth in SEQ ID NO: 1 or the sequence encoding the CRISPR nuclease has at least 95% identity to the nucleic acid sequence as set forth in SEQ ID NO: 23.

5 [00311] In an embodiment of the method:

a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 80 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:81
10 suitable to form an active complex with the CRISPR nuclease;

b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:82 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ
15 ID NO:83 suitable to form an active complex with the CRISPR nuclease; or

c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:84, SEQ ID NO:85, or SEQ ID NO:86 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide
20 RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 87-91 suitable to form an active complex with the CRISPR nuclease.

[00312] In an embodiment of the above method (c), the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:88; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group
25 consisting of AGGGNNNN, CGGGNNNN, TGGGNNNN, GGGTNNNN, NGGNNNNN, CGGTCGAA, TGGTCCGC, and AGGACCTC, to modify the nucleotide sequence at the target site in the cell.

[00313] In an embodiment of the method:

a) (i) the target site in the genome is ELANE g58; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence AGG or AGGACCCA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:92;

b) (i) the target site in the genome is ELANE g35; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GGGGAGCA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:93;

c) (i) the target site in the genome is ELANE g39; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GGGGACGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:94;

d) (i) the target site in the genome is ELANE g62; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GGGACAGA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 95;

e) (i) the target site in the genome is CXCR4 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CGGAGGAG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 96;

f) (i) the target site in the genome is CXCR4 s2; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence AGGATGGC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 97;

g) (i) the target site in the genome is PD1 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence TGGCCAGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 98; or

h) (i) the target site in the genome is PD1 s2; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence TGGGCGGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 99.

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[00314] In an embodiment of the method, the CRISPR nuclease has at least 95% identity to an amino acid sequence as set forth in SEQ ID NO:2 or the sequence encoding the CRISPR nuclease has at least 95% identity to the nucleic acid sequence as set forth in SEQ ID NO: 24.

[00315] In an embodiment of the method:

10 a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:108 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:109 suitable to form an active complex with the CRISPR nuclease;

15 b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:110 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 111 suitable to form an active complex with the CRISPR nuclease; or

20 c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 112, SEQ ID NO:113, or SEQ ID NO: 114 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group 25 consisting of SEQ ID NOs:115-116 suitable to form an active complex with the CRISPR nuclease.

[00316] In an embodiment of the above method (c) the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO: 115; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting

of NGCACNNN, NATACNNN, NGTACNNN, CGTANNNN, NRTAHNNN, TGTACTAA, TATACGAA, TGCACTAA to modify the nucleotide sequence at the target site in the cell.

[00317] In an embodiment of the method:

a) (i) the target site in the genome is 9q31.2 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CATACTTG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 117;

b) (i) the target site in the genome is 9q31.2 s3; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CCTACAAA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 118;

c) (i) the target site in the genome is HBB; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GATACCAA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 119;

d) (i) the target site in the genome is 20q11.1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence ACTACAGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:120;

e) (i) the target site in the genome is FANCF s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence ACTACCTA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:121; or

f) (i) the target site in the genome is VISTA Enhancer hs267 s2; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence TTTACAGG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:122.

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[00318] In an embodiment of the method, the CRISPR nuclease has least 95% identity to an amino acid sequence as set forth in SEQ ID NO:3 or the nucleic acid molecule comprising a sequence encoding the CRISPR nuclease has least 95% identity to the nucleic acid sequence as set forth in SEQ ID NO:25.

[00319] In an embodiment of the method:

a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:129 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:130 suitable to form an active complex with the CRISPR nuclease;

b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:131 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:132 suitable to form an active complex with the CRISPR nuclease; or

c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NOs: 133 or 134and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:135-137 suitable to form an active complex with the CRISPR nuclease.

[00320] In an embodiment of the above method (c) the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:136; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting of NNAAACNN, NCAAANNN, CGGANNNN, NNGAAGNN, NRRARNNN, TGGAAGCT, AAAAAGCT, NVVRR, NRRRR, NVVRV, NRRAV, CGGGAGAG, TAAGGTCC, TGGTCCGC and GGATGAT to modify the nucleotide sequence at the target site in the cell.

[00321] In an embodiment of the method:

a) (i) the target site in the genome is CXCR s9; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in AAGAGACC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 140;

b) (i) the target site in the genome is CXCR s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in CGGAGGAG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:141; or

c) (i) the target site in the genome is ELANE g62; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in GGGACAGA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:142.

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[00322] In an embodiment of the method, the CRISPR nuclease has least 95% identity to an amino acid sequence as set forth in SEQ ID NO:4 or the nucleic acid molecule comprising a sequence encoding the CRISPR nuclease has least 95% identity to the nucleic acid sequence as set forth in SEQ ID NO: 26.

[00323] In an embodiment of the method:

a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth SEQ ID NO:143 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:144 suitable to form an active complex with the CRISPR nuclease;

b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 145 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:146 suitable to form an active complex with the CRISPR nuclease; or

c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:147, GGCUUCGCC, or SEQ ID NO: 148 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:149-150 suitable to form an active complex with the CRISPR nuclease.

[00324] In an embodiment of the above method (c) the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:150; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting of TGCACTAA, AGGACCTC, NVNVMY, NRNACY, NVDNMY, NRKACY to modify the nucleotide sequence at the target site in the cell.

[00325] In an embodiment of the method:

a) (i) the target site in the genome is CXCR4 s3; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in GACACTCC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:151;

b) (i) the target site in the genome is CXCR4 s4; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in CCCACTAC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:152;

c) (i) the target site in the genome is PD1 s3; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in ATGACTAG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:155; or

d) (i) the target site in the genome is PD1 s4; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in GCCACTCC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 156.

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[00326] In an embodiment of the method the CRISPR nuclease has least 95% identity to an amino acid sequence as set forth in SEQ ID NO:5.

[00327] In an embodiment of the method:

5 a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth SEQ ID NO:157 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:158 suitable to form an active complex with the CRISPR nuclease;

10 b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:159 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 160 suitable to form an active complex with the CRISPR nuclease; or

15 c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 161, AUUAUUAU, or SEQ ID NO:162 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group
20 consisting of SEQ ID NOs:163-164 suitable to form an active complex with the CRISPR nuclease.

[00328] In an embodiment of the above method (c) the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:164; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting
25 of NNYVVH, NNYAAH, GGTAATAG, and GGCAAAAG to modify the nucleotide sequence at the target site in the cell.

[00329] In an embodiment of the method:

a) (i) the target site in the genome is CXCR4 s6; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3’ of a PAM

site comprising the nucleotide sequence CCCAATAT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 167;

b) (i) the target site in the genome is PD1 s5; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GACAATGG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:168; or

c) (i) the target site in the genome is PD1 s6; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CGCAATGA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:169.

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[00330] In an embodiment of the method the CRISPR nuclease has least 95% identity to an amino acid sequence as set forth in SEQ ID NO:6.

[00331] In an embodiment of the method:

a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:170 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:171 suitable to form an active complex with the CRISPR nuclease;

b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:172 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:173 suitable to form an active complex with the CRISPR nuclease; or

c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:174, AGCUUAUGC, or SEQ ID NO:175 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group

consisting of SEQ ID NOs: 176-179 suitable to form an active complex with the CRISPR nuclease.

[00332] In an embodiment of the above method (c) the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:177; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting of NGGNN, NGGNM, TGGAAGCT, TGGTCCGC, TGGTTGAT, and CGGTCGAA to modify the nucleotide sequence at the target site in the cell.

[00333] In an embodiment of the method:

a) (i) the target site in the genome is CXCR4 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CGGAGGAG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:182;

b) (i) the target site in the genome is CXCR4 s2; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence AGGATGGC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:183;

c) (i) the target site in the genome is PD1 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence TGGCCAGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:184; or

d) (i) the target site in the genome is PD1 s2; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence TGGGCGGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:185.

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[00334] In an embodiment of the method the CRISPR nuclease has least 95% identity to an amino acid sequence as set forth in SEQ ID NO: 7 or the nucleic acid molecule comprising a sequence encoding the CRISPR nuclease has least 95% identity to the nucleic acid sequence as set forth in SEQ ID NO:27.

[00335] In an embodiment of the method:

a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:186 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 187 suitable to form an active complex with the CRISPR nuclease;

b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 188 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 189 suitable to form an active complex with the CRISPR nuclease; or

c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NOs:190, 191, or 192 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 193-194 suitable to form an active complex with the CRISPR nuclease.

[00336] In an embodiment of the above method (c) the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:194; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting of NRTAN, TGCACTAA, TATACGAA to modify the nucleotide sequence at the target site in the cell.

[00337] In an embodiment of the method:

a) (i) the target site in the genome is CXCR s7; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in AATATACC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:197; or

b) (i) the target site in the genome is CXCR s8; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM

site comprising the nucleotide sequence as set forth in GATAAACA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:198.

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[00338] In an embodiment of the method the CRISPR nuclease has least 95% identity to an amino acid sequence as set forth in SEQ ID NO:8.

[00339] In an embodiment of the method:

a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:199 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:200 suitable to form an active complex with the CRISPR nuclease;

b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:201 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:202 suitable to form an active complex with the CRISPR nuclease; or

c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NOs: 203, 204, or 205 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 206-207 suitable to form an active complex with the CRISPR nuclease.

[00340] In an embodiment of the above method (c) the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:207; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting of NGGNR, NGGNG, AGGACCTC, TGGCGTTG to modify the nucleotide sequence at the target site in the cell.

[00341] In an embodiment of the method:

a) (i) the target site in the genome is CXCR4 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CGGAGGAG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:210;

5 b) (i) the target site in the genome is CXCR4 s10; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence AGGAGCGC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:211;

c) (i) the target site in the genome is PD1 s11; (ii) the CRISPR nuclease forms a complex with
10 the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GGGCGGTG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:212; or

d) (i) the target site in the genome is PD1 s12; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site
15 comprising the nucleotide sequence as set forth in CGGTGCTA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:213.

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[00342] In an embodiment of the method the CRISPR nuclease has least 95% identity to an amino acid sequence as set forth in SEQ ID NO:9.

20 [00343] In an embodiment of the method:

a) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 214 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:
25 215 suitable to form an active complex with the CRISPR nuclease;

b) the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 216 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA

sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 217 suitable to form an active complex with the CRISPR nuclease; or

c) the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NOs: 218, 219, or 220 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 221-222 suitable to form an active complex with the CRISPR nuclease.

[00344] In an embodiment of the above method (c) the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:222 optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting of NVRNH, NRRNC, NVRNC, AGGACCTC, TGGCGTTG, and TAGGCTCT to modify the nucleotide sequence at the target site in the cell.

[00345] In an embodiment of the method:

a) (i) the target site in the genome is CXCR4 s12; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in CAAACGCG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 225;

b) (i) the target site in the genome is CXCR4 s13; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in TAAACACG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 226;

c) (i) the target site in the genome is PD1 s13; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence as set forth in CAGTCGTC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 227; or

d) (i) the target site in the genome is PD1 s14; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site

comprising the nucleotide sequence as set forth in CAGACGAC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 228.

Diseases and therapies

[00346] Certain embodiments of the invention target specific genetic loci associated with disease or disease therapies.

[00347] Mutations in the ELANE gene are associated with neutropenia. Accordingly, without limitation, embodiments of the invention that target ELANE may be used in methods of treating subjects afflicted with neutropenia.

[00348] CXCR4 is a co-receptor for the human immunodeficiency virus type 1 (HIV-1) infection. Accordingly, without limitation, embodiments of the invention that target CXCR4 may be used in methods of treating subjects afflicted with HIV-1 or conferring resistance to HIV-1 infection in a subject.

[00349] Programmed cell death protein 1 (PD-1) disruption enhances CAR T cell mediated killing of tumor cells and PD-1 may be a target in other cancer therapies. Accordingly, without limitation, embodiments of the invention that target PD-1 may be used in methods of treating subjects afflicted with cancer. In an embodiment, the treatment is CAR T cell therapy with T cells that have been modified according to the invention to be PD-1 deficient.

[00350] 20q11.1 and 9q31 are genetic loci associated with cancer. Warren, H., et al. (2012). 9q31. 2-rs865686 as a susceptibility locus for estrogen receptor-positive breast cancer: evidence from the Breast Cancer Association Consortium. *Cancer Epidemiology and Prevention Biomarkers*, *21*(10), 1783-1791. Accordingly, without limitation, embodiments of the invention that target 9q31 or 20q11.1 may be used in methods of treating subjects afflicted with cancer.

[00351] HBB is a globin protein. Mutations in the gene produce several variants of the proteins which are implicated with genetic disorders such as sickle-cell disease and beta thalassemia, as well as beneficial traits such as genetic resistance to malaria. Accordingly, without limitation, embodiments of the invention that target HBB may be used in methods of treating subjects afflicted with sickle-cell disease and beta thalassemia or to confer genetic resistance to malaria.

[00352] Embodiments of the invention may also be used for studying any of the above diseases or any disease associated with the genetic locus that is targeted.

Definitions

[00353] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

[00354] In the discussion unless otherwise stated, adjectives such as “substantially” and “about” modifying a condition or relationship characteristic of a feature or features of an embodiment of the invention, are understood to mean that the condition or characteristic is defined to within tolerances that are acceptable for operation of the embodiment for an application for which it is intended. Unless otherwise indicated, the word “or” in the specification and claims is considered to be the inclusive “or” rather than the exclusive or, and indicates at least one of and any combination of items it conjoins.

[00355] It should be understood that the terms “a” and “an” as used above and elsewhere herein refer to “one or more” of the enumerated components. It will be clear to one of ordinary skill in the art that the use of the singular includes the plural unless specifically stated otherwise. Therefore, the terms “a,” “an” and “at least one” are used interchangeably in this application.

[00356] For purposes of better understanding the present teachings and in no way limiting the scope of the teachings, unless otherwise indicated, all numbers expressing quantities, percentages or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[00357] In the description and claims of the present application, each of the verbs, “comprise,” “include” and “have” and conjugates thereof, are used to indicate that the object or objects of the verb are not necessarily a complete listing of components, elements or parts of the subject or

subjects of the verb. Other terms as used herein are meant to be defined by their well-known meanings in the art.

[00358] The terms "polynucleotide", "nucleotide", "nucleotide sequence", "nucleic acid" and "Oligonucleotide" are used interchangeably. They refer to a polymeric form of nucleotides of any
5 length, either deoxyribonueleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, in Irons, messenger RNA (mRNA), transfer RNA, ribosomal RNA, short interfering RNA (siRNA), short-hairpin RNA (shR
10 A), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers, A polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence
15 of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

[00359] The term "nucleotide analog" or "modified nucleotide" refers to a nucleotide that contains one or more chemical modifications (e.g., substitutions), in or on the nitrogenous base of the nucleoside (e.g., cytosine (C), thymine (T) or uracil (U), adenine (A) or guanine (G)), in or on the
20 sugar moiety of the nucleoside (e.g., ribose, deoxyribose, modified ribose, modified deoxyribose, six-membered sugar analog, or open-chain sugar analog), or the phosphate. Each of the RNA sequences described herein may comprise one or more nucleotide analogs.

[00360] As used herein, the following nucleotide identifiers are used to represent a referenced nucleotide base(s):

Nucleotide reference	**Base(s) represented**			
A	A			
C		C		
G			G	
T				T
W	A			T

S		C	G	
M	A	C		
K			G	T
R	A		G	
Y		C		T
B		C	G	T
D	A		G	T
H	A	C		T
V	A	C	G	
N	A	C	G	T

[00361] As used herein, the term "targeting sequence" or "targeting molecule" refers a nucleotide sequence or molecule comprising a nucleotide sequence that is capable of hybridizing to a specific target sequence, e.g., the -targeting sequence has a nucleotide sequence which is at least partially complementary to the sequence being targeted along the length of the targeting sequence . The targeting sequence or -targeting molecule may be part of an RNA molecule that can form a complex with a CRISPR nuclease with the targeting sequence serving as the targeting portion of the CRISPR complex. When the molecule having the targeting sequence is present contemporaneously with the CRISPR molecule the RNA molecule is capable of targeting the CRISPR nuclease to the specific target sequence. Each possibility represents a separate embodiment. An RNA molecule can be custom designed to target any desired sequence.

[00362] The term "targets" as used herein, refers to a targeting sequence or targeting molecule's preferential hybridization to a nucleic acid having a targeted nucleotide sequence. It is understood that the term "targets" encompasses variable hybridization efficiencies, such that there is preferential targeting of the nucleic acid having the targeted nucleotide sequence, but unintentional off-target hybridization in addition to on-target hybridization might also occur. It is understood that where an RNA molecule targets a sequence, a complex of the RNA molecule and a CRISPR nuclease molecule targets the sequence for nuclease activity.

[00363] In the context targeting a DNA sequence that is present in a plurality of cells, it is understood that the targeting encompasses hybridization of the guide sequence portion of the RNA molecule with the sequence in one or more of the cells, and also encompasses hybridization of the

RNA molecule with the target sequence in fewer than all of the cells in the plurality of cells. Accordingly, it is understood that where an RNA molecule targets a sequence in a plurality of cells, a complex of the RNA molecule and a CRISPR nuclease is understood to hybridize with the target sequence in one or more of the cells, and also may hybridize with the target sequence in
5 fewer than all of the cells. Accordingly, it is understood that the complex of the RNA molecule and the CRISPR nuclease introduces a double strand break in relation to hybridization with the target sequence in one or more cells and may also introduce a double strand break in relation to hybridization with the target sequence in fewer than all of the cells. As used herein, the term "modified cells" refers to cells in which a double strand break is effected by a complex of an RNA
10 molecule and the CRISPR nuclease as a result of hybridization with the target sequence, i.e. on-target hybridization.

[00364] As used herein the term "wild type" is a term of the art understood by skilled persons and means the typical form of an organism, strain, gene or characteristic as it occurs in nature as distinguished from mutant or variant forms. Accordingly, as used herein, where a sequence of
15 amino acids or nucleotides refers to a wild type sequence, a variant refers to variant of that sequence, e.g., comprising substitutions, deletions, insertions. In embodiments of the present invention, an engineered CRISPR nuclease is a variant CRISPR nuclease comprising at least one amino acid modification (e.g., substitution, deletion and/or insertion) compared to the CRISPR nuclease of any of SEQ ID NOs:1-22.

20 [00365] The terms "non-naturally occurring" or "engineered" are used interchangeably and indicate human manipulation. The terms, when referring to nucleic acid molecules or polypeptides may mean that the nucleic acid molecule or the polypeptide is at least substantially free from at least one other component with which they are naturally associated in nature and as found in nature.

25 [00366] As used herein the term "amino acid" includes natural and/or unnatural or synthetic amino acids, including glycine and both the D or I, optical isomers, and amino acid analogs and peptidomimetics.

[00367] As used herein, "genomic DNA" refers to linear and/or chromosomal DNA and/or to plasmid or other extrachromosomal DNA sequences present in the cell or cells of interest. In some
30 embodiments, the cell of interest is a eukaryotic cell. In some embodiments, the cell of interest is

a prokaryotic cell. In some embodiments, the methods produce double-stranded breaks (DSBs) at pre-determined target sites in a genomic DNA sequence, resulting in mutation, insertion, and/or deletion of DNA sequences at the target site(s) in a genome.

[00368] "Eukaryotic" cells include, but are not limited to, fungal cells (such as yeast), plant cells, 5 animal cells, mammalian cells and human cells.

[00369] The term "nuclease" as used herein refers to an enzyme capable of cleaving the phosphodiester bonds between the nucleotide subunits of nucleic acid. A nuclease may be isolated or derived from a natural source. The natural source may be any living organism. Alternatively, a nuclease may be a modified or a synthetic protein which retains the phosphodiester bond cleaving 10 activity.#

[00370] The term “PAM” as used herein refers to a nucleotide sequence of a target DNA located in proximity to the targeted DNA sequence and recognized by the CRISPR nuclease. The PAM sequence may differ depending on the nuclease identity.

[00371] A skilled artisan will appreciate that embodiments of the present invention disclose RNA 15 molecules capable of complexing with a nuclease, e.g. a CRISPR nuclease, such as to associate with a target genomic DNA sequence of interest next to a protospacer adjacent motif (PAM). The nuclease then mediates cleavage of target DNA to create a double-stranded break within the protospacer.

[00372] In embodiments of the present invention, a CRISPR nuclease and a targeting molecule 20 form a CRISPR complex that binds to a target DNA sequence to effect cleavage of the target DNA sequence. CRISPR nucleases may form a CRISPR complex comprising a CRISPR nuclease and RNA molecule without a further tracrRNA molecule. Alternatively, CRISPR nucleases may form a CRISPR complex between the CRISPR nuclease, an RNA molecule, and a tracrRNA molecule.

[00373] The term “protein binding sequence” or “nuclease binding sequence” refers to a sequence 25 capable of binding with a CRISPR nuclease to form a CRISPR complex. An skilled artisan will understand that a tracrRNA capable of binding with a CRISPR nuclease to form a CRISPR complex comprises a protein or nuclease binding sequence.

[00374] An “RNA binding portion” of a CRISPR nuclease refers to a portion of the CRISPR nuclease which may bind to an RNA molecule to form a CRISPR complex, e.g. the nuclease

binding sequence of a tracrRNA molecule. An “activity portion” or “active portion” of a CRISPR nuclease refers to a portion of the CRISPR nuclease which effects a double strand break in a DNA molecule, for example when in complex with a DNA-targeting RNA molecule.

[00375] The term refers to a sequence sufficiently complementary to a tracrRNA molecule so as 5 to hybridize to the tracrRNA via basepairing and promote the formation of a CRISPR complex. (*See* US Patent No. 8906616). In embodiments of the present invention, the RNA molecule may further comprise a portion having a tracr mate sequence.

[00376] In embodiments of the present invention, the targeting molecule may further comprise the sequence of a tracrRNA molecule. Such embodiments may be designed as a synthetic fusion 10 of the guide portion of the RNA molecule (gRNA or crRNA) and the trans-activating crRNA (tracrRNA), together forming a single guide RNA (sgRNA). (*See* Jinek (2012) Science). Embodiments of the present invention may also form CRISPR complexes utilizing a separate tracrRNA molecule and a separate RNA molecule comprising a guide sequence portion. In such embodiments the tracrRNA molecule may hybridize with the RNA molecule via base pairing and 15 may be advantageous in certain applications of the invention described herein.

[00377] In embodiments of the present invention an RNA molecule may comprise a “nexus” region and/or “hairpin” regions which may further define the structure of the RNA molecule. (*See* Briner et al. (2014) Molecular Cell 56:333-39).

[00378] As used herein, the term “direct repeat sequence” refers to two or more repeats of a 20 specific amino acid sequence of nucleotide sequence.

[00379] As used herein, an RNA sequence or molecule capable of “interacting with” or “binding” with a CRISPR nuclease refers to the RNA sequence or molecules ability to form a CRISPR complex with the CRISPR nuclease.

[00380] As used herein, the term “operably linked” refers to a relationship (i.e. fusion, 25 hybridization) between two sequences or molecules permitting them to function in their intended manner. In embodiments of the present invention, when an RNA molecule is operably linked to a promoter, both the RNA molecule and the promotor are permitted to function in their intended manner.

[00381] As used herein, the term “heterologous promoter” refers to a promoter that does not naturally occur together with the molecule or pathway being promoted.

[00382] As used herein, a sequence or molecule has an X% “sequence identity” to another sequence or molecule if X% of bases or amino acids between the sequences of molecules are the same and in the same relative position. For example, a first nucleotide sequence having at least a 95% sequence identity with a second nucleotide sequence will have at least 95% of bases, in the same relative position, identical with the other sequence.

Nuclear Localization Sequences

[00383] The terms "nuclear localization sequence" and "NLS" are used interchangeably to indicate an amino acid sequence/peptide that directs the transport of a protein with which it is associated from the cytoplasm of a cell across the nuclear envelope barrier. The term "NLS" is intended to encompass not only the nuclear localization sequence of a particular peptide, but also derivatives thereof that are capable of directing translocation of a cytoplasmic polypeptide across the nuclear envelope barrier. NLSs are capable of directing nuclear translocation of a polypeptide when attached to the N-terminus, the C-terminus, or both the N- and C-termini of the polypeptide. In addition, a polypeptide having an NLS coupled by its N- or C-terminus to amino acid side chains located randomly along the amino acid sequence of the polypeptide will be translocated. Typically, an NLS consists of one or more short sequences of positively charged lysines or arginines exposed on the protein surface, but other types of NLS are known. Non- limiting examples of NLSs include an NLS sequence derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV (SEQ ID NO:347); the NLS from nucleoplasmin (e.g. the nucleoplasm bipartite NLS with the sequence KRPAATKKAGQAKKKK (SEQ ID NO:348); the c-myc NLS having the amino acid sequence PAA RV LD (SEQ ID NO:349) or RQRR.NELKRSP (SEQ ID NO:350); the hRNPAl M9 NLS having the sequence NQSSNFGPM GGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO:351); the sequence R RIZFK KGKDTA:ELRRRRVE 7S 7ELRKAKKDEQILKRRNV (SEQ ID NO:352) of the IBB domain from importin-alpha; the sequences VSRKRPRP (SEQ ID NO:353) and PPKKARED (SEQ ID NO:354) of the myoma T protein; the sequence PQP KKPL (SEQ ID NO:355) of human p53; the sequence SAII KKKKM AP (SEQ ID NO:356) of mouse c- abl IV; the sequences DRLRR (SEQ ID NO:357) and PKQKKRK (SEQ ID NO:358) of the influenza vims NS1; the sequence RKLKKKIKKL (SEQ ID NO:359) of the Hepatitis virus delta antigen; the sequence

REKKKFLKRR (SEQ ID NO:360) of the mouse Mx1 protein; the sequence KR GDEVDGVDEVAKKKSKK (SEQ ID NO:361) of the human poly(ADP-ribose) polymerase; and the sequence RKCLQAGMNLEARKTKK (SEQ ID NO:362) of the steroid hormone receptors (human) glucocorticoid.

5 ***Delivery***

[00384] The CRISPR nuclease or CRISPR compositions described herein may be delivered as a protein, DNA molecules, RNA molecules, Ribonucleoproteins (RNP), nucleic acid vectors, or any combination thereof. In some embodiments, the RNA molecule comprises a chemical modification. Non-limiting examples of suitable chemical modifications include 2'-0-methyl (M),
10 2'-0-methyl, 3'phosphorothioate (MS) or 2'-0-methyl, 3 'thioPACE (MSP), pseudouridine, and 1-methyl pseudo-uridine. Each possibility represents a separate embodiment of the present invention.

[00385] The CRISPR nucleases and/or polynucleotides encoding same described herein, and optionally additional proteins (e.g., ZFPs, TALENs, transcription factors, restriction enzymes) and/or nucleotide molecules such as guide RNA may be delivered to a target cell by any suitable
15 means. The target cell may be any type of cell e.g., eukaryotic or prokaryotic, in any environment e.g., isolated or not, maintained in culture, *in vitro, ex vivo, in vivo* or *in planta.*

[00386] In some embodiments, the composition to be delivered includes mRNA of the nuclease and RNA of the guide. In some embodiments, the composition to be delivered includes mRNA of the nuclease, RNA of the guide and DNA donor template. In some embodiments, the composition
20 to be delivered includes the CRISPR nuclease and guide RNA. In some embodiments, the composition to be delivered includes the CRISPR nuclease, guide RNA and DNA donor template for homology directed repair. In some embodiments, the composition to be delivered includes mRNA of the nuclease, DNA-targeting RNA and the tracrRNA. In some embodiments, the composition to be delivered includes mRNA of the nuclease, DNA-targeting RNA and the
25 tracrRNA and DNA donor template. In some embodiments, the composition to be delivered includes the CRISPR nuclease DNA-targeting RNA and the tracrRNA. In some embodiments, the composition to be delivered includes the CRISPR nuclease, DNA-targeting RNA and the tracrRNA and DNA donor template for homology directed repair.

[00387] Any suitable viral vector system may be used to deliver RNA compositions.
30 Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic

acids and/or CRISPR nuclease in cells (e.g., mammalian cells, plant cells, etc.) and target tissues. Such methods can also be used to administer nucleic acids encoding and/or CRISPR nuclease protein to cells in vitro. In certain embodiments, nucleic acids and/or CRISPR nuclease are administered for in vivo or ex vivo gene therapy uses. Non-viral vector delivery systems include naked nucleic acid, and nucleic acid complexed with a delivery vehicle such as a liposome or poloxamer. For a review of gene therapy procedures, see Anderson, Science 256:808-813 (1992); Nabel & Felgner, TIBTECH 11:211-217 (1993); Mitani & Caskey, TIBTECH 11:162-166 (1993); Dillon, TIBTECH 11:167-175 (1993); Miller, Nature 357:455-460 (1992); Van Brunt, Biotechnology 6(10):1149-1154 (1988); Vigne, Restorative Neurology and Neuroscience 8:35-36 (1995); Kremer & Perricaudet, British Medical Bulletin 51(1):31-44 (1995); Haddada et al., in Current Topics in Microbiology and Immunology Doerfler and Bohm (eds.) (1995); and Yu et al., Gene Therapy 1:13-26 (1994).

[00388] Methods of non-viral delivery of nucleic acids and/or proteins include electroporation, lipofection, microinjection, biolistics, particle gun acceleration, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, artificial virions, and agent-enhanced uptake of nucleic acids or can be delivered to plant cells by bacteria or viruses (e.g., Agrobacterium, Rhizobium sp. NGR234, Sinorhizoboiummeliloti, Mesorhizobium loti, tobacco mosaic virus, potato virus X, cauliflower mosaic virus and cassava vein mosaic virus. See, e.g., Chung et al. (2006) Trends Plant Sci. 11(1):1-4. Sonoporation using, e.g., the Sonitron 2000 system (Rich-Mar) can also be used for delivery of nucleic acids. Cationic-lipid mediated delivery of proteins and/or nucleic acids is also contemplated as an in vivo or in vitro delivery method. See Zuris et al. (2015) Nat. Biotechnol. 33(1):73-80. See also Coelho et al. (2013) N. Engl. J. Med. 369, 819-829; Judge et al. (2006) Mol. Ther. 13, 494-505; and Basha et al. (2011) Mol. Ther. 19, 2186-2200.

[00389] Additional exemplary nucleic acid delivery systems include those provided by Amaxa® Biosystems (Cologne, Germany), Maxcyte, Inc. (Rockville, Md.), BTX Molecular Delivery Systems (Holliston, Mass.) and Copernicus Therapeutics Inc., (see for example U.S. Patent No. 6,008,336). Lipofection is described in e.g., U.S. Patent No. 5,049,386, U.S. Patent No. 4,946,787; and U.S. Patent No. 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam.TM., Lipofectin.TM. and Lipofectamine.TM. RNAiMAX). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include

those of Felgner, WO 91/17424, WO 91/16024. Delivery can be to cells (ex vivo administration) or target tissues (in vivo administration).

[00390] The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Patent Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

[00391] Additional methods of delivery include the use of packaging the nucleic acids to be delivered into EnGeneIC delivery vehicles (EDVs). These EDVs are specifically delivered to target tissues using bispecific antibodies where one arm of the antibody has specificity for the target tissue and the other has specificity for the EDV. The antibody brings the EDVs to the target cell surface and then the EDV is brought into the cell by endocytosis. Once in the cell, the contents are released (see MacDiamid et al (2009) Nature Biotechnology 27(7) p. 643).

[00392] The use of RNA or DNA viral based systems for the delivery of nucleic acids take advantage of highly evolved processes for targeting a virus to specific cells in the body and trafficking the viral payload to the nucleus. Viral vectors can be administered directly to patients (in vivo) or they can be used to treat cells in vitro and the modified cells are administered to patients (ex vivo). Conventional viral based systems for the delivery of nucleic acids include, but are not limited to, retroviral, lentivirus, adenoviral, adeno-associated, vaccinia and herpes simplex virus vectors for gene transfer. However, an RNA virus is preferred for delivery of the RNA compositions described herein. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues. Nucleic acid of the invention may be delivered by non-integrating lentivirus. Optionally, RNA delivery with Lentivirus is utilized. Optionally the lentivirus includes mRNA of the nuclease, RNA of the guide. Optionally the lentivirus includes mRNA of the nuclease, RNA of the guide and DNA donor template. Optionally, the lentivirus includes the nuclease protein, guide RNA. Optionally, the lentivirus includes the nuclease protein, guide RNA and/or DNA donor template for homology directed repair. Optionally the lentivirus includes mRNA of the nuclease, DNA-targeting RNA, and the tracrRNA. Optionally the lentivirus

includes mRNA of the nuclease, DNA-targeting RNA, and the tracrRNA, and DNA donor template. Optionally, the lentivirus includes the nuclease protein, DNA-targeting RNA, and the tracrRNA. Optionally, the lentivirus includes the nuclease protein, DNA-targeting RNA, and the tracrRNA, and DNA donor template for homology directed repair.

5 [00393] The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vectors capable of transducing or infecting non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system depends on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign 10 sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations thereof (see, e.g. Buchscher et 15 al., J. Virol. 66:2731-2739 (1992); Johann et al., J. Virol. 66:1635-1640 (1992); Sommerfelt et al., Virol. 176:58-59 (1990); Wilson et al., J. Virol. 63:2374-2378 (1989); Miller et al., J. Virol. 65:2220-2224 (1991); PCT/US94/05700).

[00394] At least six viral vector approaches are currently available for gene transfer in clinical trials, which utilize approaches that involve complementation of defective vectors by genes 20 inserted into helper cell lines to generate the transducing agent.

[00395] pLASN and MFG-S are examples of retroviral vectors that have been used in clinical trials (Dunbar et al., Blood 85:3048-305 (1995); Kohn et al., Nat. Med. 1:1017-102 (1995); Malech et al., PNAS 94:22 12133-12138 (1997)). PA317/pLASN was the first therapeutic vector used in a gene therapy trial. (Blaese et al., Science 270:475-480 (1995)). Transduction efficiencies of 50% 25 or greater have been observed for MFG-S packaged vectors. (Ellem et al., Immunol Immunother. 44(1):10-20 (1997); Dranoff et al., Hum. Gene Ther. 1:111-2 (1997).

[00396] Packaging cells are used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, AAV, and psi.2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by a producer 30 cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the

minimal viral sequences required for packaging and subsequent integration into a host (if applicable), other viral sequences being replaced by an expression cassette encoding the protein to be expressed. The missing viral functions are supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess inverted terminal repeat (ITR) sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line is also infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV. Additionally, AAV can be produced at clinical scale using baculovirus systems (see U.S. Patent No. 7,479,554).

[00397] In many gene therapy applications, it is desirable that the gene therapy vector be delivered with a high degree of specificity to a particular tissue type. Accordingly, a viral vector can be modified to have specificity for a given cell type by expressing a ligand as a fusion protein with a viral coat protein on the outer surface of the virus. The ligand is chosen to have affinity for a receptor known to be present on the cell type of interest. For example, Han et al., Proc. Natl. Acad. Sci. USA 92:9747-9751 (1995), reported that Moloney murine leukemia virus can be modified to express human heregulin fused to gp70, and the recombinant virus infects certain human breast cancer cells expressing human epidermal growth factor receptor. This principle can be extended to other virus-target cell pairs, in which the target cell expresses a receptor and the virus expresses a fusion protein comprising a ligand for the cell-surface receptor. For example, filamentous phage can be engineered to display antibody fragments (e.g., FAB or Fv) having specific binding affinity for virtually any chosen cellular receptor. Although the above description applies primarily to viral vectors, the same principles can be applied to non-viral vectors. Such vectors can be engineered to contain specific uptake sequences which favor uptake by specific target cells.

[00398] Gene therapy vectors can be delivered in vivo by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, intramuscular, subdermal, or intracranial infusion) or topical application, as described below. Alternatively, vectors can be delivered to cells ex vivo, such as cells explanted from an individual patient (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by

reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector. In some embodiments, delivery of mRNA in-vivo and ex-vivo, and RNPs delivery may be utilized.

[00399] Ex vivo cell transfection for diagnostics, research, or for gene therapy (e.g., via re-infusion of the transfected cells into the host organism) is well known to those of skill in the art. In a preferred embodiment, cells are isolated from the subject organism, transfected with an RNA composition, and re-infused back into the subject organism (e.g., patient). Various cell types suitable for ex vivo transfection are well known to those of skill in the art (see, e.g., Freshney et al., Culture of Animal Cells, A Manual of Basic Technique (3rd ed. 1994)) and the references cited therein for a discussion of how to isolate and culture cells from patients).

[00400] Suitable cells include but not limited to eukaryotic and prokaryotic cells and/or cell lines. Non-limiting examples of such cells or cell lines generated from such cells include COS, CHO (e.g., CHO--S, CHO-K1, CHO-DG44, CHO-DUXB11, CHO-DUKX, CHOK1SV), VERO, MDCK, WI38, V79, B14AF28-G3, BHK, HaK, NSO, SP2/0-Ag14, HeLa, HEK293 (e.g., HEK293-F, HEK293-H, HEK293-T), and perC6 cells, any plant cell (differentiated or undifferentiated) as well as insect cells such as Spodopterafugiperda (Sf), or fungal cells such as Saccharomyces, Pichia and Schizosaccharomyces. In certain embodiments, the cell line is a CHO-K1, MDCK or HEK293 cell line. Additionally, primary cells may be isolated and used ex vivo for reintroduction into the subject to be treated following treatment with the nucleases (e.g. ZFNs or TALENs) or nuclease systems (e.g. CRISPR). Suitable primary cells include peripheral blood mononuclear cells (PBMC), and other blood cell subsets such as, but not limited to, CD4+ T cells or CD8+ T cells. Suitable cells also include stem cells such as, by way of example, embryonic stem cells, induced pluripotent stem cells, hematopoietic stem cells (CD34+), neuronal stem cells and mesenchymal stem cells.

[00401] In one embodiment, stem cells are used in ex vivo procedures for cell transfection and gene therapy. The advantage to using stem cells is that they can be differentiated into other cell types in-vitro or can be introduced into a mammal (such as the donor of the cells) where they will engraft in the bone marrow. Methods for differentiating CD34+ cells in vitro into clinically important immune cell types using cytokines such a GM-CSF, IFN-gamma. and TNF-alpha are known (as a non-limiting example see, Inaba et al., J. Exp. Med. 176:1693-1702 (1992)).

[00402] Stem cells are isolated for transduction and differentiation using known methods. For example, stem cells are isolated from bone marrow cells by panning the bone marrow cells with antibodies which bind unwanted cells, such as CD4+ and CD8+ (T cells), CD45+(panB cells), GR-1 (granulocytes), and Iad (differentiated antigen presenting cells) (as a non-limiting example see Inaba et al., J. Exp. Med. 176:1693-1702 (1992)). Stem cells that have been modified may also be used in some embodiments.

[00403] Notably, any one of the CRISPR nuclease described herein may be suitable for genome editing in post-mitotic cells or any cell which is not actively dividing, e.g., arrested cells. Examples of post-mitotic cells which may be edited using a CRISPR nuclease of the present invention include, but are not limited to, myocyte, a cardiomyocyte, a hepatocyte, an osteocyte and a neuron.

[00404] Vectors (e.g., retroviruses, liposomes, etc.) containing therapeutic RNA compositions can also be administered directly to an organism for transduction of cells in vivo. Alternatively, naked RNA or mRNA can be administered. Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood or tissue cells including, but not limited to, injection, infusion, topical application and electroporation. Suitable methods of administering such nucleic acids are available and well known to those of skill in the art, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

[00405] Vectors suitable for introduction of transgenes into immune cells (e.g., T-cells) include non-integrating lentivirus vectors. See, for example, U.S. Patent Publication No. 2009/0117617.

[00406] Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions available, as described below (see, e.g., Remington's Pharmaceutical Sciences, 17th ed., 1989).

DNA Repair by Homologous Recombination

[00407] The term "homology-directed repair" or "HDR" refers to a mechanism for repairing DNA damage in cells, for example, during repair of double-stranded and single-stranded breaks in DNA. HDR requires nucleotide sequence homology and uses a "nucleic acid template" (nucleic acid template or donor template used interchangeably herein) to repair the sequence where the double-

stranded or single break occurred (e.g., DNA target sequence). This results in the transfer of genetic information from, for example, the nucleic acid template to the DNA target sequence. HDR may result in alteration of the DNA target sequence (e.g., insertion, deletion, mutation) if the nucleic acid template sequence differs from the DNA target sequence and part or all of the nucleic
5 acid template polynucleotide or oligonucleotide is incorporated into the DNA target sequence. In some embodiments, an entire nucleic acid template polynucleotide, a portion of the nucleic acid template polynucleotide, or a copy of the nucleic acid template is integrated at the site of the DNA target sequence.

[00408] The terms "nucleic acid template" and “donor”, refer to a nucleotide sequence that is
10 inserted or copied into a genome. The nucleic acid template comprises a nucleotide sequence, e.g., of one or more nucleotides, that will be added to or will template a change in the target nucleic acid or may be used to modify the target sequence. A nucleic acid template sequence may be of any length, for example between 2 and 10,000 nucleotides in length (or any integer value there between or there above), preferably between about 100 and 1,000 nucleotides in length (or any
15 integer there between), more preferably between about 200 and 500 nucleotides in length. A nucleic acid template may be a single stranded nucleic acid, a double stranded nucleic acid. In some embodiment, the nucleic acid template comprises a nucleotide sequence, e.g., of one or more nucleotides, that corresponds to wild type sequence of the target nucleic acid, e.g., of the target position. In some embodiment, the nucleic acid template comprises a ribonucleotide sequence,
20 e.g., of one or more ribonucleotides, that corresponds to wild type sequence of the target nucleic acid, e.g., of the target position. In some embodiment, the nucleic acid template comprises modified ribonucleotides.

[00409] Insertion of an exogenous sequence (also called a "donor sequence," donor template” or "donor"), for example, for correction of a mutant gene or for increased
25 expression of a wild-type gene can also be carried out. It will be readily apparent that the donor sequence is typically not identical to the genomic sequence where it is placed. A donor sequence can contain a non-homologous sequence flanked by two regions of homology to allow for efficient HDR at the location of interest. Additionally, donor sequences can comprise a vector molecule containing sequences that are not homologous to the region of interest in cellular
30 chromatin. A donor molecule can contain several, discontinuous regions of homology to cellular chromatin. For example, for targeted insertion of sequences not normally present in a region

of interest, said sequences can be present in a donor nucleic acid molecule and flanked by regions of homology to sequence in the region of interest.

[00410] The donor polynucleotide can be DNA or RNA, single-stranded and/or double-stranded and can be introduced into a cell in linear or circular form. See, e.g., U.S. Patent Publication Nos. 20100047805; 20110281361; and 20110207221. If introduced in linear form, the ends of the donor sequence can be protected (e.g., from exonucleolytic degradation) by methods known to those of skill in the art. For example, one or more dideoxynucleotide residues are added to the 3' terminus of a linear molecule and/or self- complementary oligonucleotides are ligated to one or both ends. See, for example, Chang et al. (1987) Proc. Natl. Acad. Sci. USA 84:4959-4963; Nehls et al. (1996) Science 272:886-889. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues.

[00411] Accordingly embodiments of the present invention using a donor DNA template for HDR may use a DNA or RNA, single-stranded and/or double- stranded and can be introduced into a cell in linear or circular form. In embodiments of the present invention using: (1) an RNA molecule comprising a guide sequence to affect a double strand break in a gene prior to HDR and (2) a donor RNA template for HDR, the RNA molecule comprising the guide sequence is a first RNA molecule and the donor RNA template is a second RNA molecule.

[00412] A donor sequence may also be an oligonucleotide and be used for gene correction or targeted alteration of an endogenous sequence. The oligonucleotide may be introduced to the cell on a vector, may be electroporated into the cell, or may be introduced via other methods known in the art. The oligonucleotide can be used to `correct` a mutated sequence in an endogenous gene (e.g., the sickle mutation in beta globin), or may be used to insert sequences with a desired purpose into an endogenous locus.

[00413] A polynucleotide can be introduced into a cell as part of a vector molecule having additional sequences such as, for example, replication origins, promoters and genes encoding antibiotic resistance. Moreover, donor polynucleotides can be introduced as naked nucleic acid, as nucleic acid complexed with an agent such as a liposome or poloxamer, or can be

delivered by viruses (e.g., adenovirus, AAV, herpesvirus, retrovirus, lentivirus and integrase defective lentivirus (IDLV)).

[00414] The donor is generally inserted so that its expression is driven by the endogenous promoter at the integration site, namely the promoter that drives expression of the endogenous gene into which the donor is inserted. However, it will be apparent that the donor may comprise a promoter and/or enhancer, for example a constitutive promoter or an inducible or tissue specific promoter.

[0117] The donor molecule may be inserted into an endogenous gene such that all, some or none of the endogenous gene is expressed. For example, a transgene as described herein may be inserted into an endogenous locus such that some (N-terminal and/or C-terminal to the transgene) or none of the endogenous sequences are expressed, for example as a fusion with the transgene. In other embodiments, the transgene (e.g., with or without additional coding sequences such as for the endogenous gene) is integrated into any endogenous locus, for example a safe-harbor locus, for example a CCR5 gene, a CXCR4 gene, a PPP1R12c (also known as AAVS1) gene, an albumin gene or a Rosa gene. See, e.g., U.S. Pat. Nos. 7,951,925 and 8,110,379; U.S. Publication Nos. 20080159996; 201000218264; 20100291048; 20120017290; 20110265198; 20130137104; 20130122591; 20130177983 and 20130177960 and U.S. Provisional Application No. 61/823,689).

[00415] When endogenous sequences (endogenous or part of the transgene) are expressed with the transgene, the endogenous sequences may be full-length sequences (wild-type or mutant) or partial sequences. Preferably the endogenous sequences are functional. Non- limiting examples of the function of these full length or partial sequences include increasing the serum half-life of the polypeptide expressed by the transgene (e.g., therapeutic gene) and/or acting as a carrier.

[00416] Furthermore, although not required for expression, exogenous sequences may also include transcriptional or translational regulatory sequences, for example, promoters, enhancers, insulators, internal ribosome entry sites, sequences encoding 2A peptides and/or polyadenylation signals.

[00417] In certain embodiments, the donor molecule comprises a sequence selected from the group consisting of a gene encoding a protein (e.g., a coding sequence encoding a protein that is lacking in the cell or in the individual or an alternate version of a gene encoding a protein),

a regulatory sequence and/or a sequence that encodes a structural nucleic acid such as a microRNA or siRNA.

General

[00418] For the foregoing embodiments, each embodiment disclosed herein is contemplated as being applicable to each of the other disclosed embodiment. For example, it is understood that any of the RNA molecules or compositions of the present invention may be utilized in any of the methods of the present invention.

[00419] As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

[00420] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

[00421] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

[00422] Generally, the nomenclature used herein, and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant

DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994);
5 "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); "Bacteriophage Methods and Protocols",
10 Volume 1: Isolation, Characterization, and Interactions, all of which are incorporated by reference. Other general references are provided throughout this document.

[00423] Examples are provided below to facilitate a more complete understanding of the invention. The following examples illustrate the exemplary modes of making and practicing the invention. However, the scope of the invention is not limited to specific embodiments disclosed in
15 these Examples, which are for purposes of illustration only.

EXPERIMENTAL DETAILS

Materials for each example

DNA constructs and sequences

Table 2. Expression vectors.

Name	System	Construct elements
pbNNC	Bacterial	HA Tag-Linker-OMNI ORF
pcDNA3.1-P2A-mCherry	Mammalian	HA Tag-OMNI ORF-NLS-P2A-mCherry
pmOMNI-P2A-mCherry	Mammalian	NLS-OMNI ORF-HA tag-P2A-mCherry

20

Table 3. Details of construct elements

Element	Protein Sequence	DNA sequence
HA Tag	YPYDVPDYA (SEQ ID NO: 363)	TACCCATACGATGTTCCAGATTACGCT (SEQ ID NO: 364)
NLS	PKKKRKV (SEQ ID NO: 347)	CCAAAAAAGAAAAGAAAAGTT (SEQ ID NO: 365)
P2A	ATNFSLLKQAGDVEENPGP (SEQ ID NO: 366)	GCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCC (SEQ ID NO: 367)
mCherry	MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK* (SEQ ID NO: 368)	atggtgagcaagggcgaggaggataacatggccatcatcaaggagttcatgcgcttcaaggtgcacatggagggctccgtgaacggccacgagttcgagatcgagggcgagggcgagggccgcccctacgagggcacccagaccgccaagctgaaggtgaccaagggtggccccctgcccttcgcctgggacatcctgtcccctcagttcatgtacggctccaaggcctacgtgaagcaccccgccgacatccccgactacttgaagctgtccttccccgagggcttcaagtgggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgacccaggactcctccctgcaggacggcgagttcatctacaaggtgaagctgcgcggcaccaacttcccctccgacggccccgtaatgcagaagaagaccatgggctgggaggcctcctccgagcggatgtaccccgaggacggcgccctgaagggcgagatcaagcagaggctgaagctgaaggacggcggccactacgacgctgaggtcaagaccacctacaaggccaagaagcccgtgcagctgcccggcgcctacaacgtcaacatcaagttggacatcacctcccacaacgaggactacaccatcgtggaacagtacgaacgcgccgagggccgccactccaccggcggcatggacgagctgtacaagtag (SEQ ID NO: 369)
pbNNC-6 (E.coli optimized)		
pmOMNI-6 (human optimized)-P2A_mCherry		
pm-pGUIDE 6f2 – T2		

Example 1: OMNI 6

Materials for Example 1

Table 4. Designed RNA molecules

SEQ ID NO.	NAME of RNA molecule		Scaffold RNA sequence
	6 crRNA	crRNA forming Duplex cr:trac guide RNA	GUUUUAGAGUUAUGUAAUUUAGAAUAGUAGCAAAAC (SEQ ID NO: 79)
	6 tracRNA	tracrRNA forming Duplex cr:trac guide RNA	CAAAGAAAUUUGACUACUAUUCUAAAUUAUAUAACGAGUUAAAAUAAAGCUUUGCUUUAAUGCCAUUUUAAAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO: 78)
	6a	sg RNA designed based on Native sequence	GUUUUAGAGUUAUGUGAAAAUAUAACGAGUUAAAAUAAAGCUUUGCUUUAAUGCCAUUUUAAAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:88)
	6a1	sg RNA modification in the lower stem of the CrRNA:TracRNA duplex	GUUUAAGAGUUAUGUGAAAAUAUAACGAGUUUAAAUAAAGCUUUGCUUUAAUGCCAUUUUAAAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:89)
	6a2	6a1+modification in the loop of predicted Nexus	GUUUAAGAGUUAUGUGAAAAUAUAACGAGUUUAAAUAAAGCUUUGCUUUAAUGCCAUAUUAUAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:90)
	6f2	6a2+ elongation of the upper stem of the CrRNA:TracRNA duplex	GUUUAAGAGUUAUGUAAGAAAUUAUAUAACGAGUUUAAAUAAAGCUUUGCUUUAAUGCCAUAUUAUAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:91)
	Native	single guide RNA suitable for Spcas9	GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUU (SEQ ID NO:371)
	E+F	Guide suitable for SpCas9 (see Chen et al, Cell. 2013)	GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO:372)

Example 1A: Expression of OMNI 6 nuclease in bacterial system

[00424] An Nt-HA tag-OMNI nuclease open reading frame (Table 2) was cloned into pbNNC plasmid under the T7 promoter (Table 3). The sgRNA was cloned into bacterial plasmid under constitutive promoter. E. coli strain BW25141 (λDE3) was co-transformed with OMNI 6 and the corresponding guide and were co-expressed over-night by addition of 0.1mM IPTG during mid-exponential growth at 18 degrees Celsius. The cells were then pelleted and lysed, and the expression of OMNI 6 was detected by Western blot analysis using anti-HA antibodies.

Example 1B: PAM Depletion Assay of OMNI nucleases in bacterial system

[00425] To assay for functional PAMs, PAM depletion assay was performed. To this end, E. coli strain BW25141 (λDE3) were co-transformed with: (1) a library of plasmids containing a randomized PAM sequences of 8 N's flanked by a unique protospacer, (2) plasmids encoding the OMNI 6 nuclease, and (3) a plasmid encoding gRNA targeting the protospacer of the library or a non-targeting gRNA as control. Next, cells were selected for all three plasmids by recovering them on media containing appropriate antibiotics. Using this assay, Plasmids containing a PAM are cleaved and the cells that contain them could not grow, while cells containing plasmids with non-PAMs were able to propagate. By comparing the frequency of a sequence in the library after selection of the targeting guide relative to the non-targeting, individual PAM sequences could be identified. The most depleted PAM sites of OMNI 6 nuclease are presented in column 1 of Table 4. Based on these results the suggested consensus PAM sequence for OMNI 6 is NGGNNNNN.

Table 5. PAM sequences

PAM depleted targets	PAM Tested in bacteria	PAM Tested in vitro
AGGGNNNN	CGGTCGAA	CGGTCGAA
CGGGNNNN	TGGTCCGC	TGGTCCGC
TGGGNNNN	AGGACCTC	AGGACCTC
GGGTNNNN		

Example 1C: Activity Assay in Bacteria

[00426] Activity assay for a single target was performed as described for the depletion assay (see above) using a single PAM that was identified in the depletion assay (Table 5, PAM tested in bacteria). Activity with the premature duplex guide RNA was confirmed as well in the same format.

Example 1D: In-vitro activity assay

[00427] E. coli strain BW25141 (λDE3) co-expressing the OMNI 6 nuclease and sgRNA were lysed using BugBuster™ lysis solution. The lysate was reacted in the recommended cleavage buffer with linear DNA substrates containing the PAM sequences flanked by a unique protospacer targeted by the sgRNA or a non-targeted protospacer as a control. Tested PAMs are summarized in the 3rd column of Table 5, and editing activity in comparison to SpCas9 was demonstrated.

Example 1E: OMNI expression in mammalian cells

[00428] An expression vector carrying HA tagged OMNI nucleases linked by P2A peptide to mCherry (pm-OMNI or pcDNA3.1, see details in Table 2 and 3), was introduced into 293T cells using the TurbofectTMtransfection reagent (Thermo Fisher). The Relative level of transcription for OMNI 6 was determined using Flow cytometry. Cell were transfected with pm-OMNI OR PCDNA3.1 and a carrier plasmid expressing BFP. Transfection was done as described above and cells were subjected to Flow cytometry analysis 72 hours post transfection. The mCherry protein expressed from the P2A polycentric transcript served as an indication for OMNI 6 transcript levels. The transcription levels of OMNI 6 was about 30% and approached 40% for 6f2, compared to less than 10% for Native and E+F (pCDNA3.1 sp), as determined by the percentage of mCherry expressing cells out of the transfected population and after normalization to BFP levels to account for variation in transfection efficiency.

Example 1F: Activity in mammalian cells:

[00429] Activity of OMNI 6 nuclease in Mammalian cells was demonstrated using GFP Fluorescent gain-based reporter system. In this reporting system the PAM-target sequence is incorporated upstream to the GFP coding sequence causing a frame shift in translation leading to inactivation of the fluorescent signal. A double strand break in the target site generated by the nuclease, will lead to inaccurate DNA repair that will reestablish the GFP coding frame.

[00430] HEK 293T cells were transfected with pm-OMNI 6, sgRNA expressing vector pm-pGuide encoding for either 6a, 6a1, 6a2 or 6f2, and the reporting vector. Cells were analyzed by FACS 72 hours post transfection for GFP fluorescent quantification. OMNI 6 showed editing activity on transient target DNA in mammalian cells, measured by gain of expression of GFP. Native and E+F (pCDNA3.1 sp) showed a 60% and about 50% gain of GFP, 6a, 6a1 and 6a2 each showed about 20% or greater gain of GFP and 6f2 showed about 50% gain of GFP.

[00431] GFP disruption assay: Activity of OMNI 6 in mammalian cells was also demonstrated using disruption of genomic integrated GFP reporter (GGGCACGGGCAGCTTGCCGGTGG – SEQ ID NO:370). HEK 293T cells carrying a stably integrated GFP gene, were transfected with a mix of pm-OMNI, encoding for SpCas9 or OMNI 6 or pcDNA3-SpCas9 and pm-Guide, as
5 indicated in Fig. 4A. Cells were analyzed by FACS 72 hours post transfection for GFP fluorescent quantification. OMNI 6 with guides 6a and 6f, showed a GFP disruption of greater than 50%, similar to the disruption of the guide + pcDNA 3.1 sp and guide + pm-OMNI0 -sp, calculated as percentage loss of mean GFP intensity (compared to the initial intensity deduced from the analysis of mock transfected, GFP integrated cells).

10 [00432] Measurement of OMNI nuclease activity on an endogenous genomic location (rs3761005) was performed. To this end, Hela cells were co-transfected with a plasmid encoding SpCas9 nuclease or OMNI 6, a pm-Guide encoding for rs3761005 spacer sequence (GCTGCGGGAAAGGGATTCCC – SEQ ID NO: 92), and SpCas9 guide or improved OMNI 6 guide ('6f2'). Transient expression was achieved in a 24 well plate format using Turbofect reagent
15 (Thermo fisher scientific). For negative control, Hela cells were transfected with a plasmid encoding SpCas9 nuclease or OMNI 6 without a guide. Cells were harvested 72 hours post DNA transfection. On target activity was demonstrated by DNA capillary electrophoresis.

Example 2: OMNI 4 and OMNI 20

Materials for Example 2

Table 6. Designed RNA molecules

SEQ ID NO.	Name of RNA molecule	Scaffold RNA sequence
107	4a	GUUUGAGAGUAAUGGAAACAUUACGAGUUCAAAUA ACGAUUUAUCGACAUUACCACUUCGUGGUCAGGCGC ACGUGUGUGCGCCGUUUUUU
136	20c	GUUUGAGAGUAGUGGAAACACUACGAGUUCAAAUA AAAAUUAUUUCAAAUCGUUCUUUAUGUUCGCACAA GAGUUGUGCAUUUUAUUUGUAACUUUUGCCUGCUU UUUUAAGCAGGCUUUUUAUUUU
137	20f	GUUUGAGAGUAGUGUAAGAAAUUACACUACGAGUU CAAAUAAAAAUUAUUUCAAAUCGUUCUUUAUGUUC GCACAAGAGUUGUGCAAUUUAUUUGUAACUUAUGC CUGCUUAAUUAAGCAGGCUUUUUAUUUU
371	Native	single guide RNA suitable for Spcas9
372	E+F	Guide suitable for SpCas9 (see Chen et al, Cell. 2013)

Example 2A: Expression of OMNI 4 and OMNI 20 nuclease in bacterial system

[00433] An Nt-HA tag-OMNI nuclease open reading frame (Table 2) was cloned into pbNNC plasmid under the T7 promoter (Table 3). The sgRNA was cloned into bacterial plasmid under constitutive promoter. E. coli strain BW25141 (λDE3) was co-transformed with OMNI 4 and the corresponding guide and were co-expressed over-night by addition of 0.1mM IPTG during mid-exponential growth at 18 degrees Celsius. The cells were then pelleted and lysed, and the expression of each of OMNI 4 and OMNI 20 nucleases was detected by Western blot analysis using anti-HA antibodies.

Example 2B: PAM Depletion Assay of OMNI nucleases in bacterial system

[00434] To assay for functional PAMs, PAM depletion assay was performed. To this end, E. coli strain BW25141 (λDE3) were co-transformed with: (1) a library of plasmids containing a randomized PAM sequences of 8 N's flanked by a unique protospacer, (2) plasmids encoding the OMNI nuclease, and (3) a plasmid encoding gRNA targeting the protospacer of the library or a non-targeting gRNA as control. Next, cells were selected for all three plasmids by recovering them

on media containing appropriate antibiotics. Using this assay, Plasmids containing a PAM are cleaved and the cells that contain them could not grow, while cells containing plasmids with non-PAMs were able to propagate. By comparing the frequency of a sequence in the library after selection of the targeting guide relative to the non-targeting, individual PAM sequences could be
5 identified. The most depleted PAM sites of OMNI nuclease are presented in column 1 of Table 6B. Based on these results the suggested consensus PAM sequence for OMNI 4 is NRTAHNNN and the suggested consensus PAM sequence for OMNI 20 is NRRARNNN.

Table 6B. PAM sequences

OMNI 4		
Depleted target	Tested in bacterial	Tested in mammalian
NGCACNNN	TGCACTAA	TGCACTAA
NATACNNN	TATACGAA	TATACGAA
NGTACNNN		TGTACTAA
CGTANNNN		
OMNI 20		
Depleted target	Tested in bacterial	Tested in mammalian
NNAAACNN	TGGAAGCT	TGGAAGCT
NCAAANNN		AAAAAGCT
CGGANNNN		
NNGAAGNN		

10 ***Example 2C: Activity Assay in Bacteria***

[00435] Activity assay for a single target was performed as described for the depletion assay (see above) using a single PAM that was identified in the depletion assay (Table 6B, PAM tested in bacteria). Activity with the premature duplex guide RNA was confirmed as well in the same format.

15 ***Example 2D: In-vitro activity assay***

[00436] E. coli strain BW25141 (λDE3) co-expressing each of the OMNI nucleases and sgRNA were lysed using BugBuster™ lysis solution. The lysate was reacted in the recommended cleavage buffer with linear DNA substrates containing the PAM sequences flanked by a unique protospacer targeted by the sgRNA or a non-targeted protospacer as a control. Tested PAMs are summarized
20 in the 3rd column of Table 6B, and editing activity in comparison to SpCas9 was demonstrated.

Example 2E: OMNI expression in mammalian cells

[00437] An expression vector carrying HA tagged OMNI nucleases linked by P2A peptide to mCherry (pm-OMNI or pcDNA3.1, see details in Table 2 and 3), was introduced into 293T cells using the TurbofectTMtransfection reagent (Thermo Fisher). The Relative level of transcription for OMNI was determined using Flow cytometry. Cell were transfected with pm-OMNI OR PCDNA3.1 and a carrier plasmid expressing BFP. Transfection was done as described above and cells were subjected to Flow cytometry analysis 72 hours post transfection. The mCherry protein expressed from the P2A polycentric transcript served as an indication for OMNI nucleases transcript levels. The transcription levels of OMNI 4 and OMNI 20 was above 30% and approached 40% for 20T1-D2 (pmOMNI20), similar to the expression level of 0T1 pcDNA3.1 sp, as determined by the percentage of mCherry expressing cells out of the transfected population and after normalization to BFP levels to account for variation in transfection efficiency.

Example 3: OMNIs 4, 6-8, 10, 11, 13, 14, 16-24, 26, 27, and 29-31

[00438] CRISPR repeat (crRNA), transactivating crRNA (tracrRNA), nucleases polypeptide and PAM sequences were predicted from different metagenomic databases of sequences of environmental samples. The list of bacterial species/strains from which the CRISPR repeat, tracRNA sequence and nucleases polypeptide sequence where predicted is provided in Table 1 and designated an OMNI identification number.

Example 3A: Construction of OMNI-nuclease polypeptides.

[00439] The open reading frame of potential OMNI candidates that were codon optimized for *E.coli* and for Human cell line expression. The *E.coli* codon optimized ORF was cloned into bacterial plasmid, pb-NNC and the human codon optimized into pmOMNI plasmid as shown in Table 7 below, with column four of Table 7 showing, by way of example, the constructs for OMNI 4:

Table 7: Plasmids

Plasmid	Purpose	Elements	Example	SEQ ID NO:
pbNNC	Expressing OMNI polypeptide in the bacterial system	T7 promoter HA Tag-Linker-OMNI ORF (E.coli codon optimized) - T7 terminator	pbNNC OMNI4	

pbGuide T1/T2	Expressing OMNI sgRNA in the bacterial system	J23119 promoter - T1/T2 spacer sgRNA scaffold - rrnB T1 terminator	pbGuide OMNI4 T1 sgRNA V2	
pbPOS T1 library	Bacterial/TXTL depletion assay	T1 protospacer - 8N PAM library - chloramphenicol acetyltransferase	pbPOS T1 library	
pbPOS T1 PAM Site	Bacterial positive selection plasmid	LacYA177C - araC - araBAD promoter - CcdB - T1 ç - single PAM site	pbPOS T1 4D1 (OMNI4 PAM **TGCAC**TAA)	
pET9a	Expression and purification of OMNI proteins	T7 promoter - SV40 NLS - OMNI ORF (human codon optimized) - HA - SV40 NLS - 8 His-tag - T7 terminator	pET9a OMNI4	
pmOMNI	Expressing OMNI polypeptide in the mammalian system	CMV promoter - Kozak - SV40 NLS - OMNI ORF (human codon optimized) - HA - SV40 NLS - P2A - mCherry - bGH poly(A) signal	pmOMNI OMNI4	
pmGuide T1/Endogenic site	Expressing OMNI sgRNA in the mammalian system	U6 promoter - T1/T2 spacer sgRNA scaffold	pmGuide OMNI4 T1 sgRNA V4	
pMSS4c.2 T1	plasmid for OMNI activity in mammalian system	EF-1α core promoter - Kozak- T1 protospacer - GFP out of frame - bGH poly(A) signal - CMV promoter - BFP	pMSS4c.2 T1 4D1 (OMNI4 PAM **TGCAC**TAA)	

Example 3B: Prediction and construction of sgRNA.

[00440] For each OMNI the sgRNA was predicted by detection of the CRISPR repeat array sequence (crRNA) and a trans-activating crRNA (tracrRNA) in the bacterial genome. The native pre-mature crRNA and tracrRNA sequences were connected *in-silico* with tetra-loop 'gaaa' and

the secondary structure elements of the duplex were predicted by using an RNA Secondary Structure prediction Web Tool: available at rna.urmc.rochester.edu.

[00441] The predicted secondary structures of the full duplex RNA elements (crRNA-tracrRNA chimera) was used for identification of possible “Nexus” and “hairpins” the design of sgRNA for
5 each nuclease with various versions (*See, e.g.*, For example, **Fig. 1A**). By shortening the duplex at the upper stem at different locations, the crRNA and tracrRNA were connected with tetra-loop ‘gaaa’, thus generating possible sgRNA scaffolds (For example, **Fig. 1B**). At least 2 versions (labeled V1, V2, V3, etc.) of possible designed scaffolds for each OMNI was synthesized and 5’ connected to a 22 bps universal unique spacer sequence (SEQ ID NO:373 or 374) and was cloned
10 into a bacterial Guide expressing plasmid under the constitutive promoter and mammalian expressing plasmid under U6 promoter (pbGuide and pmGuide according to Table 7 above). #

The sgRNA designs for each OMNI are listed hereinbelow in Tables 8-12 below:

Table 8: crRNA and tracrRNA for V1 sgRNAs for each OMNI.

	crRNA:tracrRNA duplex V1		**Bacterial/Human**
OMNI Nuclease	**lower+upper stem of crRNA**	**upper+lower stem of tracrRNA**	**sgRNA V1**
OMNI 4	GUUUGAGAGUAA (SEQ ID NO: 108)	UUACGAGUUCAAAU (SEQ ID NO: 109)	GUUUGAGAGUAAgaaaUUACGAGUUCAAAUAACGAUUUAUCGACAUUACCACUUCGUGGUCAGGCGCACGUGUGUGCGCCGUUUUUUU (SEQ ID NO: 115)
OMNI 6	GUUUUAGAGUUA (SEQ ID NO:80)	UAACGAGUUAAAAU (SEQ ID NO:81)	GUUUUAGAGUUAgaaaUAACGAGUUAAAAUAAAGCUUUGCUUUAAUGCCAUUUUAAAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO: 87)
OMNI 7	GUUUGAGAGCAGUG (SEQ ID NO:230)	CACUUCGAGUUCAAAU (SEQ ID NO:231)	GUUUGAGAGCAGUGgaaaCACUUCGAGUUCAAAUAAAAGUUUACUCAAUUCACCGGUUUUUCCGGAAGCACAGUGUGUGCUAUUAAGCUUCUCGUUUGAGAAGC (SEQ ID NO:236)
OMNI 8	GUUUCGGAUGU (SEQ ID NO:239)	ACAUACAUCGAAAU (SEQ ID NO:240)	GUUUCGGAUGUgaaaACAUACAUCGAAAUAAAGCAAGACCUCGGUCGAGCUUCCUCGGACUUUAAGUCCAGCCUUAAGGCUUUU (SEQ ID NO:246)
OMNI 10	GUUGUGAAUUGCAUUCA (SEQ ID NO:249)	UGAAAGCAAUUCACAAU (SEQ ID NO:250)	GUUGUGAAUUGCAUUCAgaaaUGAAAGCAAUUCACAAUAAGGAUUAUUCCGUUGUGAAAACAUUUAAAGCGGUCUAUUUAGAUCGCUUUCUUUUUU (SEQ ID NO:256)

	crRNA:tracrRNA duplex V1		Bacterial/Human
OMNI Nuclease	**lower+upper stem of crRNA**	**upper+lower stem of tracrRNA**	**sgRNA V1**
OMNI 11	GCUUCAGAUGUC (SEQ ID NO:259)	GACAGUGUUGAAGU (SEQ ID NO:260)	GCUUCAGAUGUCgaaaGACAGUGUUGAAGUAACGCAAGGCGAAAGCCAAGCUUAUCACUUUUGGGCCCUAUGGCCAGUACAUAAAAAAGCCCCGCAAGUUUCCAACUUGCGGGGCUUUUAUUAACU (SEQ ID NO:266)
OMNI 13	GUUUGAGUGUCG (SEQ ID NO:143)	UGACGAGUUCAAAU (SEQ ID NO:144)	GUUUGAGUGUCGgaaaUGACGAGUUCAAAUAAGGCUUCGCCAAAAUCGUUGGGAAACCAACCUCACAGUGUUGUGAAAUUUAAAAAGACUUGCUUCGGCAGGUCUUUUUUUAUUUU (SEQ ID NO:149)
OMNI 14	GUUUUAUUAC (SEQ ID NO:268)	GUAAUUCAAAC (SEQ ID NO:269)	GUUUUAUUACgaaaGUAAUUCAAACCUAAUGCAAAAUAAUUAUAGCAAAUAUUUCUUAAAUAUAAAUUUAAUAAUACCUAAAUUUUU (SEQ ID NO:275)
OMNI 16	GUUUGAGAAUG (SEQ ID NO:277)	CAUUAGUUCAAAU (SEQ ID NO:278)	GUUUGAGAAUGgaaaCAUUAGUUCAAAUAAAAAUUUAUUCAAAUCGUCUUUGUGACUCCACAGUGUGUGGUAUAAGAACUUGCUUAUGCAGGUUCUUUUU (SEQ ID NO:284)
OMNI 17	GUUUGAGAGUAGUG (SEQ ID NO:157)	CACUGCAAGUUCAAAU (SEQ ID NO:158)	GUUUGAGAGUAGUGgaaaCACUGCAAGUUCAAAUAAUAUUAUUAUGAAAUCGUCGGCUAACCGACAAACACUGUGUGUGUUAUUAAAGAGUCUAUCUUUGAUAGACUUUUUAUUUUUCUA (SEQ ID NO:163)
OMNI 18	GUUUUAUAGUUG (SEQ ID NO:170)	CAACACAGUUAAAAU (SEQ ID NO:171)	GUUUUAUAGUUGgaaaCAACACAGUUAAAAUAAAGCUUAUGCUAAAGCGUCAGUAACAUGACAUCACUUAGGUGAUUAGAAAAAGUUCUUCGGAACUUUUUUU (SEQ ID NO:176)
OMNI 19	GUUUGAGAGCUA (SEQ ID NO:186)	UAGCAGGUUCAAAU (SEQ ID NO:187)	GUUUGAGAGCUAgaaaUAGCAGGUUCAAAUAAGCUUUACAGCGAACUUAUCGUCUUAGACGAAUUCACAGUGUGUGAGCAGAAACUUCCGAAAGGAAGUUUUUGUUUU (SEQ ID NO:193)
OMNI 20	GUUUGAGAGUAG (SEQ ID NO:129)	CUACGAGUUCAAAU (SEQ ID NO:130)	GUUUGAGAGUAGgaaaCUACGAGUUCAAAUAAAAAUUAUUUCAAAUCGUUCUUUAUGUUCGCACAAGAGUUGUGCAUUUUAUUUGUAACUUUUGCCUGCUUUUUUAAGCAGGCUUUUUAUUUU (SEQ ID NO:135)
OMNI 21	GUUUCGGAGCAG (SEQ ID NO:286)	CUGCAAGUCGAAAU (SEQ ID NO:287)	GUUUCGGAGCAGgaaaCUGCAAGUCGAAAUACGGCGAUAGCCACAAAAGCCCUCGGGCGCCACGGAGGUGGCAAAUUCGACUUAUUGAGAAGAUCCCCUUGAGGGUCUUCUUUUUU (SEQ ID NO:293)

	crRNA:tracrRNA duplex V1		Bacterial/Human
OMNI Nuclease	**lower+upper stem of crRNA**	**upper+lower stem of tracrRNA**	**sgRNA V1**
OMNI 22	GCUGCGGUUUGCGGCCGCA (SEQ ID NO:295)	UGUGGCGCCGGCCGUCGCG (SEQ ID NO:296)	GCUGCGGUUUGCGGCCGCAgaaaUGUGGCGCCGGCCGUCGCGGCGAAGGCAACCCUCGCCCCGCUCGCGCUCGGCCGCUUGCCGCAGGCAAAACAAAACGUCCACACCUGGUGCGGGCUGGGCCUCCCGCGGACGAUCGGGCCCGUCGACCCCCGCCUCGCGCGGGGGUCGUGUUUC (SEQ ID NO:300)
OMNI-23	GUUGCGGCUGGACCC (SEQ ID NO:199)	GGGUCCUUCCGUUAAC (SEQ ID NO:200)	GUUGCGGCUGGACCCgaaaGGGUCCUUCCGUUAACACGUCGCGGGGGUUUGCCCCUGACGGCACAAGAUGUGCGGCGGGCUUCGGCCCGCCGCGUUCCUUUU (SEQ ID NO:206)
OMNI-24	GUUGUGAAUUGCUUUCA (SEQ ID NO:214)	UGAAGCAAUUCACAAU (SEQ ID NO:215)	GUUGUGAAUUGCUUUCAgaaaUGAAGCAAUUCACAAUAAGGAUUAUUCCGUUGUGAAAACAUUCAAAGCGGCCUUAACGGGUCGCUUUUUU (SEQ ID NO:221)
OMNI-26	GUUUUAGAACU (SEQ ID NO:302)	AGUUAAGUUAAAAU (SEQ ID NO:303)	GUUUUAGAACUgaaaAGUUAAGUUAAAAUAAGCUUAAAGCGUUAAAGUGGUACUCAUACCAACACCUCACUGUGUCAGUGAGGUUUUUAUUUUUUU (SEQ ID NO:308)
OMNI-27	GUUUGAGAGUAG (SEQ ID NO:310)	CUACAAGUUCAAAU (SEQ ID NO:311)	GUUUGAGAGUAGgaaaCUACAAGUUCAAAUAACGAUUUAUCGAAAUCGUCCGAAAGGACUAAUCACAGUGUGAUUAUAAGGGACUCGCCUCUGGCGAGUUUUUU (SEQ ID NO:317)
OMNI-29	GUUGUGAAUUGAUUUCAAAUUGUAUUUUUA (SEQ ID NO:320)	UAGAACAAUUUGAAAUCAAUUCACAAU (SEQ ID NO:321)	GUUGUGAAUUGAUUUCAAAUUGUAUUUUUAgaaaUAGAACAAUUUGAAAUCAAUUCACAAUAAGGAUUAUUCCGUUGUGAAAACAUUUAAAGCGGCAUCAUAGAAAUUAUGAUGUCGCUUUCUCUUUCUUAAAUUUUU (SEQ ID NO:327)
OMNI-30	GUUUGAGAAUGG (SEQ ID NO:329)	CCAUAAGUUCAAAU (SEQ ID NO:330)	GUUUGAGAAUGGgaaaCCAUAAGUUCAAAUAAAAAUUUAUUCAAAUCGCUUUUAUAGCUUCCACGUGUGUGGAUUUAAAACUUGCAAAUGCAAGUUUUUUU (SEQ ID NO:336)
OMNI-31	GUUGUGAUUUGCUUUCA (SEQ ID NO:338)	UGAAGCAAAUCACAAU (SEQ ID NO:339)	GUUGUGAUUUGCUUUCAgaaaUGAAGCAAAUCACAAUAAGGAUUAUUCCGUUGUGAAAACAUUCAAGGUGGUACUCUAAAAAGUAUCACCUUUUUUUU (SEQ ID NO:345)

Table 9: crRNA and tracrRNA for V2 sgRNAs for each OMNI.

	crRNA:tracrRNA duplex V2		**Bacterial/Human**
OMNI Nuclease	**lower+upper stem of crRNA**	**(upper+lower stem of tracrRNA)**	**sgRNA V2**
OMNI 4	GUUUGAGAGUAAUG (SEQ ID NO:110)	CAUUACGAGUUCAAAU (SEQ ID NO:111)	GUUUGAGAGUAAUGgaaaCAUUACGAGUUCAAAUAACGAUUUAUCGACAUUACCACUUCGUGGUCAGGCGCACGUGUGUGCGCCGUUUUUUU (SEQ ID NO:116)
OMNI 6	GUUUUAGAGUUAUGU (SEQ ID NO:82)	AUAUAACGAGUUAAAAU (SEQ ID NO:83)	GUUUUAGAGUUAUGUgaaaAUAUAACGAGUUAAAAUAAAGCUUUGCUUUAAUGCCAUUUUAAAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:88)
OMNI 7	GUUUGAGAGCAGUGUA (SEQ ID NO:232)	UACACUUCGAGUUCAAAU (SEQ ID NO:233)	GUUUGAGAGCAGUGUAgaaaUACACUUCGAGUUCAAAUAAAAGUUUACUCAAUUCACCGGUUUUUCCGGAAGCACAGUGUGUGCUAUUAAGCUUCUCGUUUGAGAAGC (SEQ ID NO:237)
OMNI 8	GUUUCGGAUGUCU (SEQ ID NO:241)	AGACAUACAUCGAAAU (SEQ ID NO:242)	GUUUCGGAUGUCUgaaaAGACAUACAUCGAAAUAAAGCAAGACCUCGGUCGAGCUUCCUCGGACUUUAAGUCCAGCCUUAAGGCUUUU (SEQ ID NO:247)
OMNI 10	GUUGUGAAUUGCAUUCAAA (SEQ ID NO:251)	UUUGAAAGCAAUUCACAAU (SEQ ID NO:252)	GUUGUGAAUUGCAUUCAAAgaaaUUUGAAAGCAAUUCACAAUAAGGAUUAUUCCGUUGUGAAAACAUUUAAAGCGGUCUAUUUAGAUCGCUUUCUUUUUU (SEQ ID NO:257)
OMNI 11	GCUUCAGAUGUCUG (SEQ ID NO:261)	CAGACAGUGUUGAAGU (SEQ ID NO:262)	GCUUCAGAUGUCUGgaaaCAGACAGUGUUGAAGUAACGCAAGGCGAAAGCCAAGCUUAUCACUUUUGGGCCCUAUGGCCAGUACAUAAAAAAGCCCCGCAAGUUUCCAACUUGCGGGGCUUUUAUUAACU (SEQ ID NO:267)
OMNI 13	GUUUGAGUGUCGUG (SEQ ID NO:145)	CAUGACGAGUUCAAAU (SEQ ID NO:146)	GUUUGAGUGUCGUGgaaaCAUGACGAGUUCAAAUAAGGCUUCGCCAAAAUCGUUGGGAAACCAACCUCACAGUGUUGUGAAAUUUAAAAAGACUUGCUUCGGCAGGUCUUUUUUUAUUUU (SEQ ID NO:150)
OMNI 14	GUUUUAUUACCA (SEQ ID NO:270)	UGGUAAUUCAAAC (SEQ ID NO:271)	GUUUUAUUACCAgaaaUGGUAAUUCAAACCUAAUGCAAAAUAAUUAUAGCAAAUAUUUCUUAAAUAUAAAUUUAAUAAUACCUAAAUUUUU (SEQ ID NO:276)
OMNI 16	GUUUGAGAAUGGU (SEQ ID NO:279)	ACCAUUAGUUCAAAU (SEQ ID NO:280)	GUUUGAGAAUGGUgaaaACCAUUAGUUCAAAUAAAAAUUUAUUCAAAUCGUCUUUGUGACUCCACAGUGUGUGGUAUAAGAACUUGCUUAUGCAGGUUCUUUUU (SEQ ID NO:285)

	crRNA:tracrRNA duplex V2		Bacterial/Human
OMNI Nuclease	**lower+upper stem of crRNA**	**(upper+lower stem of tracrRNA)**	**sgRNA V2**
OMNI 17	GUUUGAGA GUAGUGUA (SEQ ID NO:159)	UACACUGC AAGUUCAA AU (SEQ ID NO:160)	GUUUGAGAGUAGUGUAgaaaUACACUGCAAG UUCAAAUAAUAUUAUUAUGAAAUCGUCGG CUAACCGACAAACACUGUGUGUGUUAUUA AAGAGUCUAUCUUUGAUAGACUUUUUAUU UUUCUA (SEQ ID NO:164)
OMNI 18	GUUUUAUA GUUGUG (SEQ ID NO:172)	CACAACAC AGUUAAAA U (SEQ ID NO:173)	GUUUUAUAGUUGUGgaaaCACAACACAGUUA AAAUAAAGCUUAUGCUAAAGCGUCAGUAA CAUGACAUCACUUAGGUGAUUAGAAAAAG UUCUUCGGAACUUUUUUU (SEQ ID NO:177)
OMNI 19	GUUUGAGA GCUAUG (SEQ ID NO:188)	CAUAGCAG GUUCAAAU (SEQ ID NO:189)	GUUUGAGAGCUAUGgaaaCAUAGCAGGUUCA AAUAAGCUUUACAGCGAACUUAUCGUCUU AGACGAAUUCACAGUGUGUGAGCAGAAAC UUCCGAAAGGAAGUUUUUGUUUU (SEQ ID NO:194)
OMNI 20	GUUUGAGA GUAGUG (SEQ ID NO:131)	CACUACGA GUUCAAAU (SEQ ID NO:132)	GUUUGAGAGUAGUGgaaaCACUACGAGUUCA AAUAAAAAUUAUUUCAAAUCGUUCUUUAU GUUCGCACAAGAGUUGUGCAUUUUAUUUG UAACUUUUGCCUGCUUUUUUAAGCAGGCU UUUUAUUUU (SEQ ID NO:136)
OMNI 21	GUUUCGGA GCAGUG (SEQ ID NO:288)	CACUGCAA GUCGAAAU (SEQ ID NO:289)	GUUUCGGAGCAGUGgaaaCACUGCAAGUCGA AAUACGGCGAUAGCCACAAAAGCCCUCGGG CGCCACGGAGGUGGCAAAUUCGACUUAUU GAGAAGAUCCCCUUGAGGGUCUUCUUUUU U (SEQ ID NO:294)
OMNI 22	GCUGCGGU UUGCGGCC GCACC (SEQ ID NO:297)	GGUGUGGC GCCGGCCG UCGCG (SEQ ID NO:298)	GCUGCGGUUUGCGGCCGCACCgaaaGGUGUG GCGCCGGCCGUCGCGGCGAAGGCAACCCUC GCCCCGCUCGCGCUCGGCCGCUUGCCGCAG GCAAAACAAAACGUCCACACCUGGUGCGGG CUGGGCCUCCCGCGGACGAUCGGGCCCGUC GACCCCCGCCUCGCGCGGGGGUCGUGUUUU C (SEQ ID NO:301)
OMNI-23	GUUGCGGC UGGACCCC G (SEQ ID NO:201)	CGGGGUCC UUCCGUUA AC (SEQ ID NO:202)	GUUGCGGCUGGACCCCGgaaaCGGGGUCCUU CCGUUAACACGUCGCGGGGGUUUGCCCCUG ACGGCACAAGAUGUGCGGCGGGCUUCGGCC CGCCGCGUUCCUUUU (SEQ ID NO:207)
OMNI-24	GUUGUGAA UUGCUUUC AGA (SEQ ID NO:216)	UCUGAAGC AAUUCACA AU (SEQ ID NO:217)	GUUGUGAAUUGCUUUCAGAgaaaUCUGAAGC AAUUCACAAUAAGGAUUAUUCCGUUGUGA AAACAUUCAAAGCGGCCUUAACGGGUCGCU UUUUU (SEQ ID NO:222)
OMNI-26	GUUUUAGA ACUAU	AUAGUUAA GUUAAAAU	GUUUUAGAACUAUgaaaAUAGUUAAGUUAA AAUAAGCUUAAAGCGUUAAAGUGGUACUC

	crRNA:tracrRNA duplex V2		Bacterial/Human
OMNI Nuclease	**lower+upper stem of crRNA** (SEQ ID NO:304)	**(upper+lower stem of tracrRNA)** (SEQ ID NO:305)	**sgRNA V2** AUACCAACACCUCACUGUGUCAGUGAGGUUUUUAUUUUUUU (SEQ ID NO:309)
OMNI-27	GUUUGAGAGUAGUG (SEQ ID NO:312)	CACUACAAGUUCAAAU (SEQ ID NO:313)	GUUUGAGAGUAGUGgaaaCACUACAAGUUCAAAUAACGAUUUAUCGAAAUCGUCCGAAAGGACUAAUCACAGUGUGAUUAUAAGGGACUCGCCUCUGGCGAGUUUUUU (SEQ ID NO:318)
OMNI-29	GUUGUGAAUUGAUUUCAAAUUGUAUUUUUACG (SEQ ID NO:322)	UGUAGAACAAUUUGAAAUCAAUUCACAAU (SEQ ID NO:323)	GUUGUGAAUUGAUUUCAAAUUGUAUUUUUACGgaaaUGUAGAACAAUUUGAAAUCAAUUCACAAUAAGGAUUAUUCCGUUGUGAAAACAUUUAAAGCGGCAUCAUAGAAAUUAUGAUGUCGCUUUCUCUUUCUUAAAUUUUU (SEQ ID NO:328)
OMNI-30	GUUUGAGAAUGGUG (SEQ ID NO:331)	CACCAUAAGUUCAAAU (SEQ ID NO:332)	GUUUGAGAAUGGUGgaaaCACCAUAAGUUCAAAUAAAAAUUUAUUCAAAUCGCUUUUAUAGCUUCCACGUGUGUGGAUUUAAAACUUGCAAAUGCAAGUUUUUUU (SEQ ID NO:337)
OMNI-31	GUUGUGAUUUGCUUUCAGA (SEQ ID NO:340)	UCUGAAGCAAAUCACAAU (SEQ ID NO:341)	GUUGUGAUUUGCUUUCAGAgaaaUCUGAAGCAAAUCACAAUAAGGAUUAUUCCGUUGUGAAAACAUUCAAGGUGGUACUCUAAAAAGUAUCACCUUUUUUUU (SEQ ID NO:346)

Table 10: Additional sgRNA designs for OMNI 6

OMNI 6 sgRNA	Sequence
sgRNA v3	GUUU**A**AGAGUUAUGUgaaaAUAUAACGAGUU**U**AAAUAAAGCUUUGCUUUAAUGCCAUUUUAAAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:89)
sgRNA v4	GUUU**A**AGAGUUAUGUgaaaAUAUAACGAGUU**U**AAAUAAAGCUUUGCUUUAAUGCCAU**A**UUA**U**AUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:90)
sgRNA v5	GUUU**A**AGAGUUAUG<u>UAA</u>gaaa<u>UUA</u>UAUAACGAGUU**U**AAAUAAAGCUUUGCUUUAAUGCCAU**A**UUA**U**AUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:91)

Table 11: Additional sgRNA designs for OMNI 18.

OMNI 18 sgRNA	Sequence

sgRNA v3	GUUU**A**AUAGUUGUGgaaaCACAACACAGUU**U**AAAUAAAGCUUAUGCUAAAGCGUCAGUAACAUGACAUCACUUAGGUGAUUAGAAAAAGUUCUUCGGAACUUUUUUU (SEQ ID NO:178)
sgRNA v4	GUUU**A**AUAGUUGUG**UUA**gaaa**UAA**CACAACACAGUU**U**AAAUAAAGCUUAUGCUAAAGCGUCAGUAACAUGACAUCACUUAGGUGAUUAGAAAAAGUUCUUCGGAACUUUUUUU (SEQ ID NO:179)

Table 12: Additional sgRNA designs for OMNI 20.

OMNI 20 sgRNA	Sequence
sgRNA v3	GUUUGAGAGUAGUG**UAA**gaaa**UUA**CACUACGAGUUCAAAUAAAAAUUAUUUCAAAUCGUUCUUUAUGUUCGCACAAGAGUUGUGCA**A**UUUAUUUGUAACUU**A**UGCCUGCUU**AA**UUAAGCAGGCUUUUUAUUUU (SEQ ID NO:137)

[00442] Additionally, conserved nexus and hairpin regions for sgRNAs for OMNI nucleases were identified as indicated in Table 13 below, with each nexus region indicated in bold and each hairpin region indicated in underline.

5 Table 13: nexus and hairpin regions for sgRNAs for OMNI nucleases

OMNI Nuclease	sgRNA nexus region	sgRNA hairpin region	nexus and hairpins identified on tracr
OMNI 4	**CGAUUUAUCG** (SEQ ID NO:113)	ACCACUUCGUGGUCAGGCGCACGUGUGUGCGCCG (SEQ ID NO:114)	AA**CGAUUUAUCG**ACAUUACCACUUCGUGGUCAGGCGCACGUGUGUGCGCCGUUUUUUU (SEQ ID NO:112)
OMNI 6	**AAGCUUUGCUU** (SEQ ID NO:85)	AUGCCAUUUUAAAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUA (SEQ ID NO:86)	A**AAGCUUUGCUU**UAAUGCCAUUUUAAAUGGUAUCACAUAGGUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU (SEQ ID NO:84)
OMNI 7	**AGUUUACU**	CCGGUUUUUCCGGAAGCACAGUGUGUGCUAUUAAGCUUCUCGUUUGAGAAGC (SEQ ID NO:235)	AAA**AGUUUACU**CAAUUCACCGGUUUUUCCGGAAGCACAGUGUGUGCUAUUAAGCUUCUCGUUUGAGAAGC (SEQ ID NO:234)

OMNI Nuclease	sgRNA nexus region	sgRNA hairpin region	nexus and hairpins identified on tracr
OMNI 8	**AAGCAAGACCUCGGUCGAGCUU** (SEQ ID NO:244)	GGACUUUAAGUCCAGCCUUAAGGCU (SEQ ID NO:245)	A**AAGCAAGACCUCGGUCGAGCUU**CCUCGGACUUUAAGUCCAGCCUUAAGGCUUUUU (SEQ ID NO:243)
OMNI 10	**GGAUUAUUCC** (SEQ ID NO:254)	UGUGAAAACAUUUAAAGCGGUCUAUUUAGAUCGCUUU (SEQ ID NO:255)	AA**GGAUUAUUCC**GUUGUGAAAACAUUUAAAGCGGUCUAUUUAGAUCGCUUUCUUUUUUU (SEQ ID NO:253)
OMNI 11	**GCAAGGCGAAAGC** (SEQ ID NO:264)	GGCCCUAUGGCCAGUACAUAAAAAAGCCCCGCAAGUUUCCAACUUGCGGGGCUUUU (SEQ ID NO:265)	AAC**GCAAGGCGAAAGC**CAAGCUUAUCACUUUUGGGCCCUAUGGCCAGUACAUAAAAAAGCCCCGCAAGUUUCCAACUUGCGGGGCUUUUAUUAACU (SEQ ID NO:263)
OMNI 13	**GGCUUCGCC**	GUUGGGAAACCAACCUCACAGUGUUGUGAAAUUUAAAAAGACUUGCUUCGGCAGGUCUUUUUUUAU (SEQ ID NO:148)	AA**GGCUUCGCC**AAAAUCGUUGGGAAACCAACCUCACAGUGUUGUGAAAUUUAAAAAGACUUGCUUCGGCAGGUCUUUUUUUAUUUU (SEQ ID NO:147)
OMNI 14	**UAAUGCAAAAUAA** (SEQ ID NO:273)	AUAUUUCUUAAAUAUAAAUUUAAUAAUACCUAAAU (SEQ ID NO:274)	**CUAAUGCAAAAUAA**UUAUAGCAAAUAUUUCUUAAAUAUAAAUUUAAUAAUACCUAAAUUUUU (SEQ ID NO:272)
OMNI 16	**AUUUAUUCAAAU** (SEQ ID NO:282)	GUCUUUGUGACUCCACAGUGUGUGGUAUAAGAACUUGCUUAUGCAGGUUCUU (SEQ ID NO:283)	AAAA**AUUUAUUCAAAU**CGUCUUUGUGACUCCACAGUGUGUGGUAUAAGAACUUGCUUAUGCAGGUUCUUUUU (SEQ ID NO:281)
OMNI 17	**AUUAUUAU**	GUCGGCUAACCGACAAACACUGUGUGUGUUAUUAAAGAGUCUAUCUUUGAUAGACUUUUUA (SEQ ID NO:162)	AAU**AUUAUUAU**GAAAUCGUCGGCUAACCGACAAACACUGUGUGUGUUAUUAAAGAGUCUAUCUUUGAUAGACUUUUUAUUUUUCUA (SEQ ID NO:161)
OMNI 18	**AGCUUAUGC**	GUCAGUAACAUGACAUCACUUAGGUGA	AA**AGCUUAUGC**UAAAGCGUCAGUAACAUGACAUCACUUAGGUGAUUA

OMNI Nuclease	sgRNA nexus region	sgRNA hairpin region	nexus and hairpins identified on tracr
		UUAGAAAAAGUUCUUCGGAACUUUUUUU (SEQ ID NO:175)	GAAAAAGUUCUUCGGAACUUUUUUU (SEQ ID NO:174)
OMNI 19	**GCUUUACAGC** (SEQ ID NO:191)	UCGUCUUAGACGAAUUCACAGUGUGUGAGCAGAAACUUCCGAAAGGAAGUUUUUGU (SEQ ID NO:192)	AA**GCUUUACAGC**GAACUUAUCGUCUUAGACGAAUUCACAGUGUGUGAGCAGAAACUUCCGAAAGGAAGUUUUUGUUUU (SEQ ID NO:190)
OMNI 20	--	GCACAAGAGUUGUGCAUUUUAUUUGUAACUUUUGCCUGCUUUUUUAAGCAGGC (SEQ ID NO:134)	AAAAAUUAUUUCAAAUCGUUCUUUAUGUUCGCACAAGAGUUGUGCAUUUUAUUUGUAACUUUUGCCUGCUUUUUUAAGCAGGCUUUUUAUUUU (SEQ ID NO:133)
OMNI 21	**GGCGAUAGCC** (SEQ ID NO:291)	GCCCUCGGGCGCCACGGAGGUGGCAAAUUCGACUUAUUGAGAAGAUCCCCUUGAGGGUCUUCUU (SEQ ID NO:292)	AC**GGCGAUAGCC**ACAAAAGCCCUCGGGCGCCACGGAGGUGGCAAAUUCGACUUAUUGAGAAGAUCCCCUUGAGGGUCUUCUUUUUU (SEQ ID NO:290)
OMNI 22	--	--	GCGAAGGCAACCCUCGCCCCGCUCGCGCUCGGCCGCUUGCCGCAGGCAAAACAAAACGUCCACACCUGGUGCGGGCUGGGCCUCCCGCGGACGAUCGGGCCCGUCGACCCCCGCCUCGCGCGGGGGUCGUGUUUUC (SEQ ID NO:299)
OMNI-23	**GUCGCGGGGGUUUGCCCCUGACGGC** (SEQ ID NO:204)	GAUGUGCGGCGGGCUUCGGCCCGCCGCGUUC (SEQ ID NO:205)	AC**GUCGCGGGGGUUUGCCCCUGACGGC**ACAAGAUGUGCGGCGGGCUUCGGCCCGCCGCGUUCCUUUU (SEQ ID NO:203)
OMNI-24	**GGAUUAUUCC** (SEQ ID NO:219)	GAAAACAUUCAAAGCGGCCUUAACGGGUCGCUU (SEQ ID NO:220)	AA**GGAUUAUUCC**GUUGUGAAAACAUUCAAAGCGGCCUUAACGGGUCGCUUUUUU (SEQ ID NO:218)
OMNI-26	**GCUUAAAGC**	UGGUACUCAUACCAACACCUCACUGUGU	AA**GCUUAAAGC**GUUAAAGUGGUACUCAUACCAACACCUCACUGUGUC

OMNI Nuclease	sgRNA nexus region	sgRNA hairpin region	nexus and hairpins identified on tracr
		CAGUGAGGU (SEQ ID NO:307)	AGUGAGGUUUUUAUUUUUUU (SEQ ID NO:306)
OMNI-27	**CGAUUUAUCG** (SEQ ID NO:315)	UCCGAAAGGACUAAUCACAGUGUGAUUAUAAGGGACUCGCCUCUGGCGAGUUUUUU (SEQ ID NO:316)	AA**CGAUUUAUCG**AAAUCGUCCGAAAGGACUAAUCACAGUGUGAUUAUAAGGGACUCGCCUCUGGCGAGUUUUUU (SEQ ID NO:314)
OMNI-29	**GGAUUAUUCC** (SEQ ID NO:325)	UGUGAAAACAUUUAAAGCGGCAUCAUAGAAAUUAUGAUGUCGCUUUCUCUUUCUUAA (SEQ ID NO:326)	AA**GGAUUAUUCC**GUUGUGAAAACAUUUAAAGCGGCAUCAUAGAAAUUAUGAUGUCGCUUUCUCUUUCUUAAAUUUUU (SEQ ID NO:324)
OMNI-30	**AUUUAUUCAAAU** (SEQ ID NO:334)	GCUUCCACGUGUGUGGAUUUAAAACUUGCAAAUGCAAGUUUU (SEQ ID NO:335)	AAAA**AUUUAUUCAAAU**CGCUUUUAUAGCUUCCACGUGUGUGGAUUUAAAACUUGCAAAUGCAAGUUUUUUU (SEQ ID NO:333)
OMNI-31	**GGAUUAUUCC** (SEQ ID NO:343)	GAAAACAUUCAAGGUGGUACUCUAAAAAGUAUCACCU (SEQ ID NO:344)	AA**GGAUUAUUCC**GUUGUGAAAACAUUCAAGGUGGUACUCUAAAAAGUAUCACCUUUUUUUU (SEQ ID NO:342)

[00443] At least 2 versions of possible designed scaffolds for each OMNI were synthesized and the 5' connected of each scaffold connected to a 22 bps universal unique spacer sequence (T1 "GGTGCGGTTCACCAGGGTGTCG" - SEQ ID NO:373 and T2 "GGAAGAGCAGAGCCTTGGTCTC" – SEQ ID NO:374) and were cloned into a bacterial Guide expressing plasmids under the constitutive promoter and mammalian expressing plasmid under U6 promoter (pbGuide and pmGuide accordantly, Table 7 above). #

Example 3C: Bacterial PAM Depletion Assay.

[00444] To confirm that each of the identified loci are functional CRISPR–OMNI nuclease systems and to identify their PAMs, *E.coli* strain BW25141 (λDE3) were co-transformed with: (1) a library of plasmid pool containing a randomized PAM sequences of 8 N's flanking a unique protospacer (pbPOS T1 library, Table 7 above), (2) plasmids encoding *E.coli* codon-optimized OMNI nucleases, pbNNC (Table 7) and (3) a plasmid encoding designed sgRNA targeting the protospacer of the library or a non-targeting gRNA as control (pbGuide, T1 and T2 respectively,

Table 7). Next, cells were selected for all three plasmids by recovering them on media containing appropriate antibiotics. In this assay, plasmids containing a PAM are cleaved and the cells that contain them cannot grow, while cells containing plasmids with non-PAMs are able to propagate. Survived plasmid DNA pool was isolated, and the library was sequenced using a 75-cycle NextSeq kit (Illumina). PAM representation in the library was determined using a custom script and compared between OMNI and control. By comparing the frequency of a sequence in the library after selection of the targeting guide relative to a non-targeting guide, individual PAM sequences could be identified (**Fig. 2A – Fig. 13B**). The presented data reflects a condensed 4N window library with all possible locations along the 8bp sequence. Sequence motifs were generated using the Weblogo tool (weblogo.berkeley.edu). Activity of the OMNI nuclease was estimated based on the average of the two most depleted sequences and was calculated as:1 – Depletion score (Depletion score - Average of the ratios from 2 most depleted sites). OMNI nucleases with scores that are higher than 0.6 were considered as active. Following deep sequencing we detected depletion in 12 of the 22 tested OMNI systems, indicating functional DNA interference in a heterologous host (**Fig. 2A – Fig. 13B**). PAM sites and depletion assay results for each OMNI nuclease are shown in Table 14 below.

Table 14: OMNI Bacterial PAM Depletion Assay results.

	Bacterial Depletion		
Name	**PAM General**	**PAM Specific**	**Activity (1-Depletion score)***
OMNI 4	NVYAH	YGTAM	1.00
OMNI 6	NGGNN	NGGNM	1.00
OMNI 7	Unknown		
OMNI 8	NGGNN	NGGYK	0.99
OMNI 10	NVVHHY	NRRTTT	0.77
OMNI 11	NNGWWB	NHGWWY	0.96
OMNI 13	NVNVMY	NRNACY	0.99
OMNI 14	Unknown		

OMNI 16	Unknown		
OMNI 17	NNYVVH	NNYAAH	0.95
OMNI 18	NGGNN	NGGNM	0.994
OMNI 19	NRTAN	NRTAN	1.00
OMNI 20	NVVRR	NRRRR	0.63
OMNI 21	Unknown		
OMNI 22	Unknown		
OMNI 23	NGGNR	NGGNG	0.98
OMNI 24	NVRNH	NRRNC	0.978
OMNI 26	Unknown		
OMNI 27	Unknown		
OMNI 29	Unknown		
OMNI 30	Unknown		
OMNI 31	Unknown		

Example 3D: In-vitro Depletion assay by TXTL.

[00445] Depletion of PAM sequences in-vitro was followed by Maxwell *et al.* (2018) Methods. Briefly, linear DNA expressing the OMNI nucleases and the sgRNAs under T7 promoter were added to a TXTL mix (Arbor Bioscience) together with linear construct expressing T7 polymerase. RNA expression and protein translation by the TXTL mix result in the formation of the RNP complex. Since linear DNA was used, Chi6 sequences, a RecBCD inhibitor were added to protect the DNA from degradation. The sgRNA spacer is designed to target a library of plasmids containing the targeting protospacer (pbPOS T1 library, Table 7) flanked by an 8N' randomized set of potential PAM sequences. Depletion of PAM sequences from the library is measured by adding the adapters and indices necessary for high-throughput sequencing using PCR to both the

cleaved library and to a control library expressing a non-targeting gRNA. Following deep sequencing we confirm the activity in-vitro by depletion in OMNI 4, OMNI 6, OMNI 8, OMNI 10, OMNI 13, OMNI 17, OMNI 18, OMNI 19, OMNI 20, OMNI 23, and OMNI 24 showing similar PAM pattern as discovered in *E.coli* (**Figure 14 – Figure 25**). Furthermore, we discover activity in 4 new OMNI nucleases (OMNI 16, OMNI 21, OMNI 27 and OMNI 30), indicating functional DNA interference by in-vitro system (**Fig. 26A – Fig. 29B**). PAM sites and depletion assay results for each tested OMNI nuclease are shown in Table 15 below.

Table 15: OMNI TXTL PAM Depletion Assay results.

Name	**PAM General**	**PAM Specific**	**Activity (1-Depletion score)***	**sgRNA**
OMNI 4	NNNWH	NNCAM	0.92	V2
OMNI 6	NGG	NGG	0.98	V2
OMNI 8	NGGN	NGGY	0.79	V2
OMNI 10	NVVHHY	NRRTTT	0.62	V2
OMNI 13	NVDVMY	NRKACY	0.98	V2
OMNI 16	NRHRNY	NRHAAC	0.88	V2
OMNI 17	NNYAAH	NNYAAH	0.95	V2
OMNI 18	NGG	NGG	0.99	V2
OMNI 19	NRTAN	NRTAN	0.95	V2
OMNI 20	NVVRV	NRRAV	0.85	V2
OMNI 21	NRAVR	NAARG	0.60	V1
OMNI 23	NGGNR	NGGNG	0.98	V2
OMNI 24	NVRNC	NRRNC	0.86	V2
OMNI 27	NNDVYY	NNDAYT	0.95	V1, V2
OMNI 30	NNNVYT	NNNACT	0.97	V1, V2

Example 3D: Verification of PAM by bacterial survival assay and in vitro activity assay in crude lysate.

[00446] The identified PAM sites of OMNI nucleases were further cloned downstream to the protospacer T1 into a positive selection plasmid harboring a toxic gene CcdB under control of BAD promoter (pbPOS T1 PAM site, Table 7). Electro-competent cells were prepared from Escherichia coli strain BW25141 (λDE3) co-transformed with each OMNI nuclease and the positive selection plasmid. Thus, upon transformation of the targeting guide (T1 spacer, pbGuide T1), OMNI nucleases that cleave the positive plasmid can survive in the presence of Arabinose, whereas non-targeting guide (pbGuide T2) cannot. Following a 60 min recovery in TB media with 1mM IPTG, transformations were plated on selective TB plates containing Carbomycin and 15 mM arabinose and non-selective plates. The plates were incubated over night at 37C and the next morning single colonies were counted. A correct composition of the identified PAM sites with the targeting guide and each OMNI nuclease was represented by growth on selective plates in which the positive selection plasmid was cleaved together with the toxic gene (**Fig. 30A-30C**). Results of the bacterial survival assay are shown in Table 16 below.

[00447] OMNI nucleases were also tested for *in vitro* cleavage of different PAM sites. *E. coli* strain BW25141 (λDE3) co-expressing the OMNI nucleases and sgRNA were lysed using BugBuster lysis solution. The lysate was reacted in the recommended cleavage buffer with linear DNA substrates containing the PAM sequences flanked by a unique protospacer targeted by the sgRNA (T1) or a non-targeted protospacer (T2) as a control (**Fig. 31A-C**). Results of the bacterial survival assay and *in vitro* activity assay are shown in Table 16 below.

Table 16: Bacterial survival assay and in vitro activity assay results.

		PAM verification			
Name	**sgRNA**	**Seq name**	**PAM**	**Survival**	***In vitro* cleavage assay**
OMNI-4	V2	4D1	**TGCAC**TAA	active	
		4D2	**TATAC**GAA	active	active
OMNI-6	V2	6D1	T**GG**TCCGC	active	active
		6D2	A**GG**ACCTC	active	active
		1	C**GG**TCGAA		
OMNI-8	V2	8D1	T**GGTT**GAT	active	active

OMNI-10	V2	10D1	CAGTTTAA	active	not-active
		10D2	CAATTTAA	active	
OMNI-11	V2	11D1	GTGTACTC	active	active
		11D2	GTGTTCTC	active	active
OMNI-13	V2	4D1	TGCACTAA	active	
		6D2	AGGACCTC	active	
OMNI-17	V2	17D1	GGTAATAG	active	
		17D2	GGCAAAAG	active	
OMNI-18	V2	0	TGGAAGCT	active	
		6D1	TGGTCCGC		active
		8D1	TGGTTGAT		active
		1	CGGTCGAA		
OMNI-19	V2	4D1	TGCACTAA	active	
		4D2	TATACGAA	active	
OMNI-20	V2	0	TGGAAGCT	active	active
		20D2	AAAAAGCT		
		2D4	CGGGAGAG		active
		3D2	TAAGGTCC		active
		6D1	TGGTCCGC		partially
		10	GGATGAT		partially
OMNI-23	V2	6D2	AGGACCTC		
		8D2	TGGCGTTG	active	
OMNI-24	V2	6D2	AGGACCTC		
		8D2	TGGCGTTG	active	
		3D1	TAGGCTCT	active	

[00448] The PAM site identified for OMNI 20, for example was verified as NRRRR by full *in vitro* cleavage of T**GGAA**, C**AAGG**, T**AAGG**, partial cleavage of C**GGAT**, G**GAAT**, and no cleavage of T**GG**TC (**Fig. 31B**). This activity is dependent on the expression and transcription of the OMNI nucleases and their guide.

5

Example 3E: Purification of OMNI proteins.

[00449] OMNI 4 and OMNI 6 open-reading frames were cloned into bacterial expression plasmids (T7-NLS-OMNI-NLS-HA-His-tag, pET9a, Table 8) and expressed in G10 cells (HI-Control™ 10G Competent Cells, Lucigen). Cells were grown in Terrific Broth to mid-log phase

and the temperature lowered to 18 °C. Expression was induced at 0.6 OD with 0.1mM IPTG for 16–20 h before harvesting and freezing cells at –80 °C. Cell paste was resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl,10 mM imidazole pH8.0, 1mM TCEP) supplemented with EDTA-free complete protease inhibitor cocktail set III (Calbiochem). Cells were lysed using
5 Constant systems TS-75 and cleared lysate was incubated with Ni-NTA resin. The resin was loaded onto gravity column and washed with wash buffer (50 mM NaH_2PO_4,300 mM NaCl, 50 mM imidazole pH8.0, 1mM TCEP) and OMNI protein eluted with wash buffer supplemented with 100-500mM Imidazole. Fractions containing OMNI protein were pooled and concentrated and loaded onto a centricone (Amicon Ultra 15ml 100K, Merck), and buffer exchanged to GF buffer
10 (50mM Tris-HCl pH 7.5, 500mM NaCl, 10% glycerol, 0.4M Arginine). The concentrated OMNI protein was further purified by SEC on HiLoad 16/600 Superdex 200 pg-SEC, AKTA Pure (GE Healthcare Life Sciences) with a 50mM Tris-HCl pH 7.5, 500mM NaCl, 10% glycerol, 0.4M Arginine. Fractions containing OMNI protein were pooled and concentrated and loaded onto a centricone (Amicon Ultra 15ml 100K, Merck) with a final storage buffer of 10mM Tris-HCl, pH
15 7.5, 150mM NaCl, 10% glycerol and 1mM TCEP. Purified OMNI protein was concentrated to 1 - 4ug/ul stocks and flash-frozen in liquid nitrogen and stored at -80 °C. Purity of OMNI proteins was measured by SDS-PAGE analysis (**Fig. 32A**).

Example 3F: In vitro activity assay by RNP.

20 [00450] Synthetic sgRNA of OMNI 4 and OMNI 6 were synthesized with three 2'-O-methyl 3'-phosphorothioate at the 3' and 5' ends (Agilent). RNPs were formed by incubating 1 mg/mL protein with 1µM synthetic sgRNA at Room-temp for 10 min. RNPs were stored on ice until reacted with target DNA. The RNPs were reacted in the recommended cleavage buffer with 100ng of linear DNA substrates containing the protospacer targeted by the sgRNA flanking each OMNI
25 PAM sequence (on-targets shown in **Fig. 32B**). The RNP of OMNI 6 was 5-fold diluted in storage buffer and reacted with both On and Off targets (single mismatch).

[00451] To biochemically characterize OMNI nucleases, we tested for *in vitro* DNA cleavage activity by RNP of purified OMNI 4 and OMNI 6 nucleases with corresponding synthetic sgRNA. Full cleavage of OMNI 4 and OMNI 6 with their "On-target" protospacers was observed. OMNI
30 6 was further tested for specificity. We observed remarkable level of discrimination for

protospacer with single nucleotide mismatch (**Fig. 32**). Tested protospacer and PAMs are summarized in Table 17 below.

Table 17: OMNI 4 and OMNI 6 Protospacer and PAM sites tested *in vitro*

Nuclease	Name	Spacer	Scaffold	sgRNA	Full sequence	Protospacer (with PAM) – On target	Protospacer (with PAM) – Off target
OMNI 4	EMX1_Site4_OMNI 4	GGGCCTGATTCCCACCTCTC (SEQ ID NO: 123)	GTTTGAGAGTAATGgaaaCATTACGAGTTCAAATAACGATTTATCGACATTACCACTTCGTGGTCAGGCGCACGTGTGTGCGCCGTTTTTTT (SEQ ID NO: 387)	V1	GGGCCTGATTCCCACCTCTCGTTTGAGAGTAATGgaaaCATTACGAGTTCAAATAACGATTTATCGACATTACCACTTCGTGGTCAGGCGCACGTGTGTGCGCCGTTTTTTT (SEQ ID NO:124)	GGGCCTGATTCCCACCTCTCAATACG (SEQ ID NO:125)	
OMNI 6	ELANEg58_OMNI 6	GCTGCGGGAAAGGGATTCCC (SEQ ID NO: 92)	CCCGTTTAAGAGTTATGTAAgaaaTTATATAACGAGTTTAAATAAAGCTTTGCTTTAATGCCATATTATATGGTATCACATAGGTGATATACTAATAGTTGCTATGCAACTATTTTT (SEQ ID NO:388)	V4	GCTGCGGGAAAGGGATTCCCGTTTAAGAGTTATGTAAgaaaTTATATAACGAGTTTAAATAAAGCTTTGCTTTAATGCCATATTATATGGTATCACATAGGTGATATACTAATAGTTGCTATGCAACTATTTTT (SEQ ID NO: 100)	GCTGCGGGAAAGGGATTCCCAGGACCCA (SEQ ID NO: 101)	GCTGCGGGAAAGGGTTTCCCAGGACCCA (SEQ ID NO:102)

Example 3G: Expression of OMNI nucleases coded by a codon optimized DNA sequence in mammalian cells.

[00452] First, expression of each of the codon optimized DNA sequences coding for each of the OMNI proteins OMNI 4, OMNI 6, OMNI 13, OMNI 18, OMNI 19, OMNI 20, and OMNI 23 in mammalian cells was validated. To this end, an expression vector coding for an HA tagged OMNI nuclease or Streptococcus Pyogenes Cas9 (SpCas9) linked by P2A peptide to mCherry (pmOMNI, Table 7) was introduced into HEK-293T cells using the Turbofect™transfection reagent (Thermo Fisher). 72 hours post transfection cells were lysed and subjected to Western blot analysis. Primary antibody against the HA tag was used to detect each of the OMNI proteins expression level. Results demonstrated expression of each of the tested OMNIs. P2A peptide is a self-cleaving peptide which can induce the cleaving of the recombinant protein in cell, such that the OMNI nucleases and the mCherry are separated upon expression and the mCherry can serve as indicator for transcription efficiency of the OMNI from expression vector. The level of transcription for OMNI in the activity assays was determined using Flow cytometry.

Example 3H: Activity in mammalian cells on plasmid target.

[00453] Activity of OMNI nucleases in Mammalian cells was first assayed using GFP Fluorescent gain-based reporter system (pMSS4c.2, Table 7). In this reporting system the PAM-target sequence flanking the T1 unique protospacer is incorporated upstream to the GFP coding sequence causing a frame-shift in translation leading to inactivation of the fluorescent signal. A double strand brake in the target site generated by the nuclease will lead to inaccurate DNA repair that will reestablish the GFP coding frame. To this end, HEK 293T cells were transfected with the expression vector coding for an HA tagged OMNI nuclease linked by P2A to mCherry which serves as indicator for transfection efficiency of the vector. An sgRNA expressing vector with a T1 spacer (pmGuide, Table 7), and the reporting vector expressing silenced GFP that is expressed upon editing and BFP which is constantly expressed and serves as indicator for transfection efficiency of the target plasmid (pMSS4c.2, Table 7). For negative control cells were transfected with the reporter vector with only the OMNI nuclease or only the guide. Cells were analyzed by FACS 72 hours post transfection for GFP fluorescent quantification BFP and mCherry fluorescent quantification. The % editing presented in Figures 33 and 34 are determined by quantification of GFP signal as fraction of BFP signal and mCherry signal. As demonstrated in Figures 33 and 34

all of the OMNIs exhibited editing activity in HEK293T cells to various degrees, indicated by the normalized GFP signal which is significantly higher compared to the relevant control.

[00454] In order to overcome potential transcriptional and structural constrains and to assess the plasticity of the sgRNA scaffold in the human cellular environmental context, several versions of sgRNA were tested. In each case the modifications represent small variations in the nucleotide sequence within the duplex and/or hairpins that were introduced to several synthetic sgRNA as shown in Fig. 35A-D and as described in Table 10 above.

[00455] The effect of the modifications on the OMNI nuclease activity was tested as described above. As can be seen in **Fig. 34A-C**, the use of the modified versions of sgRNAs ether significantly increased (**Fig.34A** black bar V3 - V5 *vs*. V2) or had no negative effect on activity of the different OMNIs (**Fig.34B** and **Fig. 34C** V3 or V4 *vs*. V2). Results of the mammalian transient activity assays for various sgRNAs for each OMNI are shown in Table 18 below.

Table 18: Bacterial survival assay and in vitro activity assay results.

		PAM verification		
Name	**sgRNA**	**Seq name**	**Mammalian transient**	**Mammalian sgRNA**
OMNI 4	V2	4D2	active	V2
OMNI 6	V2	1	active	V2, V3, V4
OMNI 13	V2	4D1	active	V2
OMNI 17	V2	17D2	active	V2
OMNI 18	V2	1	active	V4
OMNI 19	V2	4D2	active	V2
OMNI 20	V2	0	active	V2, V3
		20D2	active	V2, V3
OMNI 23	V2	6D2	active	V2
OMNI 24	V2	8D2	active	V2

Example 3I: Activity in human cells on endogenous genomic targets.

[00456] OMNIs were also assayed for their ability to promote editing on specific genomic locations in human cells. To this end, per each OMNI the corresponding OMNI-P2A-mCherry expression vector (pmOMNI, Table 7) was transfected into HEK293 or Hela cells together with sgRNA designed to target specific location in the human genome (pmGuide, Table 7). At 72h, cells were harvested, and half were used for quantification of transfection efficiency by FACS using mCherry fluorescence as marker. The rest of the cells were lysed, and their genomic DNA content was used in PCR reaction, amplifying the corresponding putative genomic targets. Amplicons were subjected to NGS and the resulting sequences were then used calculate the percentage of editing events in each target site. Short Insertions or deletions (InDels) around the cut site are the typical outcome of repair of DNA ends following nuclease induced DNA cleavage. The calculation of % editing was therefore deduced from the fraction of Indels containing sequences within each amplicon. With the exception of OMNI 4, all editing values were normalized to the transfection and translation eficasy obtained for each experimen and deduced from the percentage of mCherry expressing cells. The normalized values represent the effective editing levels within the population of cells that expressed the nucleases.

[00457] Genomic activity of each ONMI was assessed using a panel of 4 to 10 unique sgRNA each design to target a different genomic location. Each respective sgRNA target is shown in Table 20 below.

Table 20: sgRNA targets for mammalian assays.

OMNI	Genomic site	Corresponding Spacer name	Spacer sequence	3' (PAM containing) genomic seq
OMNI 4	EMX1 Site 3	EMX1 Site 3_OMNI4	ggGTTCCAGAACCGGAGGACAA (SEQ ID NO:126)	AGTACAAA
	EMX1 s4	EMX1_Site4_OMNI4	GGGCCTGATTCCCACCTCTC (SEQ ID NO:123)	AATACGTT
	9q31.2 Site 1	9q31.2 Site 1_OMNI4	gGCTGGTCTAGAGACCCAGCT (SEQ ID NO:117)	CATACTTG

OMNI	Genomic site	Corresponding Spacer name	Spacer sequence	3' (PAM containing) genomic seq
	9q31.2 Site 3	9q31.2 Site 3_OMNI4	ggAGCAGAAAAAAGCATCAAG (SEQ ID NO:118)	CCTACAAA
	HBB	HBB_OMNI4	TAAACCTGTCTTGTAACCTT (SEQ ID NO:119)	GATACCAA
	20q11.1	20q11.1_OMNI4	GGAGACTCTGGTGCTGTGTG (SEQ ID NO:120)	ACTACAGT
	FANCF Site 1	FANCF_6 Site 1_OMNI4	ggAGCTTCTGGCGGTCTCAAGC (SEQ ID NO:121)	ACTACCTA
	VISTA Enhancer hs267 Site 2	VISTA Enhancer hs267 Site 2_OMNI4	ggTGCTTTGTGTTCCAGTTTCC (SEQ ID NO:122)	TTTACAGG
OMNI 6	Elane g35	ELANEg35_OMNI6	AGTCCGGGCTGGGAGCGGGT (SEQ ID NO:93)	GGGGAGCA
	Elane g39	ELANEg39_OMNI6	CAGCGGGTGTAGACTCCGAG (SEQ ID NO:94)	GGGGACGT
	Elane g58	ELANEg58_OMNI6	GCTGCGGGAAAGGGATTCCC (SEQ ID NO:92)	AGGACCCA
	Elane g62	ELANEg62_OMNI6	GTCAAGCCCCAGAGGCCACA (SEQ ID NO:95)	GGGACAGA
	EMX1 s1	EMX1g1_OMNI6	TTCTGTGAATGTTAGACCCA (SEQ ID NO:103)	TGGGAGCA
	EMX1 s2	EMX1g2_OMNI6	TCTGTGAATGTTAGACCCAT (SEQ ID NO:104)	GGGAGCAG

OMNI	Genomic site	Corresponding Spacer name	Spacer sequence	3' (PAM containing) genomic seq
	CXCR4 s1	CXCR4g1_OMNI6	CGCGCCAAGTGATAAACACG (SEQ ID NO:96)	CGGAGGAG
	CXCR4 s2	CXCR4g2_OMNI6	ATGGCAAGAGACCCACACAC (SEQ ID NO:97)	AGGATGGC
	PD1 s1	PDCD1g1_OMNI6	TGCAGATCCCACAGGCGCCC (SEQ ID NO:98)	TGGCCAGT
	PD1 s2	PDCD1g2_OMNI6	AGGCGCCCTGGCCAGTCGTC (SEQ ID NO:99)	TGGGCGGT
OMNI 13	CXCR4 s3	CXCR4g1_OMNI13	GTGCCGTTTGTTCATTTTCT (SEQ ID NO:151)	GACACTCC
	CXCR4 s4	CXCR4g2_OMNI13	TTAAGACCGCATTCTCTTTA (SEQ ID NO:152)	CCCACTAC
	EMX1 s3	EMX1g1_OMNI13	GGACATCGATGTCACCTCCA (SEQ ID NO:153)	ATGACTAG
	EMX1 s4	EMX1g2_OMNI13	TGGGCCCAAGCTGGACTCTG (SEQ ID NO:154)	GCCACTCC
	PD1 s3	PDCD1g1_OMNI13	CCAGTCGTCTGGGCGGTGCT (SEQ ID NO:155)	ACAACTGG
	PD1 s4	PDCD1g2_OMNI13	CCAGTTGTAGCACCGCCCAG (SEQ ID NO:156)	ACGACTGG
OMNI 17	EMX1 s5	EMX1g1_OMNI17	GGCGCATTGCCACGAAGCAG (SEQ ID NO:165)	GCCAATGG
	EMX1 s6	EMX1g2_OMNI17	GGGAGGACATCGATGTCACC (SEQ ID NO:166)	TCCAATGA

OMNI	Genomic site	Corresponding Spacer name	Spacer sequence	3' (PAM containing) genomic seq
	CXCR4 s6	CXCR4g1_OMNI17	TTCATTTTCTGACACTCCCG (SEQ ID NO:167)	CCCAATAT
	PD1 s5	PDCD1g2_OMNI17	GTGCCCATTCCGCTAGGAAA (SEQ ID NO:168)	GACAATGG
	PD1 s6	PDCD1g1_OMNI17	TGAGCGTGGTCAGGGCCCGG (SEQ ID NO:169)	CGCAATGA
OMNI 18	EMX1 s1	EMX1g1_OMNI18	TTCTGTGAATGTTAGACCCA (SEQ ID NO:180)	TGGGAGCA
	EMX1 s2	EMX1g2_OMNI18	TCTGTGAATGTTAGACCCAT (SEQ ID NO:181)	GGGAGCAG
	CXCR4 s1	CXCR4g2_OMNI18	ATGGCAAGAGACCCACACAC (SEQ ID NO:182)	CGGAGGAG
	CXCR4 s2	CXCR4g1_OMNI18	CGCGCCAAGTGATAAACACG (SEQ ID NO:183)	AGGATGGC
	PD1 s1	PDCD1g1_OMNI18	TGCAGATCCCACAGGCGCCC (SEQ ID NO:184)	TGGCCAGT
	PD1 s2	PDCD1g2_OMNI18	AGGCGCCCTGGCCAGTCGTC (SEQ ID NO:185)	TGGGCGGT
OMNI 19	EMX1 s7	EMX1g1_OMNI19	GTTCCAGAACCGGAGGACAA (SEQ ID NO:195)	AGTACAAA
	EMX1 s8	EMX1g2_OMNI19	CTCCTCCAGCTTCTGCCGTT (SEQ ID NO:196)	TGTACTTT
	CXCR4 s7	CXCR4g2_OMNI19	ATTTTCTGACACTCCCGCCC (SEQ ID NO:197)	AATATACC

OMNI	Genomic site	Corresponding Spacer name	Spacer sequence	3' (PAM containing) genomic seq
	CXCR4 s8	CXCR4g1_OMNI19	ACGTCCCAAACGCGCCAAGT (SEQ ID NO:198)	GATAAACA
OMNI 20	EMX1 s2	EMX1g1_OMNI20	TCTGTGAATGTTAGACCCAT (SEQ ID NO:138)	GGGAGCAG
	EMX1 s9	EMX1g2_OMNI20	GACCCATGGGAGCAGCTGGT (SEQ ID NO:139)	CAGAGGGG
	CXCR4 s9	CXCR4g1_OMNI20	GTGATAAACACGAGGATGGC (SEQ ID NO:140)	AAGAGACC
	CXCR4 s1	CXCR4g2_OMNI20	ATGGCAAGAGACCCACACAC (SEQ ID NO:141)	CGGAGGAG
	Elane g62	ELANEg62_OMNI20	GTCAAGCCCCAGAGGCCACA (SEQ ID NO:142)	GGGACAGA
OMNI 23	EMX1 s2	EMX1g1_OMNI23	TCTGTGAATGTTAGACCCAT (SEQ ID NO:208)	GGGAGCAG
	EMX1 s10	EMX1g2_OMNI23	CCATGGGAGCAGCTGGTCAG (SEQ ID NO:209)	AGGGGACC
	CXCR4 s1	CXCR4g1_OMNI23	ATGGCAAGAGACCCACACAC (SEQ ID NO:210)	CGGAGGAG
	CXCR4 s10	CXCR4g2_OMNI23	GCAAGAGACCCACACACCGG (SEQ ID NO:211)	AGGAGCGC
	PD1 s11	PDCD1g1_OMNI23	GGCGCCCTGGCCAGTCGTCT (SEQ ID NO:212)	GGGCGGTG
	PD1 s12	PDCD1g2_OMNI23	GCCCTGGCCAGTCGTCTGGG (SEQ ID NO:213)	CGGTGCTA

OMNI	Genomic site	Corresponding Spacer name	Spacer sequence	3' (PAM containing) genomic seq
OMNI 24	EMX1 s11	EMX1g1_OMNI24	GTTAGACCCATGGGAGCAGC (SEQ ID NO:223)	TGGTCAGA
	EMX1 s12	EMX1g2_OMNI24	ATGGGAGCAGCTGGTCAGAG (SEQ ID NO:224)	GGGACCCC
	CXCR4 s12	CXCR4g1_OMNI24	TGCCCCGCTCCCTAACGTCC (SEQ ID NO:225)	CAAACGCG
	CXCR4 s13	CXCR4g2_OMNI24	GTCCCAAACGCGCCAAGTGA (SEQ ID NO:226)	TAAACACG
	PD1 s13	PDCD1g1_OMNI24	GATCCCACAGGCGCCCTGGC (SEQ ID NO:227)	CAGTCGTC
	PD1 s14	PDCD1g2_OMNI24	AGCCCAGTTGTAGCACCGCC (SEQ ID NO:228)	CAGACGAC

[00458] The results of these experiments are summarized in Table 20 below.

Table 20: Genomic activity of each OMNI in mammalian cells.

OMNI	Genomic site	% indels	% transfection	Norm. % editing	% editing in neg control	% transfection in neg control	Norm. % editing in neg control
OMNI 4	EMX1 Site 3	29.71%	N/A	N/A	0.00%	N/A	N/A
	EMX1 s4	47.69%	N/A	N/A	0.07%	N/A	N/A
	9q31.2 Site 1	9.77%	N/A	N/A	0.06%	N/A	N/A
	9q31.2 Site 3	23.80%	N/A	N/A	0.02%	N/A	N/A
	HBB	13.13%	N/A	N/A	0.10%	N/A	N/A
	20q11.1	31.90%	N/A	N/A	0.20%	N/A	N/A

OMNI	Genomic site	% indels	% transfection	Norm. % editing	% editing in neg control	% transfection in neg control	Norm. % editing in neg control
	FANCF Site 1	26.03%	N/A	N/A	0.30%	N/A	N/A
	VISTA Enhancer hs267 Site 2	3.85%	N/A	N/A	0.20%	N/A	N/A
OMNI 6	Elane g35	24.67%	90%	27%	2.17%	25%	8.6667%
	Elane g39	0.23%	92%	0%	0.27%	25%	1.0667%
	Elane g58	33.80%	85%	40%	0.10%	25%	0.4000%
	Elane g62	0.02%	91%	0%	0.03%	25%	0.1200%
	EMX1 s1	11.17%	17%	67%	0.10%	25%	0.3932%
	EMX1 s2	0.05%	16%	0%	0.10%	25%	0.3932%
	CXCR4 s1	1.49%	12%	12%	0.06%	25%	0.2536%
	CXCR4 s2	8.68%	13%	69%	0.04%	25%	0.1690%
	PD1 s1	17.33%	11%	156%	0.00%	25%	0.0000%
	PD1 s2	4.66%	12%	39%	0.00%	25%	0.0000%
OMNI 13	CXCR4 s3	0.00%	54%	0.00%	0.07%	46%	0.1437%
	CXCR4 s4	0.00%	58%	0.00%	N/A	46%	N/A
	EMX1 s3	0.06%	57%	0.10%	0.03%	46%	0.0731%
	EMX1 s4	0.21%	54%	0.39%	N/A	46%	N/A
	PD1 s3	3.86%	51%	7.62%	0.05%	46%	0.1132%
	PD1 s4	0.06%	60%	0.09%	N/A	46%	N/A

OMNI	Genomic site	% indels	% transfection	Norm. % editing	% editing in neg control	% transfection in neg control	Norm. % editing in neg control
OMNI 17	EMX1 s5	0.05%	23%	0.23%	0.04%	15%	0.2976%
	EMX1 s6	0.08%	24%	0.32%	0.03%	15%	0.2314%
	CXCR4 s6	0.14%	19%	0.72%	0.03%	15%	0.2042%
	PD1 s5	0.32%	23%	1.37%	0.03%	15%	0.2037%
	PD1 s6	0.20%	22%	0.93%	0.05%	15%	0.3097%
OMNI 18	EMX1 s1	0.90%	16%	5.6%	0.05%	17%	0.2854%
	EMX1 s2	0.11%	16%	0.7%	0.05%	17%	0.2854%
	CXCR4 s1	1.12%	12%	9.3%	0.11%	17%	0.6198%
	CXCR4 s2	1.09%	9%	12.1%	0.05%	17%	0.2908%
	PD1 s1	3.00%	9%	33.3%	0.05%	17%	0.3025%
	PD1 s2	1.36%	21%	6.5%	0.05%	17%	0.3025%
OMNI 19	EMX1 s7	0.04%	17%	0.3%	0.04%	31%	0.1187%
	EMX1 s8	0.05%	26%	0.2%	0.06%	31%	0.2078%
	CXCR4 s7	0.19%	40%	0.5%	0.08%	31%	0.2721%
	CXCR4 s8	0.10%	41%	0.2%	0.04%	31%	0.1365%
OMNI 20	EMX1 s2	0.04%	18%	0.2%	0.02%	21%	0.1003%
	EMX1 s9	0.65%	27%	2.4%	0.05%	21%	0.2508%
	CXCR4 s9	8.86%	19%	46.7%	0.07%	21%	0.3496%
	CXCR4 s1	0.53%	17%	3.2%	0.07%	21%	0.3496%

OMNI	Genomic site	% indels	% transfection	Norm. % editing	% editing in neg control	% transfection in neg control	Norm. % editing in neg control
	Elane g62	19.67%	22%	89.4%	5.49%	21%	26.1665%
OMNI 23	EMX1 s2	0.62%	50%	1.2%	0.05%	38%	0.1349%
	EMX1 s10	0.16%	47%	0.3%	0.11%	38%	0.2922%
	CXCR4 s1	0.06%	45%	0.1%	0.05%	38%	0.1254%
	CXCR4 s10	0.13%	47%	0.3%	0.12%	38%	0.3136%
	PD1 s11	0.06%	40%	0.2%	0.07%	38%	0.1719%
	PD1 s12	0.04%	54%	0.1%	0.07%	38%	0.1719%
OMNI 24	EMX1 s11	0.11%	40%	0.3%	0.12%	35%	0.3420%
	EMX1 s12	0.14%	35%	0.4%	0.17%	35%	0.4806%
	CXCR4 s12	0.09%	38%	0.2%	0.13%	35%	0.3602%
	CXCR4 s13	4.07%	38%	10.7%	0.09%	35%	0.2502%
	PD1 s13	0.08%	41%	0.2%	0.01%	35%	0.0322%
	PD1 s14	0.08%	30%	0.3%	0.05%	35%	0.1289%

[00459] As can be seen in column 3 of Table 20 “% indels”. Some OMNIs, for example OMNI 4 and OMNI 6 exhibit high and significant editing levels compared to the negative control as seen in column 6 of Table 20 “% editing in neg control”. In all or most target sites tested (3.85%-47.9%, on 8/8 sites; 1.5%-33.8%, on 7/10 sites, OMNI 4 and OMNI 6 respectively). OMNI 20 exhibited
5 high and significant editing levels in 2/5 sites tested, OMNI 18 exhibit low yet significant activity across most tested sites (4/5) and OMNI 13, OMNI 23, and OMNI 24 showed significant yet low activity on one of the six sites tested. For OMNI 17 and OMNI 19 no editing above control levels was detected in any of the genomic site tested. As demonstrated in **Figures 33** and **34**, all tested OMNI nucleases are active on plasmid targets. Nevertheless, the results presented in Table 20
10 show that some OMNI nucleases are not active or are only partially active on genomic targets.

Taken together, these two observations demonstrate that while all the components required for the catalytic cleavage reaction for all tested nuclease are available and optimized to support activity in the human cells environment the activity level is not always sufficient to promote cleavage of genomic DNA.

5 ***Example 3J: Intrinsic fidelity in human cell.***

[00460] The intrinsic fidelity of a nuclease is a measure of its cleavage specificity. A high-fidelity nuclease is a nuclease that promotes cleavage on the intended target ("on-target") with minimal or no cleavage of the unintended target ("off-target"). For a CRISPR nuclease the target is acquired based on sequence complementarity to the spacer element of the guide RNA. Off-targeting results 10 from similarity of the unintended target to the spacer sequence. The intrinsic fidelity of OMNIs at the genomic level in human cells was measured by conducting an activity assay as described in section xi, following PCR amplification NGS and InDel analysis for both the on-target region and a pre validated off target region. A measurement of intrinsic fidelity for OMNI 6 is provided in **Figure 36**. In this example, OMNI 6 fidelity was measured using two guide RNAs independently. 15 In each case a side-by-side measurement of spCas9 is provided for reference. The first site was targeted using the ELANE g35 gRNA targeting the ELANE g35 site specified in Table 19, which has a defined on-target upstream to the ELANE gene on chr19 and an off-target on chr15. As can be seen in **Fig 36B**, the on/off target editing efficiency ratio obtained by OMNI 6 was 17:1 while spCas9 on/off ratio is 1.6:1 (24.7%/1.4%; 64.8%/41% respectively). The second site was targeted 20 using the ELANE g58 gRNA targeting the ELANE g25 site specified in Table 19. This gRNA spacer sequence has a defined off-target in the genome in the form of heterozygous single nucleotide polymorphism (SNP) in the tested cell line. As a result, one allele is identical to the spacer (on-target) and the other allele contains a single mismatch and makes an extremely challenging off-target. In this case, the On/Off ratio obtained by OMNI 6 was 2.43:1 compared to 25 the 1:1 ratio obtained by spCas9 (**Fig. 36A**). As noted above, the intrinsic high fidelity of OMNI-6 *in vitro* was already at the RNP level (**Fig. 32C**). Taken together these results demonstrate that OMNI 6 has a significantly higher intrinsic fidelity in comparison to spCas9 in both *in vitro* and human cellular environment.

Example 3K: Activity in human cells as RNPs.

30 [00461] Synthetic sgRNAs for OMNI 4 and OMNI 6 were synthesized with three 2'-O-methyl 3'-phosphorothioate at the 3' and 5' ends (Agilent). RNPs were formed by incubating 1 mg/mL

protein with 1μM synthetic sgRNA at room-temp for 10 min. RNPs were stored on ice until reacting with cells. U2OS cells (ATCC HTB-96) were suspend, wash, and electroporate using Lonza SE Cell Line 4D-Nucleofector™ X Kit with DN100 program, according to the manufacture protocol. Negative control cells were electroporate with the OMNI nucleases only. Cells were lysed and their genomic DNA content was used in PCR reaction, amplifying the corresponding putative genomic targets. Amplicons were subjected to NGS and the resulting sequences were then used calculate the percentage of editing events. As can be seen in **Figure 37**, both OMNI 4 and OMNI 6 RNPs are active in mammalian endogenous context.

CLAIMS

What is claimed is:

1. A method of modifying a nucleotide sequence at a target site in the genome of a mammalian cell comprising introducing into the cell (i) a composition comprising a CRISPR nuclease having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding a CRISPR nuclease which sequence has at least 95% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs:23-27 and (ii) a DNA-targeting RNA molecule, or a DNA polynucleotide encoding a DNA-targeting RNA molecule, comprising a nucleotide sequence that is complementary to a sequence in the target DNA.

2. The method of claim 1, further comprising introducing into the cell: (iii) an RNA molecule comprising a nuclease-binding RNA sequence or a DNA polynucleotide encoding an RNA molecule comprising a nuclease-binding RNA that interacts with the CRISPR nuclease.

3. The method of claim 1 or 2, wherein the DNA targeting RNA molecule is a crRNA molecule suitable to form an active complex with the CRISPR nuclease.

4. The method of any one of claims 2-3, wherein the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule suitable to form an active complex with the CRISPR nuclease.

5. The method of any one of claims 2-4, wherein the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-binding RNA sequence are fused in the form of a single guide RNA molecule.

6. The method of any one of claims 1-5, comprising introducing into the cell: (iv) an RNA molecule comprising a sequence complementary to a protospacer sequence.

7. The method of any one of claims 1-6, wherein the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a Protospacer Adjacent Motif (PAM).

8. The method of any one of claims 1-7, wherein the CRISPR nuclease has at least 95% identity to an amino acid sequence as set forth in SEQ ID NO:1 or the sequence encoding the CRISPR nuclease has at least 95% identity to the nucleic acid sequence as set forth in SEQ ID NO: 23.

9. The method of claim 8, wherein:

   a. the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 80 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:81 suitable to form an active complex with the CRISPR nuclease;

   b. the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:82 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:83 suitable to form an active complex with the CRISPR nuclease; or

   c. the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:84, SEQ ID NO:85, or SEQ ID NO:86 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs: 87-91 suitable to form an active complex with the CRISPR nuclease.

10. The method of claim 9(c), wherein;

   i. the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO:88; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting of AGGGNNNN, CGGGNNNN, TGGGNNNN, GGGTNNNN, NGGNNNNN,

CGGTCGAA, TGGTCCGC, and AGGACCTC, to modify the nucleotide sequence at the target site in the cell.

11. The method of claim 10, wherein:

a. (i) the target site in the genome is ELANE g58; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence AGG or AGGACCCA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:92;

b. (i) the target site in the genome is ELANE g35; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GGGGAGCA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:93;

c. (i) the target site in the genome is ELANE g39; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GGGGACGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:94;

d. (i) the target site in the genome is ELANE g62; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GGGACAGA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 95;

e. (i) the target site in the genome is CXCR4 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CGGAGGAG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 96;

f. (i) the target site in the genome is CXCR4 s2; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence AGGATGGC; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 97;

g. (i) the target site in the genome is PD1 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence TGGCCAGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 98; or

h. (i) the target site in the genome is PD1 s2; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence TGGGCGGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 99.

12. The method of any one of claims 1-7, wherein the CRISPR nuclease has at least 95% identity to an amino acid sequence as set forth in SEQ ID NO:2 or the sequence encoding the CRISPR nuclease has at least 95% identity to the nucleic acid sequence as set forth in SEQ ID NO: 24.

13. The method of claim 12, wherein:

a. the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:108 suitable to form an active complex with the CRISPR nuclease and/or the RNA molecule comprising a nuclease-binding RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:109 suitable to form an active complex with the CRISPR nuclease;

b. the DNA targeting RNA molecule is a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:110 suitable to form an active complex with the CRISPR nuclease; and/or the RNA molecule comprising a nuclease-binding

RNA sequence is a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 111 suitable to form an active complex with the CRISPR nuclease; or

c. the DNA-targeting RNA molecule and the RNA molecule comprising a nuclease-biding RNA sequence are fused in the form of a single guide RNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 112, SEQ ID NO:113, or SEQ ID NO: 114 and is suitable to form an active complex with the CRISPR nuclease; optionally the single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NOs:115-116 suitable to form an active complex with the CRISPR nuclease.

14. The method of claim 13(c), wherein;

i. the single guide RNA molecule comprises the nucleotide sequence as set forth in SEQ ID NO: 115; optionally the active complex of the CRISPR nuclease may use a PAM site or variant comprising a sequence selected from the group consisting of NGCACNNN, NATACNNN, NGTACNNN, CGTANNNN, NRTAHNNN, TGTACTAA, TATACGAA, TGCACTAA to modify the nucleotide sequence at the target site in the cell.

15. The method of claim 14, wherein:

a. (i) the target site in the genome is 9q31.2 s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CATACTTG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 117;

b. (i) the target site in the genome is 9q31.2 s3; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence CCTACAAA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 118;

c. (i) the target site in the genome is HBB; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence GATACCAA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO: 119;

d. (i) the target site in the genome is 20q11.1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence ACTACAGT; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:120;

e. (i) the target site in the genome is FANCF s1; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence ACTACCTA; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:121; or

f. (i) the target site in the genome is VISTA Enhancer hs267 s2; (ii) the CRISPR nuclease forms a complex with the one or more RNA molecules and effects a double strand break in the 3' of a PAM site comprising the nucleotide sequence TTTACAGG; and (iii) the protospacer region comprises a nucleotide sequence as set forth in SEQ ID NO:122.

16. The method of any one of claims 1-15, for treating a subject afflicted with a disease associated with a genomic mutation comprising modifying a nucleotide sequence at a target site in the genome of the subject.

17. The method of claim 16, comprising first selecting a subject afflicted with a disease associated with a genomic mutation, and obtaining the cell from the subject.

18. A modified cell or cells obtained by the method of any one of claims 1-15.

19. The modified cell or cells of claim 18, capable of giving rise to progeny cells.

20. The modified cell or cells of claim 19, capable of giving rise to progeny cells after engraftment.

21. A composition comprising the modified cells of any one of claims 18-20 and a pharmaceutically acceptable carrier.

22. An *in vitro* or *ex vivo* method of preparing the composition of claim 21, comprising mixing the cells with the pharmaceutically acceptable carrier.

23. A non-naturally occurring composition comprising a CRISPR nuclease comprising a sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding the CRISPR nuclease.

24. The composition of claim 23, wherein the CRISPR nuclease further comprises an RNA-binding portion capable of interacting with a DNA-targeting RNA molecule (gRNA) and an activity portion that exhibits site-directed enzymatic activity.

25. The composition of claim 24 further comprising a DNA-targeting RNA molecule or a DNA polynucleotide encoding a DNA-targeting RNA molecule, wherein the DNA-targeting RNA molecule comprises a nucleotide sequence that is complementary to a sequence in a target region, wherein the DNA-targeting RNA molecule and the CRISPR nuclease do not naturally occur together.

26. The composition of claim 24 or claim 25, wherein the DNA-targeting RNA molecule further comprises a nucleotide sequence that can form a complex with a CRISPR nuclease.

27. The composition of any one of claims 25 or 26 further comprising an RNA molecule comprising a nucleotide sequence that can form a complex with a CRISPR nuclease (tracrRNA) or a DNA polynucleotide comprising a sequence encoding an RNA molecule that can form a complex with the CRISPR nuclease.

28. The composition of any one of claims 25-27 further comprising a donor template for homology directed repair (HDR).

29. The composition of any one of claims 25-28, wherein the composition is capable of editing the target region in the genome of a cell.

30. The composition of any one of claims 23-29, wherein:

a. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:1 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO: 73;

b. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:2 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:105.

c. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:3 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO: 127.

d. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:10 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:229;

e. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:11 the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:238;

f. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:12 and the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:248; or

g. the CRISPR nuclease comprises a sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:13 the nucleotide sequence that can form a complex with the CRISPR nuclease in the DNA-targeting RNA molecule is SEQ ID NO:258.

31. A non-naturally occurring composition comprising a CRISPR associated system comprising:

    one or more RNA molecules comprising a guide sequence portion linked to a direct repeat sequence, wherein the guide sequence is capable of hybridizing with a target sequence, or one or more nucleotide sequences encoding the one or more RNA molecules; and

    an CRISPR nuclease comprising an amino acid sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding the CRISPR nuclease; and

    wherein the one or more RNA molecules hybridize to the target sequence, wherein the target sequence is 3' of a Protospacer Adjacent Motif (PAM), and the one or more RNA molecules form a complex with the RNA-guided nuclease.

32. The composition of claim 31 further comprising an RNA molecule comprising a nucleotide molecule that can form a complex with the RNA nuclease (tracrRNA) or a DNA polynucleotide encoding an RNA molecule comprising a nucleotide sequence that can form a complex with the CRISPR nuclease.

33. The composition of claim 31 or 32 further comprising a donor template for homology directed repair (HDR).

34. A non-naturally occurring composition comprising:

    a CRISPR nuclease comprising a sequence having at least 95% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NOs:4-22 or a nucleic acid molecule comprising a sequence encoding the CRISPR nuclease; and

    one or more RNA molecules, or one or more DNA polynucleotide encoding the one or more RNA molecules, comprising at least one of:

    (i) a nuclease-binding RNA nucleotide sequence capable of interacting with/binding to the CRISPR nuclease; and

(ii) a DNA-targeting RNA nucleotide sequence comprising a sequence complementary to a sequence in a target DNA sequence,

wherein the CRISPR nuclease is capable of complexing with the one or more RNA molecules to form a complex capable of hybridizing with the target DNA sequence.

35. The composition of claim 34, wherein the CRISPR nuclease and the one or more RNA molecules form a CRISPR complex that is capable of binding to the target DNA sequence to effect cleavage of the target DNA sequence.

36. The composition of any one of claims 34 or 35, wherein the CRISPR nuclease and at least one of the one or more RNA molecules do not naturally occur together.

37. The composition of any one of claims 34-36, wherein:

the CRISPR nuclease comprises an RNA-binding portion and an activity portion that exhibits site-directed enzymatic activity;

the DNA-targeting RNA nucleotide sequence comprises a nucleotide sequence that is complementary to a sequence in a target DNA sequence; and

the nuclease-binding RNA nucleotide sequence comprises a sequence that interacts with the RNA-binding portion of the CRISPR nuclease.

38. The composition of any one of claims 34-37, wherein the nuclease-binding RNA nucleotide sequence and the DNA-targeting RNA nucleotide sequence are on a single guide RNA molecule (sgRNA), wherein the sgRNA molecule can form a complex with the CRISPR nuclease and serve as the DNA targeting module.

39. The non-naturally occurring composition of claim 38, wherein the sgRNA has a length of up to 1000 bases, 900 bases, 800 bases, 700 bases, 600 bases, 500 bases, 400 bases, 300 bases, 200 bases, 100 bases, 50 bases.

40. The composition of any one of claims 34-39, wherein the nuclease-binding RNA nucleotide sequence is on a first RNA molecule and the DNA-targeting RNA nucleotide sequence is on a single guide RNA molecule, and wherein the first and second RNA sequence interact by base-paring or are fused together to form one or more RNA molecules or sgRNA that complex with the CRISPR nuclease and serve as the targeting module.

41. The composition of any one of claims 23-40 further comprising a donor template for homology directed repair (HDR).

42. The composition of any one of claims 23-41, wherein the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:1 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO:29 or SEQ ID NO:23.

43. The composition of any one of claims 23-41, wherein the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:1 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO: 51.

44. The composition of claims 42 or 43, wherein the composition comprises an RNA molecule comprising a nuclease-binding RNA nucleotide sequence wherein the nucleotide binding RNA sequence is selected from the group consisting of SEQ ID NOs:73-79 and is suitable to form an active complex with the CRISPR nuclease.

45. The composition of claims 42 or 43, wherein the composition comprises an RNA molecule comprising a nuclease-binding RNA nucleotide sequence wherein the nucleotide binding RNA sequence is selected from the group consisting of SEQ ID NOs: 88-91 and is suitable to form an active complex with the CRISPR nuclease.

46. The composition of any one of claims 43-45, wherein the CRISPR nuclease may use a PAM site or variant selected from the group consisting of AGGGNNNN, CGGGNNNN, TGGGNNNN, GGGTNNNN, NGGNNNNN, CGGTCGAA, TGGTCCGC, and AGGACCTC.

47. The composition of claims 42 or 43, further comprising a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 80 suitable to form an active complex with the CRISPR nuclease.

48. The composition of any one of claims 42, 43, or 47, further comprising a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:81 suitable to form an active complex with the CRISPR nuclease.

49. The composition of claims 42 or 43, further comprising a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:82 suitable to form an active complex with the CRISPR nuclease.

50. The composition of any one of claims 42, 43, or 49, further comprising a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:83 suitable to form an active complex with the CRISPR nuclease.

51. The composition of claims 42 or 43, further comprising a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86 and is suitable to form an active complex with the CRISPR nuclease.

52. The composition of claim 51, wherein the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 87-91 suitable to form an active complex with the CRISPR nuclease.

53. The composition of claim 52, wherein the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO:88].

54. The composition of claim 53, wherein the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of AGGGNNNN, CGGGNNNN, TGGGNNNN, GGGTNNNN, NGGNNNNN, CGGTCGAA, TGGTCCGC, AGGACCTC, NGGNN, NGGNM, and NGG.

55. The composition of any one of claims 23-41, wherein the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:2 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO: 30 or SEQ ID NO: 24.

56. The composition of any one of claims 23-41, wherein the CRISPR nuclease has at least 95% identity to the amino acid sequence as set forth in SEQ ID NO:2 or the sequence encoding the CRISPR nuclease has at least a 95% sequence identity to SEQ ID NO: 52.

57. The composition of claims 55 or 56, wherein the composition comprises an RNA molecule comprising a nuclease-binding RNA nucleotide sequence wherein the nucleotide binding

RNA sequence is selected from the group consisting of SEQ ID NOs:105-107 and is suitable to form an active complex with the CRISPR nuclease.

58. The composition of any one of claims 55-57, wherein the CRISPR nuclease may use a PAM site or variant selected from the group consisting of NGCACNNN, NATACNNN, NGTACNNN, CGTANNNN, NRTAHNNN, TGTACTAA, TATACGAA, TGCACTAA.

59. The composition of claims 55 or 56, further comprising a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:108 suitable to form an active complex with the CRISPR nuclease.

60. The composition of any one of claims 55 or 56, or 59, further comprising a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:109 suitable to form an active complex with the CRISPR nuclease.

61. The composition of claims 55 or 56, further comprising a crRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO:110 suitable to form an active complex with the CRISPR nuclease.

62. The composition of any one of claims 55, 56, or 61, further comprising a tracrRNA molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 111 suitable to form an active complex with the CRISPR nuclease.

63. The composition of claims 55 or 56, further comprising a single guide RNA molecule comprising the nucleotide sequence selected from the group consisting of SEQ ID NO: 112, SEQ ID NO:113, and SEQ ID NO: 114 and is suitable to form an active complex with the CRISPR nuclease.

64. The composition of claim 63, wherein the single guide RNA molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:115-116 suitable to form an active complex with the CRISPR nuclease.

65. The composition of claim 64, wherein the single guide RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 115.

66. The composition of claim 65, wherein the CRISPR nuclease may use a PAM site or variant as selected from the group consisting of NGCACNNN, NATACNNN, NGTACNNN,

CGTANNNN, NRTAHNNN, TGTACTAA, TATACGAA, TGCACTAA, NVYAH, and YGTAM.

67. The composition of any one of claims 23-66, wherein the CRISPR nuclease comprises 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130,130-140, or 140-150 amino acid substitutions, deletions, and/or insertions compared to the amino acid sequence of the wild-type of the CRISPR nuclease.

68. The composition of claim 67, wherein the CRISPR nuclease exhibits at least 2%, 5%, 7% 10%, 15%, 20%, 25%, 30, or 35% increased specificity compared the wild-type of the CRISPR nuclease.

69. The composition of claim 67 or 68, wherein the CRISPR nuclease exhibits at least 2%, 5%, 7% 10%, 15%, 20%, 25%, 30, or 35% increased activity compared the wild-type of the CRISPR nuclease.

70. The composition of any one of claims 67-69, wherein the CRISPR nuclease has altered PAM specificity compared to the wild-type of the CRISPR nuclease.

71. The composition of any one of claims 23-70, wherein the CRISPR nuclease is non-naturally occurring.

72. The composition of claim 71, wherein the CRISPR nuclease is engineered and comprises unnatural or synthetic amino acids.

73. The composition of claim 71 or 72, wherein the CRISPR nuclease is engineered and comprises one or more of a nuclear localization sequences (NLS), cell penetrating peptide sequences, and/or affinity tags.

74. The composition of claim 73, wherein the CRISPR nuclease comprises one or more nuclear localization sequences of sufficient strength to drive accumulation of a CRISPR complex comprising the CRISPR nuclease in a detectable amount in the nucleus of a eukaryotic cell.

75. The composition of claim 74, wherein the CRISPR nuclease comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the amino-terminus, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near carboxy-terminus, or a combination of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the amino-terminus and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near carboxy-terminus.

76. The composition of claim 75, wherein the one or more NLSs are in tandem repeats.

77. The composition of claim 76, wherein the one or more NLSs are considered in proximity to the N- or C-terminus when the nearest amino acid of the NLS is within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, or more amino acids along the polypeptide chain from the N- or C-terminus.

78. The composition of any one of claims 67-77, wherein the CRISPR nuclease exhibits increased specificity to a target site compared to the wild-type of the CRISPR nuclease when complexed with the one or more RNA molecules.

79. The composition of claim 78, wherein the complex of the CRISPR nuclease and one or more RNA molecules exhibits at least maintained on-target editing activity of the target site and reduced off-target activity compared to the wild-type of the CRISPR nuclease.

80. The composition of any one of claims 23-79 further comprising a recombinant nucleic acid molecule comprising a heterologous promoter operably linked to the nucleotide acid molecule comprising the sequence encoding the CRISPR nuclease.

81. The composition of any one of claims 23-80, wherein the CRISPR nuclease or nucleic acid molecule comprising a sequence encoding the CRISPR nuclease is non-naturally occurring or engineered.

82. A non-naturally occurring or engineered composition comprising a vector system comprising the nucleic acid molecule comprising a sequence encoding the CRISPR nuclease of any one of claims 23-80.

83. A method of modifying a nucleotide sequence at a target site in a cell-free system or the genome of a cell comprising introducing into the cell the composition of any one of claims 23-82.

84. The method of claim 83, wherein the cell is a eukaryotic cell.

85. Use of the compositions of any one of claims 21 or 23-82 for the treatment of a subject afflicted with a disease associated with a genomic mutation comprising modifying a nucleotide sequence at a target site in the genome of the subject.

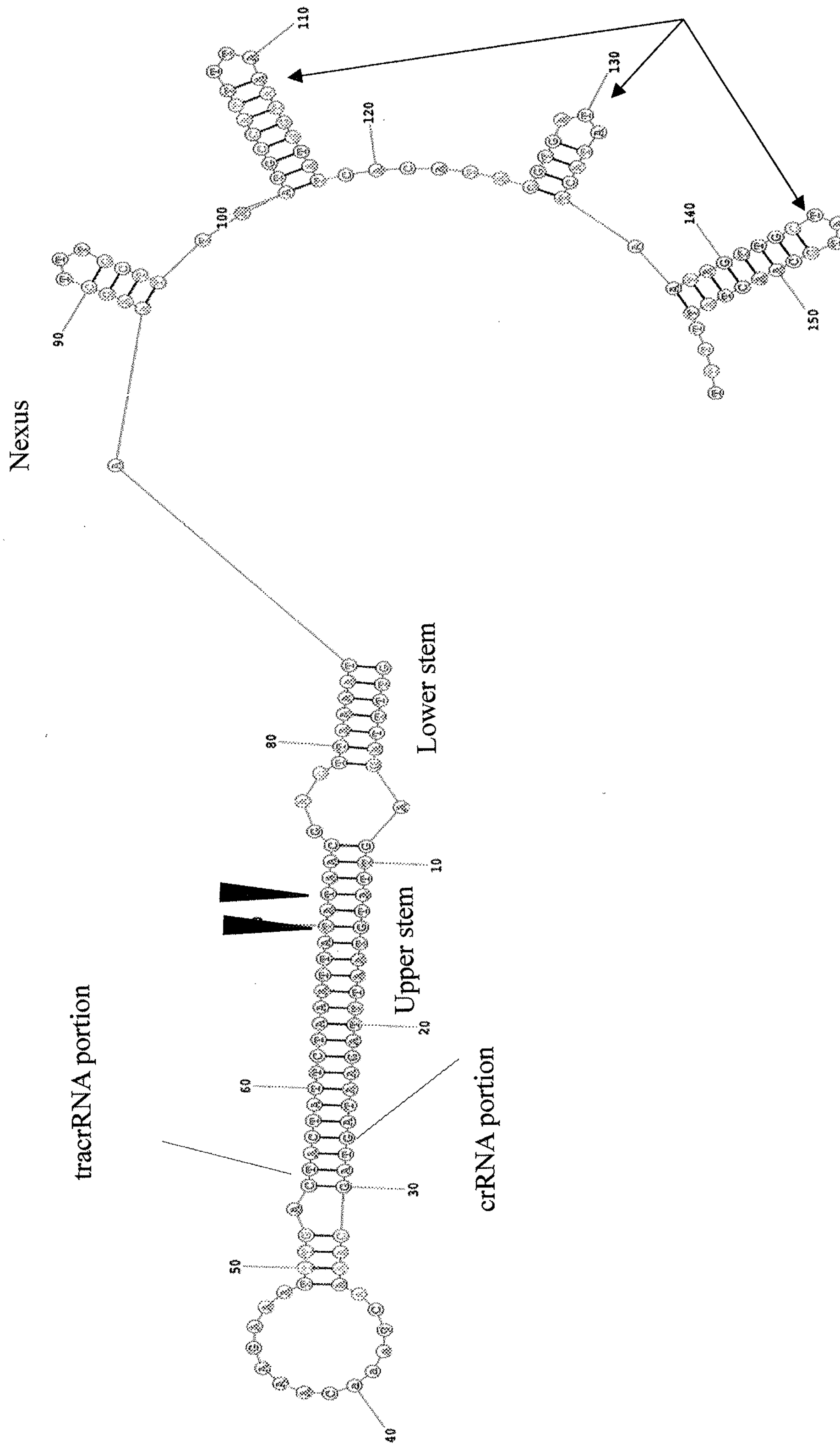


Fig. 1A

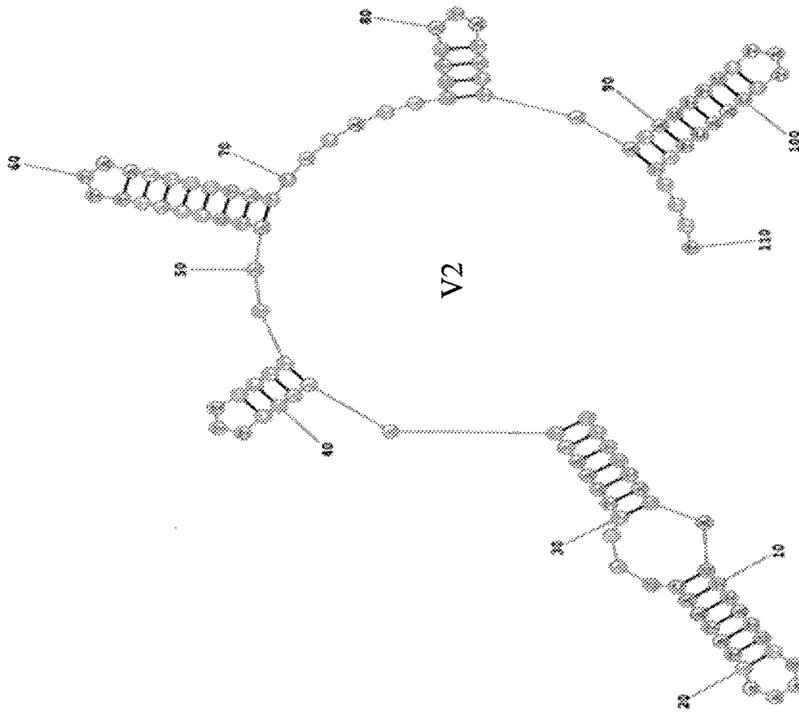
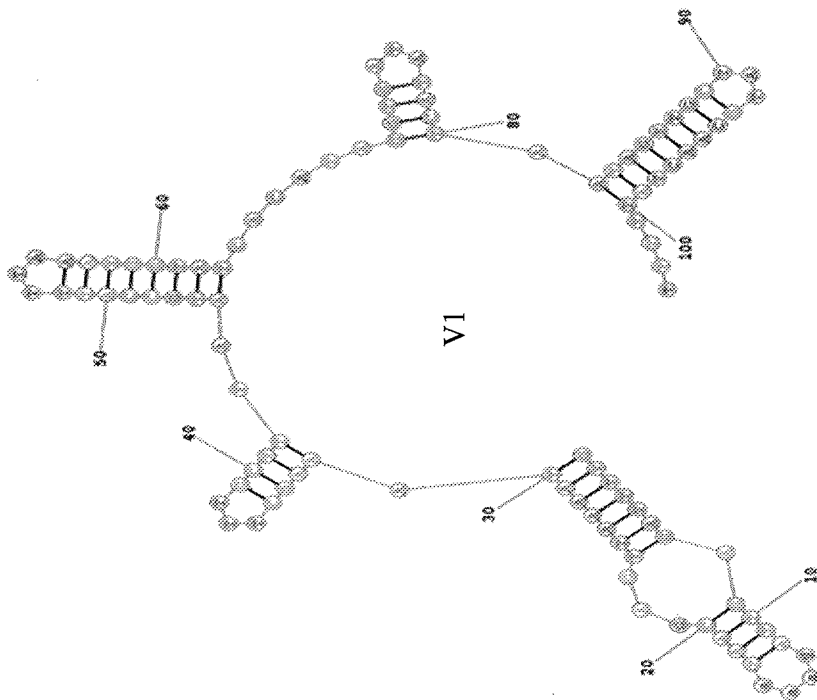
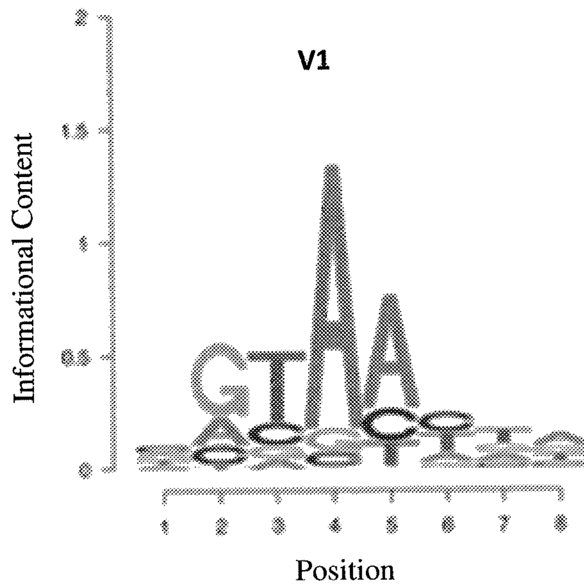
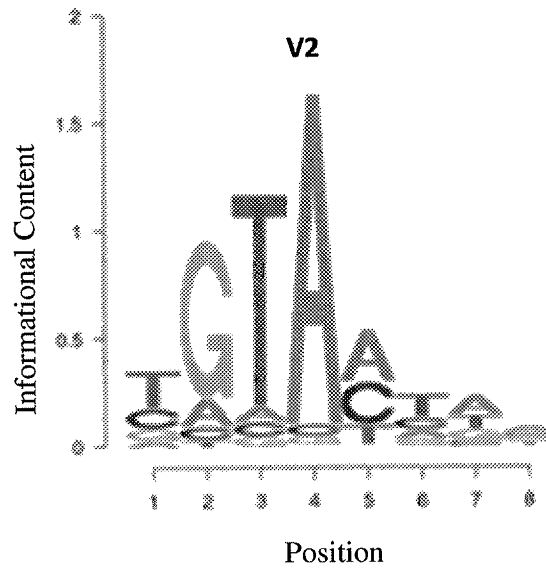


Fig. 1B



OMNI 4





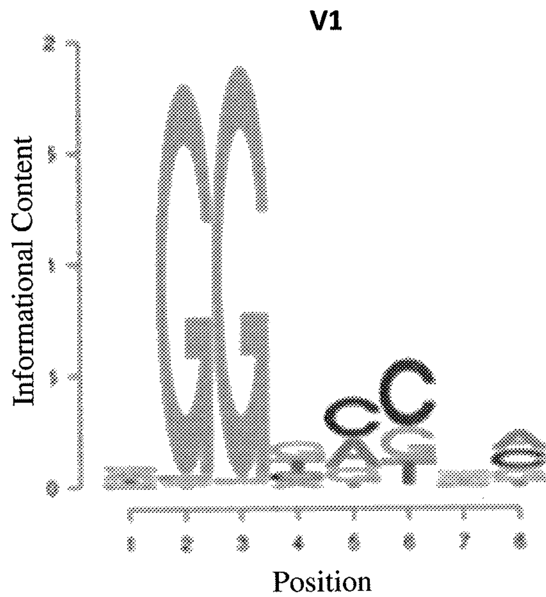
1	2	3	4	5	6	7	8	Ratio
	A	T	A	C				0.000
		T	A	C	G			0.000
		T	A	C	T			0.012
	G	T	A	C				0.012
			A	C	G	A		0.016
	C	T	A	C				0.018
	G	T	A	T				0.020
			A	C	T	C		0.031
G	G	T	A					0.032
		T	A	A	T			0.032
			A	C	T	A		0.034
	A	T	A	T				0.035
	G	T	A	A				0.039
T	A	T	A					0.041
	A	T	A	A				0.043
		A	A	C	T			0.047
		C	A	C	G			0.054
C	G	T	A					0.057
	G	C	A	C				0.069
			A	C	G	C		0.072
T	G	T	A					0.076
	C	T	A	A				0.084
	A	C	A	C				0.086
			A	C	G	G		0.092

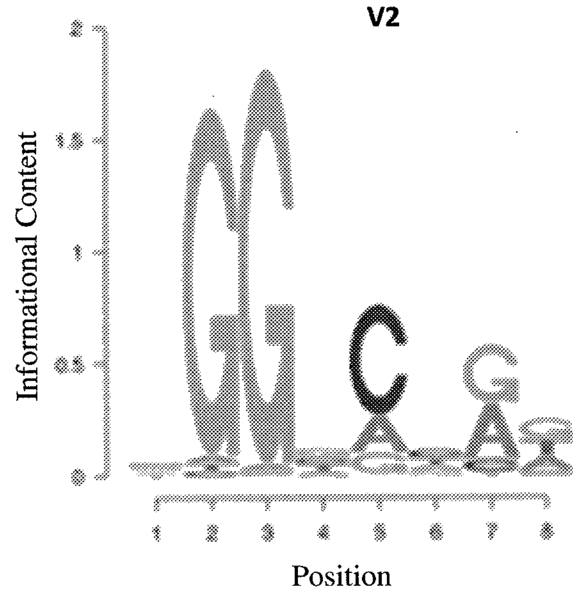
Fig. 2A

1	2	3	4	5	6	7	8	Ratio
	G	C	A	C				0.000
T	G	T	A					0.005
C	G	T	A					0.008
			A	C	T	/		0.012
	G	T	A	C				0.013
	C	T	A	A				0.016
	G	T	A	T				0.017
	G	T	A	A				0.017
		T	A	C	G			0.017
		T	A	C	T			0.019
	C	T	A	C				0.019
	A	T	A	C				0.022
	A	T	A	A				0.022
		T	A	A	T			0.033
			A	C	G	/		0.034
T	A	T	A					0.038
G	G	T	A					0.039
			A	C	T	1		0.044
	A	T	A	T				0.045
A	G	T	A					0.054
			A	A	T	/		0.055
		G	A	C	T			0.070
	G	T	A	G				0.073
	C	T	A	T				0.075

Fig. 2B

OMNI 6





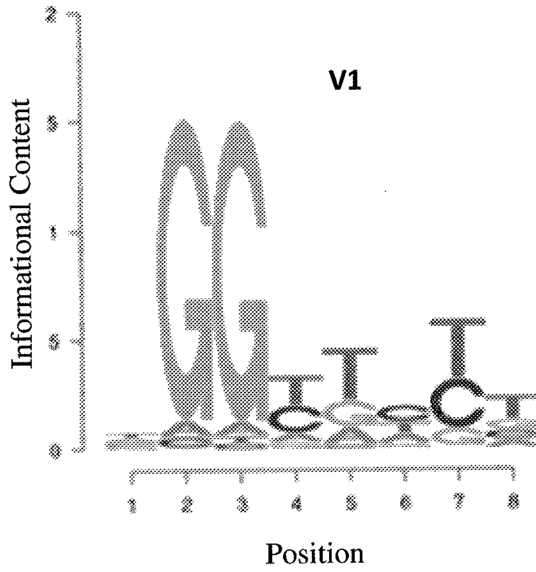
1	2	3	4	5	6	7	8	Ratio
	G	G	T	C				0.000
	G	G	A	C				0.000
	G	G	G	G				0.005
	G	G	C	T				0.005
	G	G	C	C				0.013
A	G	G	G					0.013
G	G	G	G					0.014
T	G	G	T					0.014
G	G	G	T					0.015
	G	G	T	A				0.015
	G	G	G	A				0.020
A	G	G	C					0.020
	G	G	G	T				0.021
T	G	G	G					0.023
C	G	G	G					0.029
	G	G	G	C				0.030
	G	G	A	A				0.031
G	G	G	C					0.041
G	G	G	A					0.042
C	G	G	C					0.050
T	G	G	C					0.059
C	G	G	T					0.069
	G	G	C	G				0.069
	G	G	C	A				0.069

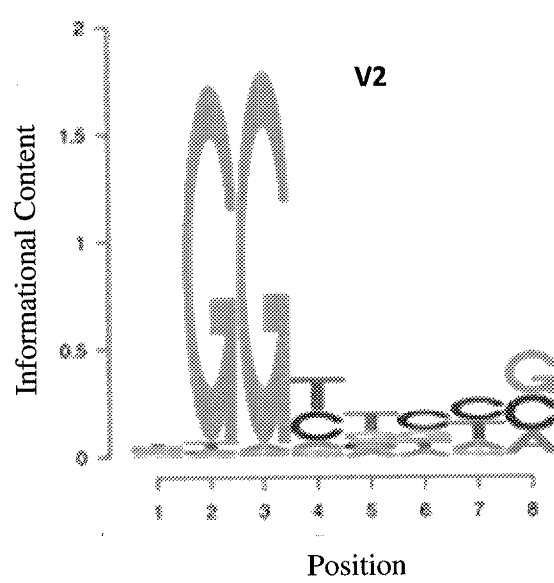
Fig. 3A

1	2	3	4	5	6	7	8	Ratio
	G	G	C	C				0.000
	G	G	A	C				0.007
	G	G	T	A				0.012
	G	G	G	C				0.014
C	G	G	G					0.018
G	G	G	G					0.020
A	G	G	T					0.025
	G	G	G	A				0.026
C	G	G	C					0.033
T	G	G	G					0.034
	G	G	T	C				0.035
G	G	G	C					0.036
	G	G	G	G				0.039
	G	G	G	T				0.039
C	G	G	A					0.040
T	G	G	C					0.040
	G	G	C	T				0.044
A	G	G	G					0.045
	G	G	C	A				0.049
C	G	G	T					0.051
A	G	G	C					0.055
T	G	G	T					0.055
	G	G	A	A				0.061
	G	G	T	G				0.062

Fig. 3B

OMNI 8





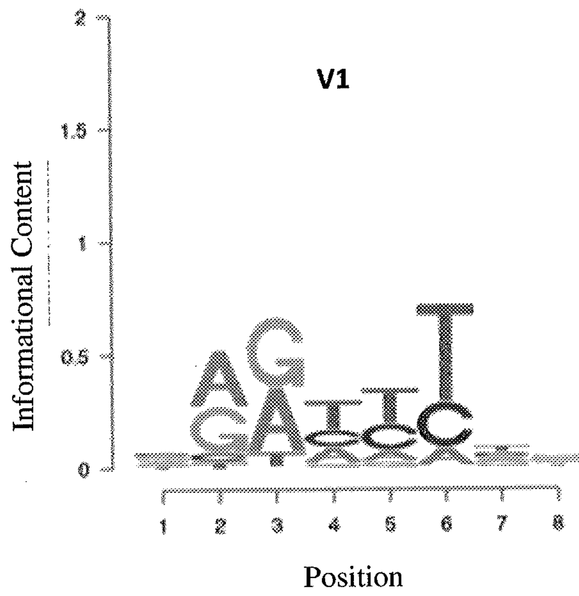
1	2	3	4	5	6	7	8	Ratio
	G	G	T	T				0.007
	G	G	T	G				0.016
	G	G	C	G				0.069
T	G	G	C					0.093
A	G	G	T					0.105
T	G	G	T					0.150
	G	G	C	T				0.166
C	G	G	T					0.175
	G	G	G	G				0.182
	G	G	A	G				0.193
	G	G	C	C				0.204
C	G	G	C					0.206
G	G	G	T					0.212
A	G	G	C					0.235
G	G	G	C					0.245
	G	G	T	A				0.265
	G	G	A	T				0.302
	G	G	C	A				0.308
T	G	G	A					0.312

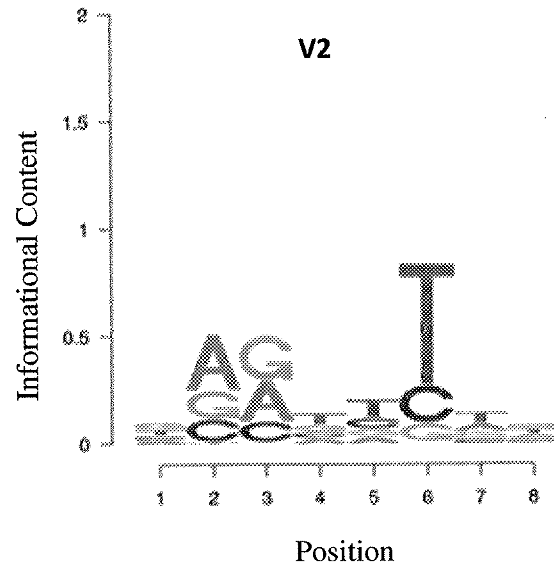
Fig. 4A

1	2	3	4	5	6	7	8	Ratio
	G	G	C	G				0.027
	G	G	T	T				0.032
	G	G	T	G				0.033
	G	G	C	T				0.053
	G	G	G	G				0.053
T	G	G	T					0.061
	G	G	A	G				0.071
	G	G	T	A				0.071
C	G	G	C					0.083
C	G	G	T					0.087
T	G	G	C					0.089
A	G	G	T					0.098
A	G	G	C					0.099
G	G	G	T					0.107
	G	G	C	C				0.137
G	G	G	C					0.148
	G	G	C	A				0.186
	G	G	G	T				0.243
T	G	G	A					0.254

Fig. 4B

OMNI 10





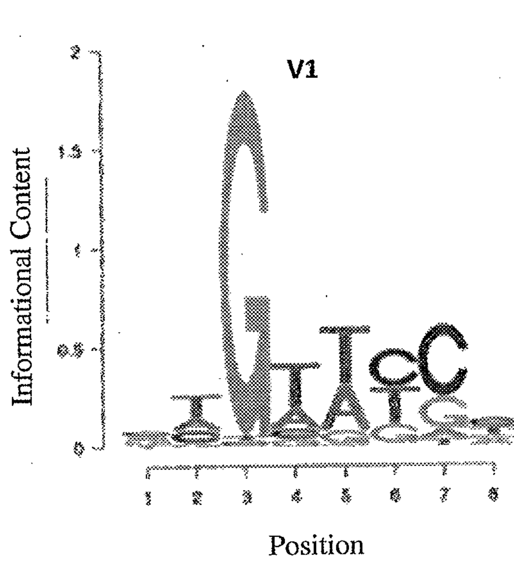
1	2	3	4	5	6	7	8	Ratio
		G	T	T	T			0.221
		A	T	T	T			0.246
	A	G	T	T				0.377
C	A	G	T					0.419
			T	T	T	A		0.437
	A	A	T	T				0.468
			T	T	T	T		0.487
		G	C	T	T			0.488
	G	A	T	T				0.496
		A	A	T	T			0.514
	G	G	T	T				0.520
	A	G	T	C				0.531
T	A	A	T					0.537
		G	A	T	T			0.548
	A	A	T	C				0.549

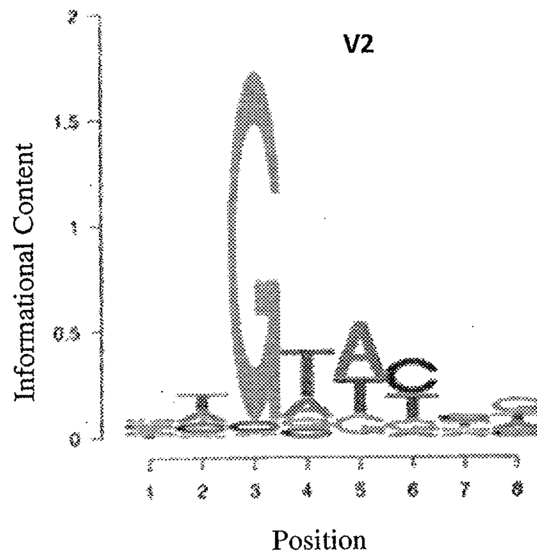
Fig. 5A

1	2	3	4	5	6	7	8	Ratio
		G	T	T	T			0.223
		A	T	T	T			0.269
		A	C	T	T			0.359
	A	G	T	T				0.476
		G	C	T	T			0.476
			T	T	T	T		0.488
	A	A	T	T				0.490
		G	A	T	T			0.513
C	G	G	T					0.516
				T	T	A	T	0.518
				T	T	A	A	0.541
				T	T	C	G	0.547
			T	T	T	A		0.548
		A	A	T	T			0.554
			C	T	T	C		0.555

Fig. 5B

OMNI 11





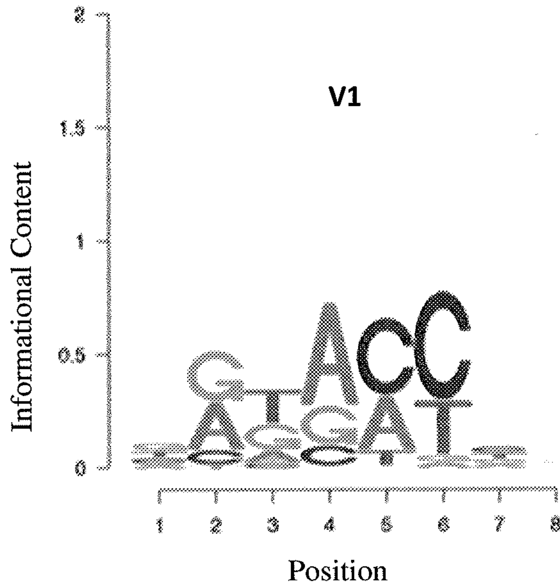
1	2	3	4	5	6	7	8	Ratio
	T	G	T	T				0.071
		G	T	A	C			0.075
C	T	G	T					0.077
		G	A	T	C			0.080
	T	G	T	A				0.083
		G	T	A	T			0.096
	C	G	T	A				0.098
	A	G	T	A				0.109
		G	T	T	C			0.110
	T	G	T	G				0.116
A	T	G	T					0.118
		G	A	A	T			0.124
G	T	G	T					0.127
	T	G	A	A				0.133
	C	G	T	T				0.148
	A	G	A	A				0.158
		G	T	G	T			0.159
	T	G	A	T				0.166
	A	G	T	T				0.171

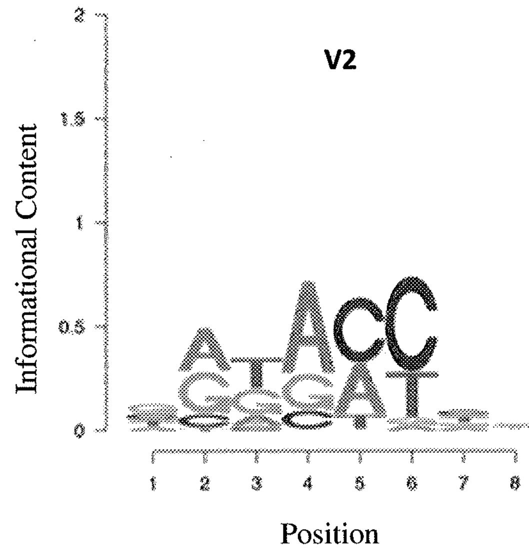
Fig. 6A

1	2	3	4	5	6	7	8	Ratio
		G	A	T	C			0.038
	T	G	T	A				0.051
G	T	G	T					0.082
		G	T	A	C			0.094
		G	T	A	T			0.097
	C	G	T	A				0.114
C	T	G	T					0.135
		G	A	A	C			0.141
	T	G	T	T				0.145
		G	T	T	T			0.147
	A	G	T	A				0.151
		G	T	A	G			0.158
A	T	G	T					0.166
G	A	G	T					0.174
	C	G	A	A				0.190
T	T	G	T					0.197
	T	G	T	C				0.200
		G	T	G	C			0.200
G	C	G	T					0.207

Fig. 6B

OMNI 13





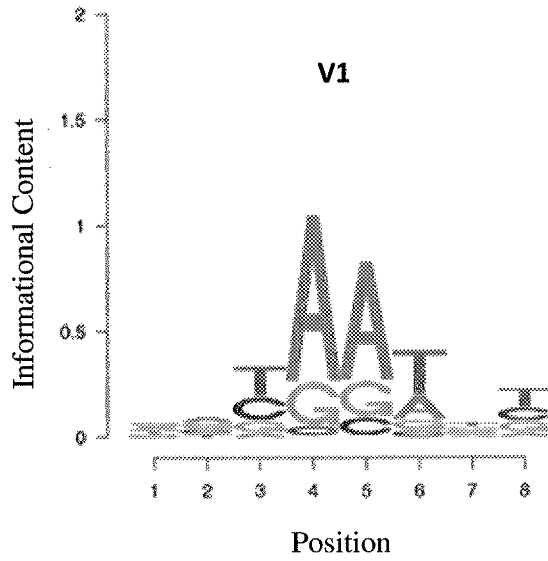
1	2	3	4	5	6	7	8	Ratio
	G	T	A	C				0.012
		T	A	C	T			0.015
		G	A	C	T			0.016
		T	A	C	C			0.017
		G	A	C	C			0.019
	A	T	A	C				0.021
		A	A	C	T			0.021
			A	C	T	A		0.023
			A	C	C	A		0.024
			A	C	T	G		0.030
			A	C	C	T		0.036
			A	C	T	T		0.037
	A	G	A	C				0.037
	G	G	A	C				0.048
	G	T	A	T				0.049
		A	A	C	C			0.054
			A	C	T	C		0.056
	C	T	A	C				0.057
	A	T	A	T				0.059
			A	C	C	G		0.062
			A	C	C	C		0.068
T	G	T	A					0.077
	G	G	A	T				0.083
	G	T	G	C				0.085

Fig. 7A

1	2	3	4	5	6	7	8	Ratio
		G	A	C	T			0.004
		T	A	C	T			0.008
			A	C	T	A		0.009
		T	A	C	C			0.010
	A	T	A	C				0.013
	G	T	A	C				0.017
		A	A	C	T			0.017
	A	G	A	C				0.019
			A	C	T	G		0.023
	G	T	A	T				0.025
			A	C	T	T		0.029
	G	G	A	C				0.031
			A	C	C	A		0.035
	A	T	A	T				0.039
		G	A	C	C			0.041
			A	C	C	C		0.047
			A	C	C	T		0.048
			A	C	C	G		0.061
			A	C	T	C		0.062
		A	A	C	C			0.068
	G	T	G	C				0.068
		T	A	T	C			0.077
	G	A	A	C				0.079
		C	A	C	T			0.082

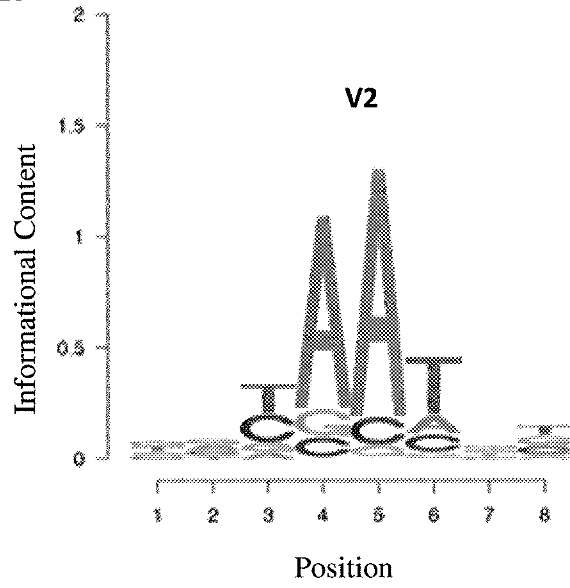
Fig. 7B

OMNI 17



1	2	3	4	5	6	7	8	Ratio
		T	A	A	T			0.013
		C	A	A	T			0.095
		T	A	A	A			0.139
	G	T	A	A				0.140
	A	C	A	A				0.152
	A	T	A	A				0.163
	C	T	A	A				0.205
			A	A	T	A		0.216
	T	C	A	A				0.285
		C	A	A	A			0.286
		T	A	G	T			0.358
			A	A	T	G		0.362
		T	A	A	C			0.365
			A	A	T	T		0.382
T	G	T	A					0.394
			A	A	T	C		0.430
		T	A	A	G			0.435
		C	A	A	C			0.440
	G	C	A	A				0.441

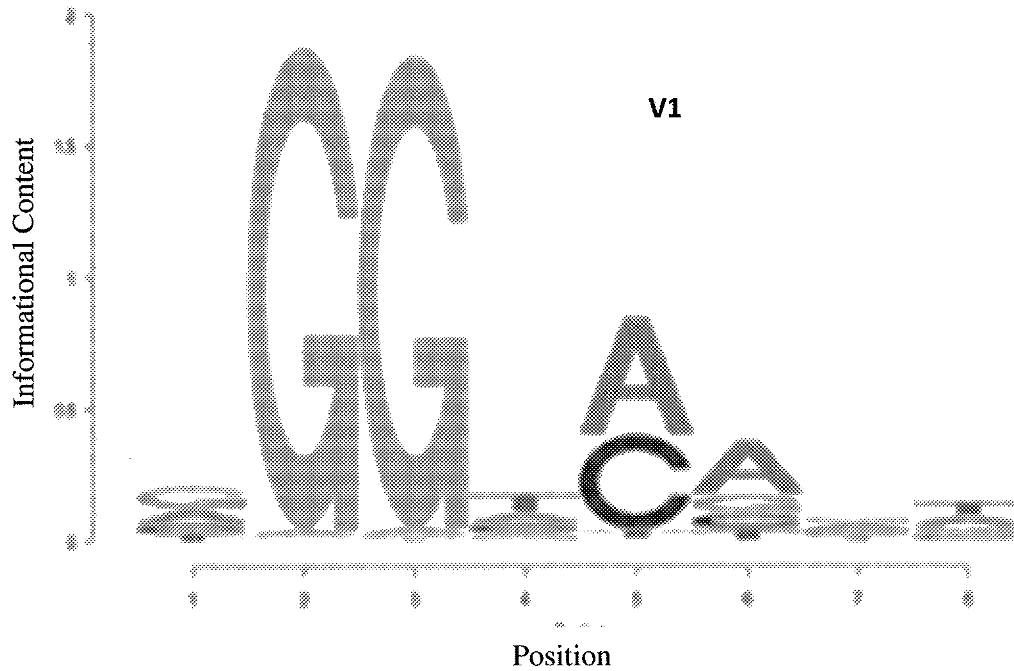
Fig. 8A



1	2	3	4	5	6	7	8	Ratio
		T	A	A	T			0.060
		C	A	A	T			0.062
	G	T	A	A				0.086
		C	A	A	A			0.138
	G	C	A	A				0.165
	A	C	A	A				0.166
			A	A	T	A		0.176
	A	T	A	A				0.190
	C	T	A	A				0.209
		T	A	A	A			0.238
	T	C	A	A				0.248
			A	A	T	T		0.255
		C	A	A	C			0.302
		A	A	A	T			0.321
		T	A	A	G			0.348
			A	A	T	C		0.370
	C	C	A	A				0.388
			A	A	T	G		0.397
		T	A	A	C			0.403

Fig. 8B

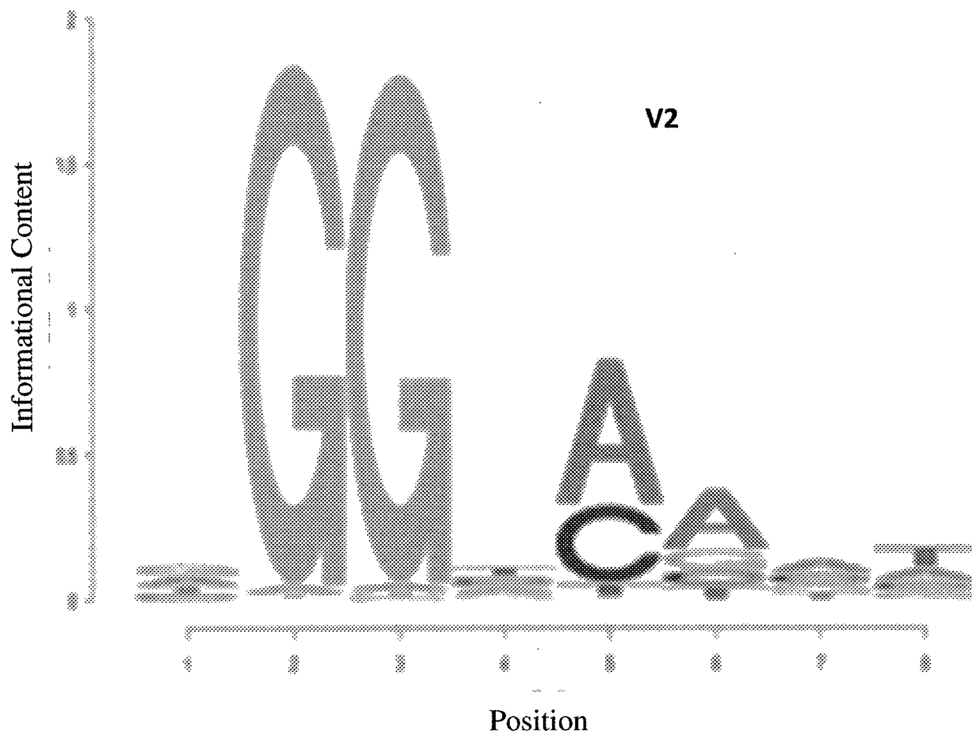
OMNI 18



1	2	3	4	5	6	7	8	Ratio
	G	G	T	C				0.006
	G	G	A	A				0.007
G	G	G	T					0.007
	G	G	T	G				0.008
	G	G	T	A				0.008
	G	G	A	C				0.011
A	G	G	T					0.012
G	G	G	A					0.013
C	G	G	T					0.015
	G	G	G	A				0.016
	G	G	C	C				0.017
G	G	G	C					0.017
A	G	G	C					0.020
	G	G	C	A				0.021
G	G	G	G					0.024
A	G	G	A					0.027
	G	G	T	T				0.030
A	G	G	G					0.035
	G	G	C	T				0.039
	G	G	G	C				0.041
C	G	G	A					0.045
	G	G	A	T				0.046
C	G	G	G					0.050

Fig. 9A

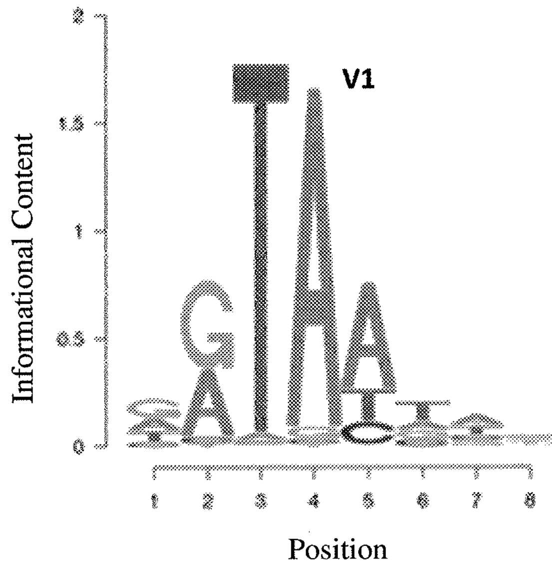
OMNI 18



1	2	3	4	5	6	7	8	Ratio
	G	G	A	A				0.008
	G	G	C	A				0.010
A	G	G	C					0.011
	G	G	A	C				0.011
G	G	G	T					0.012
G	G	G	A					0.013
	G	G	T	A				0.013
T	G	G	T					0.014
G	G	G	C					0.015
	G	G	T	G				0.016
	G	G	C	T				0.018
	G	G	T	T				0.019
	G	G	G	A				0.020
C	G	G	T					0.023
A	G	G	T					0.023
	G	G	T	C				0.024
A	G	G	A					0.026
	G	G	C	G				0.027
G	G	G	G					0.031
	G	G	C	C				0.035
C	G	G	C					0.035
T	G	G	C					0.039

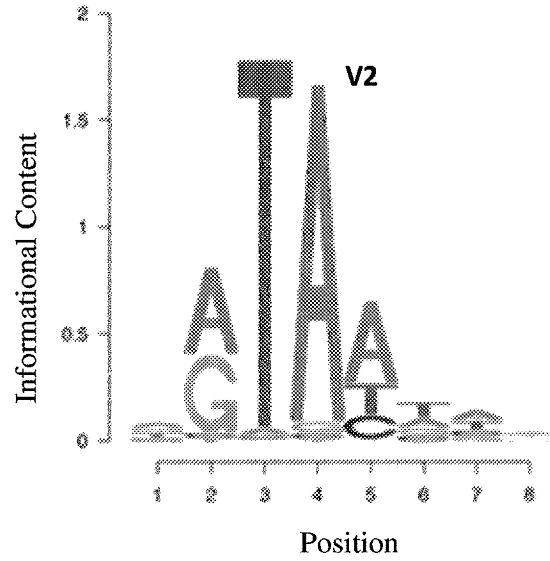
Fig. 9B

OMNI 19



1	2	3	4	5	6	7	8	Ratio
	A	T	A	T				0.000
	G	T	A	G				0.000
T	A	T	A					0.000
C	A	T	A					0.000
	A	T	A	A				0.002
	A	T	A	G				0.003
G	A	T	A					0.003
G	G	T	A					0.003
T	G	T	A					0.003
	A	T	A	C				0.003
A	A	T	A					0.004
	G	T	A	C				0.004
A	G	T	A					0.007
	G	T	A	A				0.008
	G	T	A	T				0.009
C	G	T	A					0.009
		T	A	A	T			0.026
	C	T	A	A				0.034
G	C	T	A					0.044
		T	A	T	T			0.053
	C	T	A	T				0.056
T	C	T	A					0.057
		T	A	A	G			0.061

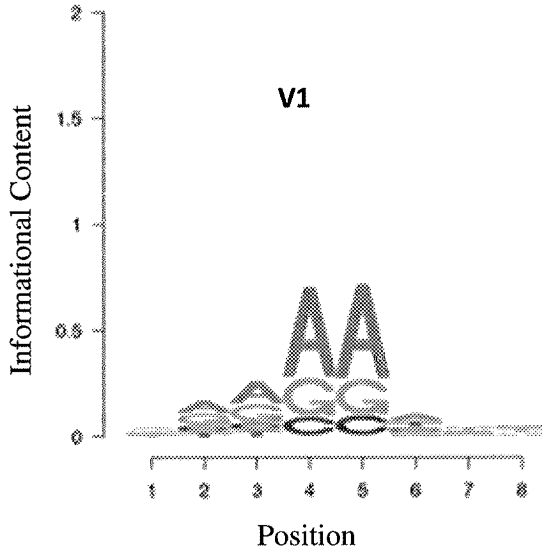
Fig. 10A

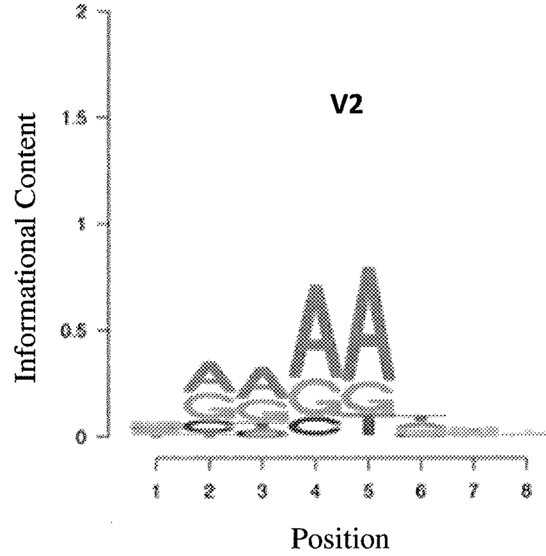


1	2	3	4	5	6	7	8	Ratio
	G	T	A	A				0.000
C	A	T	A					0.000
	G	T	A	T				0.000
G	C	T	A					0.000
	A	T	A	G				0.000
T	G	T	A					0.000
A	G	T	A					0.002
	A	T	A	T				0.003
T	A	T	A					0.003
G	A	T	A					0.003
	G	T	A	G				0.003
	A	T	A	A				0.004
C	G	T	A					0.004
A	A	T	A					0.009
G	G	T	A					0.009
	A	T	A	C				0.013
		T	A	A	T			0.015
	G	T	A	C				0.017
	C	T	A	A				0.033
		T	A	T	T			0.036
	C	T	A	T				0.040
T	C	T	A					0.051
	C	T	A	G				0.068

Fig. 10B

OMNI 20





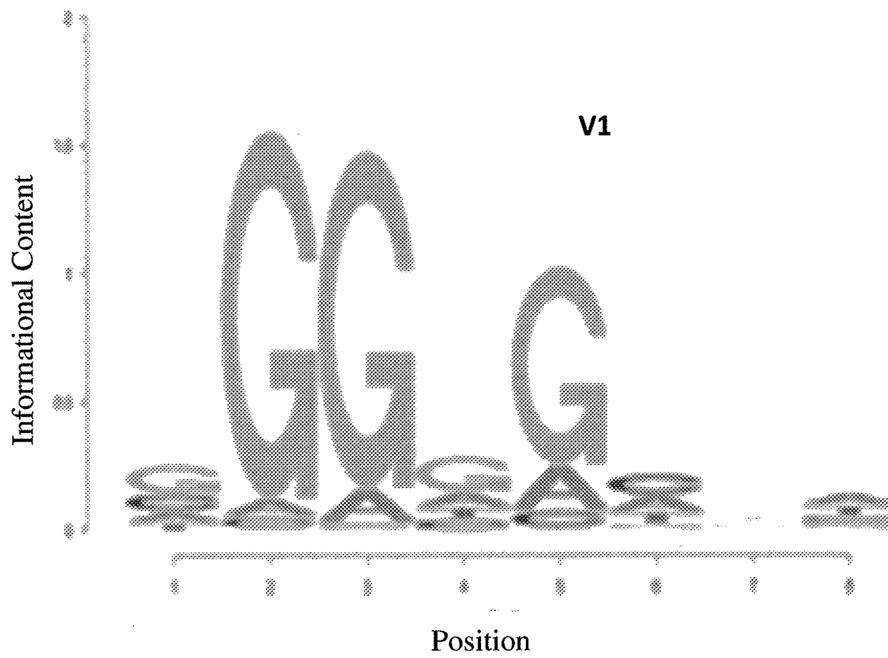
1	2	3	4	5	6	7	8	Ratio
	A	A	A	C				0.7
		A	A	A	T			0.73
	A	G	A	A				0.73
G	A	A	A					0.73
			A	A	T	C		0.73
			A	A	A	G		0.74
C	G	A	A					0.74
A	A	A	A					0.74
		A	A	A	A			0.74
	G	A	A	A				0.75
A	T	A	A					0.75
		A	A	G	C			0.75
	G	C	A	G				0.75
	T	A	A	A				0.75
	C	A	A	A				0.75
A	G	A	A					0.75
		A	A	C	A			0.76
	G	T	A	A				0.76

Fig. 11A

1	2	3	4	5	6	7	8	Ratio
	A	C	A	A				0.347
			A	A	A	G		0.384
		A	A	G	T			0.385
	A	G	A	A				0.394
		G	A	A	G			0.398
			A	A	T	G		0.406
		A	G	A	T			0.410
		A	A	G	A			0.412
	C	G	A	G				0.416
	A	A	A	G				0.416
	A	A	A	A				0.423
	G	G	A	A				0.428
G	A	G	A					0.433
		G	A	A	A			0.437
	A	A	G	A				0.441
			A	A	G	C		0.441
	G	A	A	G				0.442
	C	A	A	A				0.445

Fig. 11B

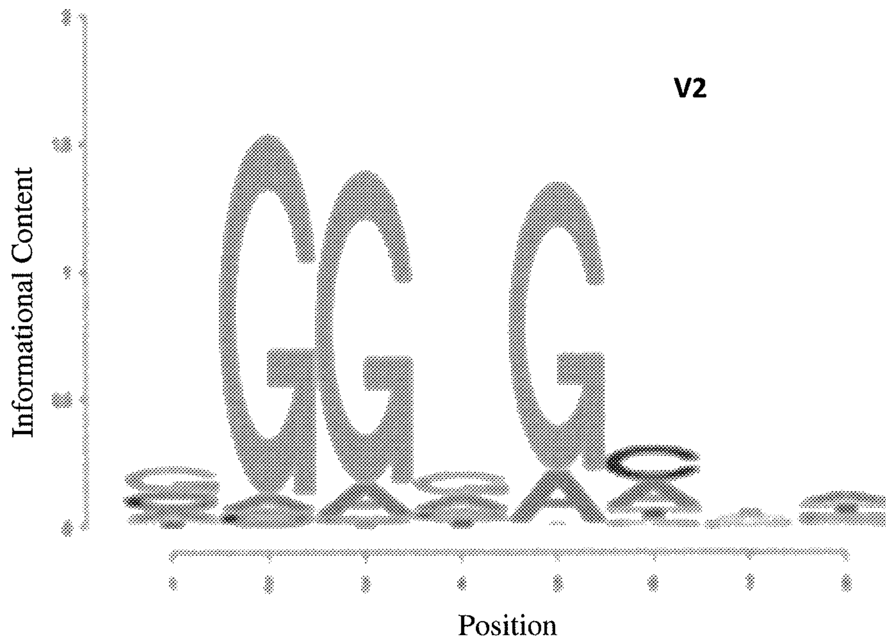
OMNI 23



1	2	3	4	5	6	7	8	Ratio
	G	G	G	G				0.02
G	G	G	G					0.02
	G	G	C	G				0.03
	G	G	A	G				0.03
	G	G	T	G				0.04
C	G	G	G					0.05
	G	G	G	A				0.06
G	G	G	A					0.07
A	G	G	G					0.07
	G	G	A	A				0.1
G	G	G	T					0.1
C	G	G	A					0.11
	G	G	G	C				0.11
C	G	G	C					0.12
	G	A	G	G				0.13
G	G	G	C					0.13
C	G	G	T					0.13
	G	G	G	T				0.14
T	G	G	G					0.16
A	G	G	C					0.17
	G	G	C	A				0.18
A	G	G	A					0.18
	G	G	A	C				0.2
	G	G	C	T				0.22

Fig. 12A

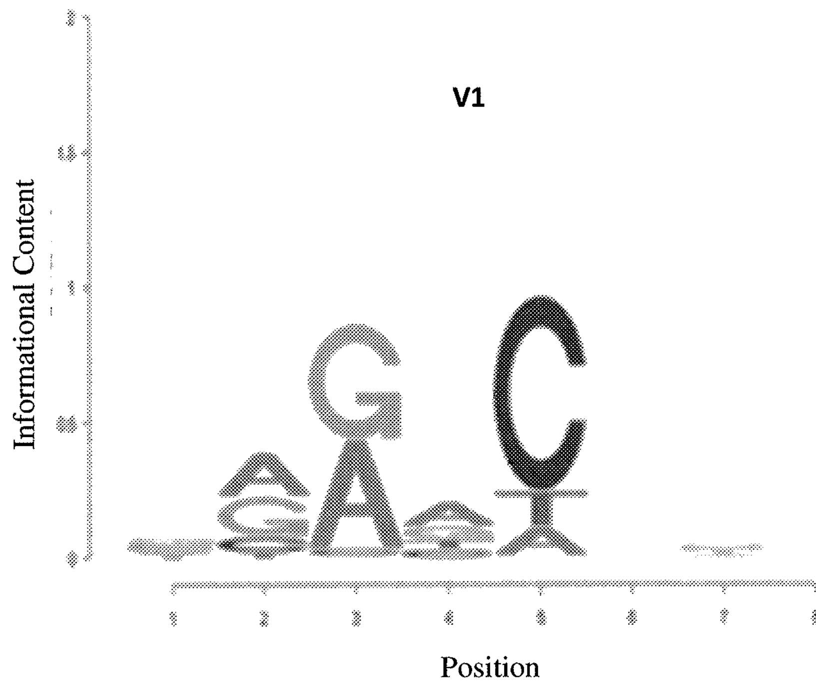
OMNI 23



1	2	3	4	5	6	7	8	Ratio
	G	G	G	G				0.02
	G	G	C	G				0.03
	G	G	A	G				0.03
G	G	G	G					0.04
	G	G	T	G				0.04
G	G	G	A					0.06
C	G	G	G					0.06
C	G	G	A					0.08
	G	G	G	A				0.09
A	G	G	G					0.1
G	G	G	C					0.1
	G	G	A	A				0.13
G	G	G	T					0.14
	G	G	G	C				0.15
	G	G	G	T				0.16
C	G	G	C					0.17
	G	A	G	G				0.17
C	G	G	T					0.18
	G	G	C	A				0.18
T	G	G	G					0.18
A	G	G	A					0.19
G	G	A	G					0.21
	G	G	A	T				0.21
	G	G	A	C				0.23

Fig. 12B

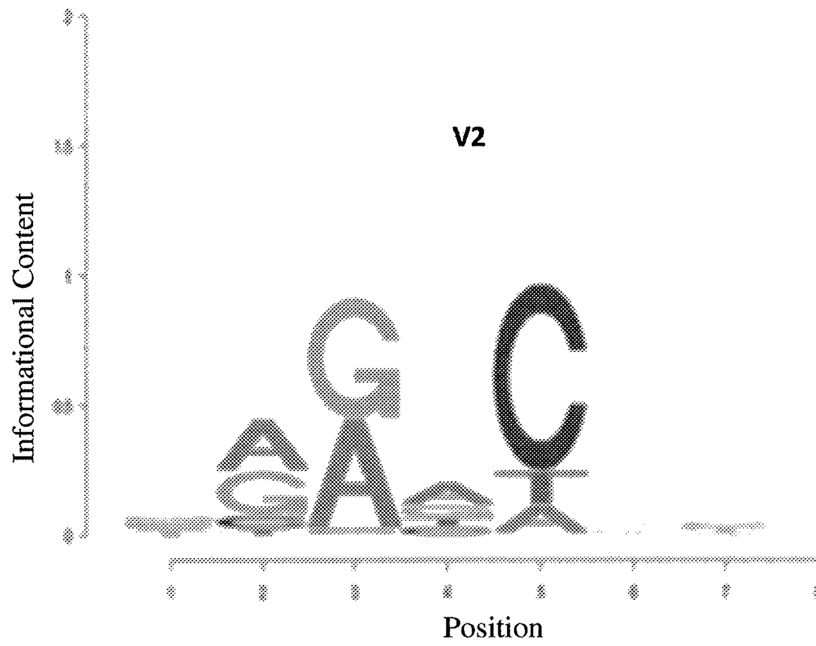
OMNI 24



1	2	3	4	5	6	7	8	Ratio
	A	G	T	C				0.01
	A	A	A	C				0.01
	G	G	A	C				0.01
	A	G	A	C				0.01
	G	A	A	C				0.01
		A	A	C	G			0.02
	G	A	G	C				0.02
	G	G	G	C				0.02
	C	G	A	C				0.03
		G	A	C	G			0.03
	G	G	C	C				0.03
		A	A	C	A			0.03
	A	G	G	C				0.03
		G	A	C	A			0.04
		G	A	C	T			0.04
	A	G	C	C				0.05
	A	A	G	C				0.05
		A	A	C	T			0.05
		G	A	C	C			0.08
		A	A	C	C			0.1
		G	C	C	G			0.11
		A	G	C	A			0.13
		G	C	C	A			0.14
		G	T	C	G			0.14

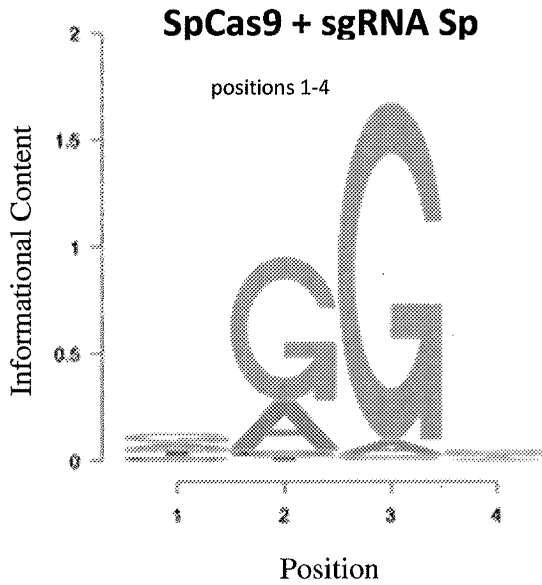
Fig. 13A

OMNI 24



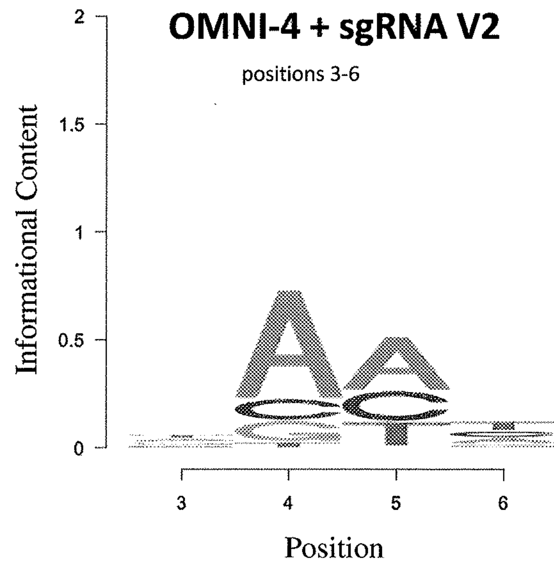
1	2	3	4	5	6	7	8	Ratio
	A	G	A	C				0
	A	G	T	C				0.01
	G	A	A	C				0.01
		A	A	C	G			0.01
	A	A	A	C				0.01
	G	G	A	C				0.02
	C	G	A	C				0.02
		G	A	C	G			0.02
	G	G	C	C				0.02
	G	G	G	C				0.03
	A	A	G	C				0.03
		G	A	C	A			0.03
		A	A	C	A			0.03
	G	A	G	C				0.03
	A	G	C	C				0.03
		G	A	C	T			0.04
	A	A	T	C				0.05
		A	A	C	T			0.06
		G	A	C	C			0.08
		G	C	C	G			0.08
		A	A	C	C			0.11
		A	G	C	A			0.11
		G	G	C	A			0.14
		G	C	C	A			0.14

Fig. 13B



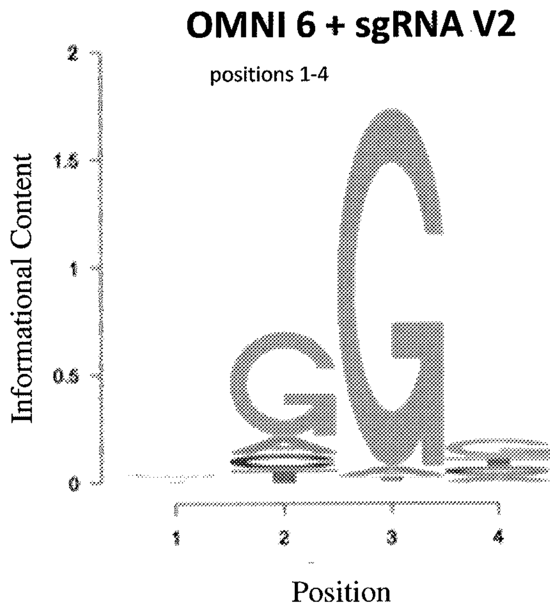
1	2	3	4	5	6	7	8	Ratio
A	G	G	C					0.056
	G	G	G	C				0.057
A	G	G	G					0.057
	G	G	C	C				0.059
G	G	G	C					0.061
	G	G	G	A				0.065
G	A	G	C					0.066
T	G	G	G					0.068
	G	G	A	T				0.068
	G	G	T	C				0.071
	G	G	C	A				0.074
A	G	G	T					0.077
		G	G	C	G			0.241
		G	G	C	A			0.285
		G	G	T	G			0.301
		G	G	C	T			0.305
		G	G	T	C			0.324
		G	G	A	T			0.349

Figure 14



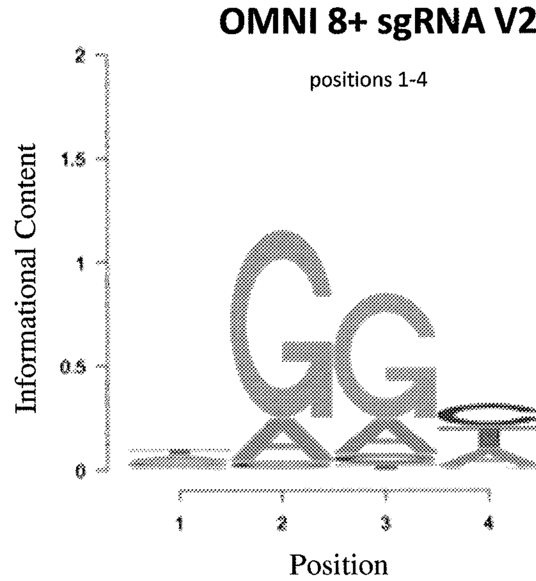
1	2	3	4	5	6	7	8	Ratio
		G	A	A	T			0.078
	A	T	A	C				0.081
		G	A	C	T			0.082
C	G	A	A					0.083
			T	A	C	T		0.083
	G	C	A	C				0.087
			A	C	T	G		0.088
		G	T	A	C			0.088
C	G	C	A					0.089
			A	A	T	C		0.091
		T	A	A	T			0.091
G	A	T	A					0.092
	C	C	A	A				0.092
	A	T	A	T				0.092
		A	A	C	T			0.093
		C	A	A	T			0.093
			A	C	T	A		0.094
	G	C	A	T				0.095
		C	A	C	T			0.095

Figure 15



1	2	3	4	5	6	7	8	Ratio
	G	G	C	C				0.019
	G	G	G	G				0.020
G	G	G	C					0.020
A	G	G	G					0.021
C	G	G	G					0.021
	G	G	T	A				0.021
	G	G	G	A				0.023
		G	G	C	C			0.025
T	G	G	T					0.026
	G	G	G	C				0.027
A	G	G	T					0.027
		G	T	A	C			0.027
	G	G	T	C				0.028
		G	C	C	A			0.029
C	G	G	C					0.029
		G	G	T	A			0.031
		G	G	G	C			0.033
		G	G	C	T			0.034

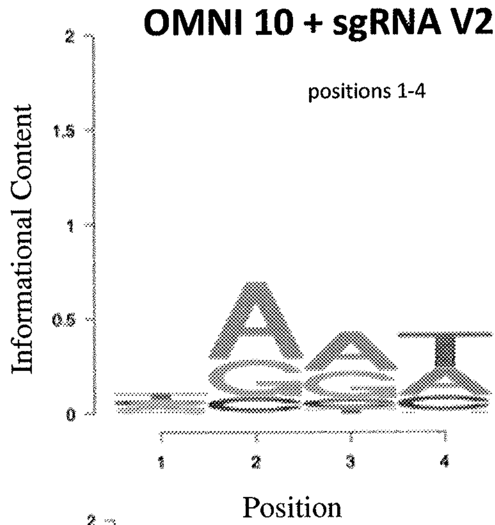
Figure 16

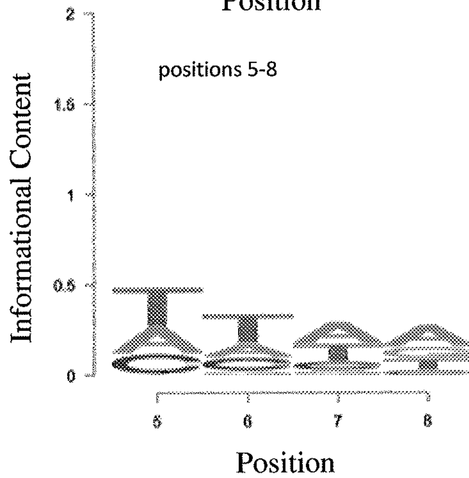


1	2	3	4	5	6	7	8	Ratio
	G	G	C	T				0.198
	G	G	T	T				0.216
C	G	G	C					0.231
T	G	G	C					0.234
	G	G	C	G				0.245
G	G	G	T					0.251
	G	G	A	G				0.267
A	G	G	T					0.272
T	G	G	T					0.279
G	G	G	C					0.285
	G	G	T	A				0.286
	G	G	C	C				0.291
				A	A	A	A	0.609
				T	A	A	A	0.624
		A	A	A	A			0.646
				G	A	A	A	0.649
				A	G	A	A	0.672
			A	A	A	A		0.695

Figure 17

OMNI 10 + sgRNA V2

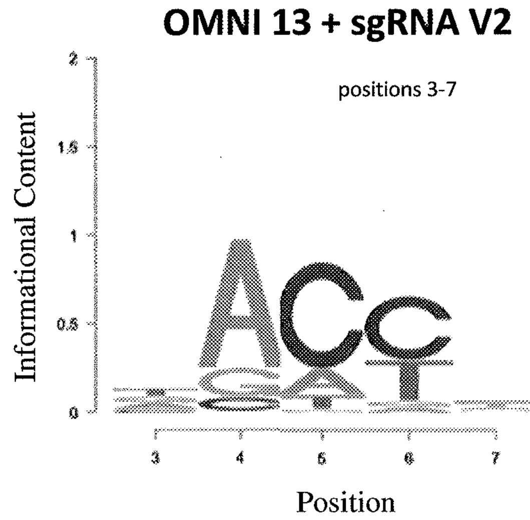




1	2	3	4	5	6	7	8	Ratio
		A	T	T	T			0.361
		G	T	T	T			0.403
			T	T	T	T		0.527
			T	T	T	A		0.551
	G	G	T	T				0.628
	A	A	T	T				0.645
		G	C	T	T			0.651
			T	T	T	G		0.659
C	A	A	A					0.664
	A	G	T	C				0.681
		A	A	T	T			0.684
C	A	A	T					0.685
	A	G	T	T				0.688
			C	T	T	A		0.701
	A	A	A	T				0.704
			T	C	T	T		0.707
				T	T	T	T	0.708
		G	T	C	T			0.710

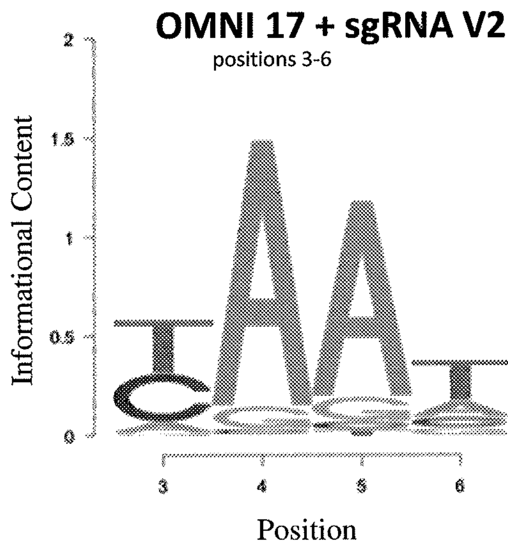
Figure 18

OMNI 13 + sgRNA V2



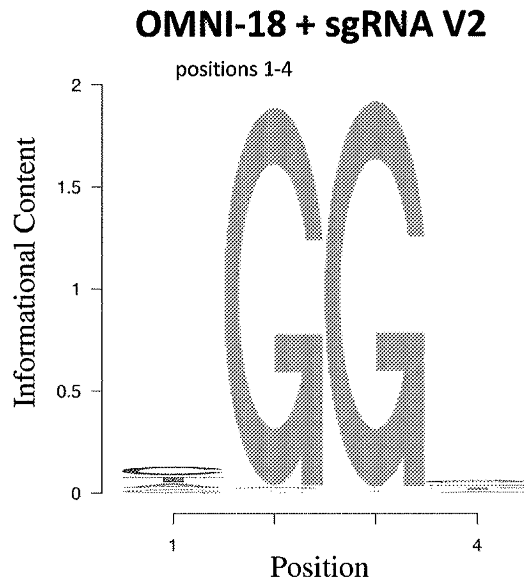
1	2	3	4	5	6	7	8	Ratio
			A	C	C	C		0.020
		T	A	C	C			0.026
		A	A	C	T			0.027
	G	T	A	C				0.030
	C	T	A	C				0.038
	C	G	A	C				0.040
			A	C	T	A		0.042
		G	A	C	T			0.043
			A	C	T	G		0.043
			A	C	C	G		0.044
C	G	T	A					0.047
	A	T	A	C				0.048
			A	C	T	T		0.048
		T	A	C	T			0.051
	G	T	A	T				0.052
			A	C	T	C		0.053
		A	A	C	C			0.055
		G	A	C	C			0.056

Figure 19



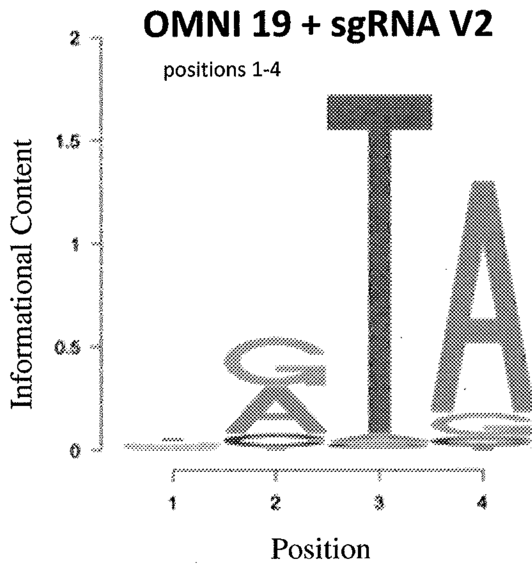
1	2	3	4	5	6	7	8	Ratio
	A	C	A	A				0.046
		C	A	A	T			0.049
	G	C	A	A				0.051
	C	T	A	A				0.056
	G	T	A	A				0.060
		T	A	A	T			0.062
	A	T	A	A				0.065
		T	A	A	A			0.088
		C	A	A	A			0.089
			A	A	T	A		0.105
		A	A	A	T			0.130
		T	A	A	C			0.161
			A	A	T	T		0.209
	T	C	A	A				0.217
			A	A	T	C		0.219
			A	A	T	G		0.237
G	G	T	A					0.423
T	G	T	A					0.443

Figure 20



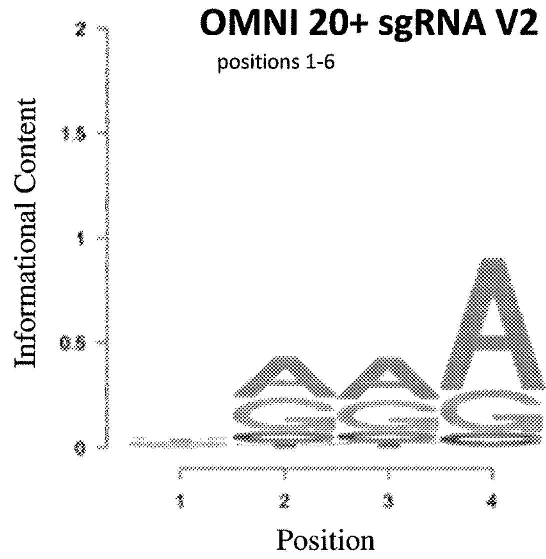
1	2	3	4	5	6	7	8	Ratio
C	G	G	C					0.012
	G	G	C	G				0.020
C	G	G	G					0.021
C	G	G	T					0.023
T	G	G	C					0.025
	G	G	T	C				0.028
	G	G	A	A				0.034
	G	G	A	T				0.034
C	G	G	A					0.037
A	G	G	A					0.037
T	G	G	T					0.039
	G	G	G	A				0.040
	G	G	T	A				0.041
	G	G	C	C				0.042
	G	G	C	T				0.042
G	G	G	G					0.043
	G	G	T	G				0.043
T	G	G	A					0.043
	G	G	G	T				0.043
A	G	G	T					0.045

Figure 21



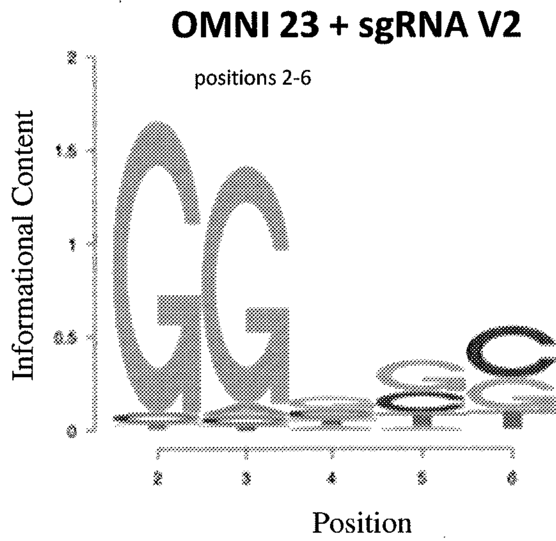
1	2	3	4	5	6	7	8	Ratio
	G	T	A	T				0.052
	A	T	A	G				0.052
C	G	T	A					0.054
T	A	T	A					0.054
A	G	T	A					0.056
T	G	T	A					0.057
	A	T	A	T				0.057
	G	T	A	A				0.059
G	A	T	A					0.061
	G	T	A	C				0.063
	G	T	A	G				0.069
C	A	T	A					0.069
		T	A	A	T			0.133
		T	A	A	G			0.150
		T	A	T	T			0.169
		T	A	C	T			0.247
		T	A	T	G			0.256
		T	A	T	C			0.260

Figure 22



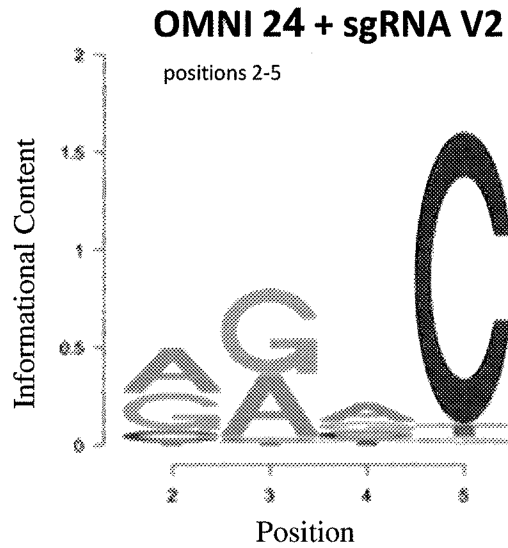
1	2	3	4	5	6	7	8	Ratio
	A	G	A	G				0.149
	C	A	A	A				0.150
	G	A	A	A				0.151
	G	A	G	A				0.152
	A	A	A	C				0.157
	G	G	A	G				0.157
A	G	A	A					0.167
G	A	G	A					0.174
		G	A	A	C			0.177
G	A	A	A					0.177
G	G	A	A					0.183
C	A	A	A					0.192
T	G	A	A					0.199
		A	A	A	T			0.203
		A	A	A	A			0.211
		G	G	A	A			0.212
		A	A	A	C			0.212
		A	G	A	A			0.217
			A	A	T	C		0.255
			A	A	A	G		0.299

Figure 23



1	2	3	4	5	6	7	8	Ratio
	G	G	G	G				0.132
G	G	G	G					0.171
	G	G	C	G				0.180
	G	G	A	G				0.184
	G	G	T	G				0.207
A	G	G	G					0.219
C	G	G	G					0.226
	G	G	G	A				0.239
G	G	G	A					0.311
G	G	G	C					0.335
	G	G	G	C				0.340
C	G	G	A					0.365
		G	T	G	C			0.643
		G	C	G	C			0.666
		G	A	G	C			0.689
		G	G	G	A			0.689
				G	C	G	G	0.710
			G	G	T	G		0.717
				T	C	A	G	0.723
		G	C	T	C			0.729

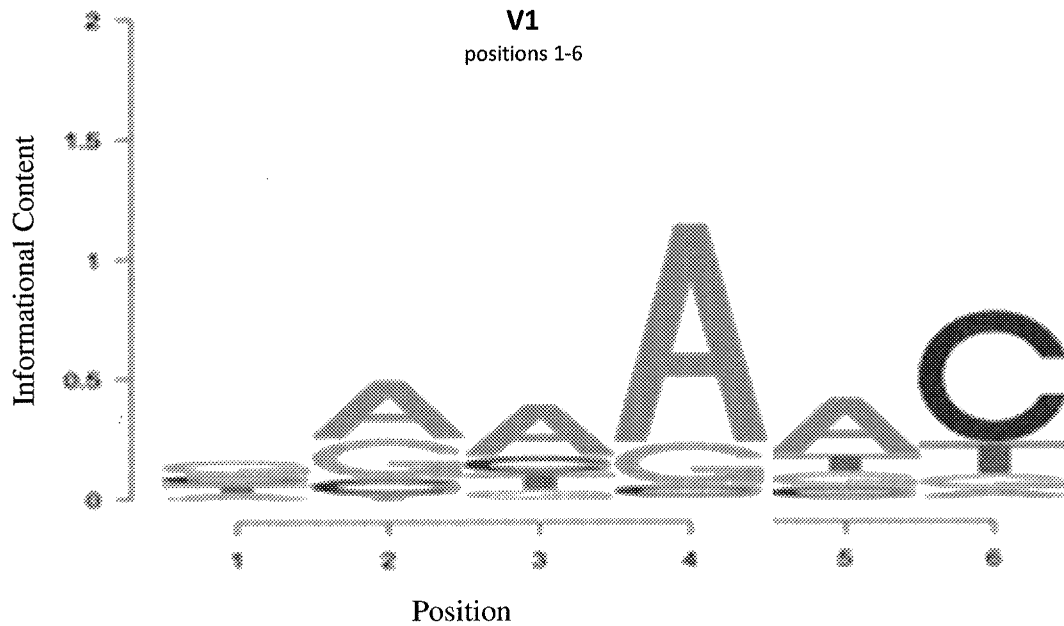
Figure 24



1	2	3	4	5	6	7	8	Ratio
	A	G	A	C				0.138
	A	A	A	C				0.145
	G	A	A	C				0.146
	G	G	A	C				0.172
	A	G	C	C				0.190
		G	A	C	A			0.208
		A	A	C	A			0.234
	A	G	G	C				0.245
		A	A	C	G			0.267
		G	A	C	G			0.298
		G	A	C	T			0.328
		A	A	C	T			0.345
			A	C	G	G		0.544
			A	C	A	C		0.548
			A	C	A	G		0.564
			A	C	G	T		0.566
			A	C	T	G		0.570
			A	C	G	C		0.575
G	A	G	A					0.612
				C	G	A	G	0.635

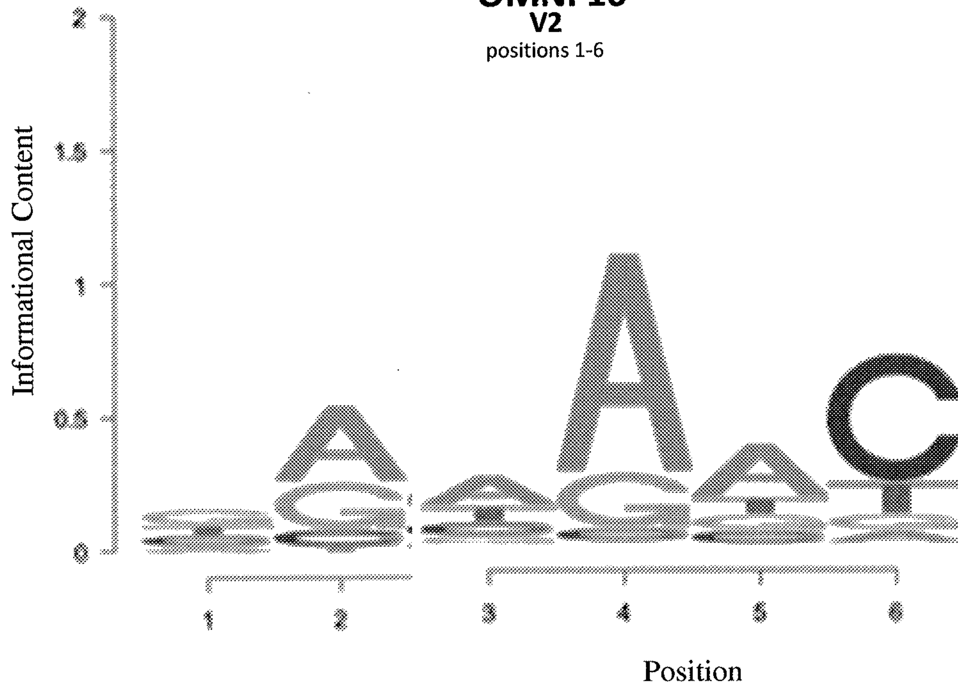
Figure 25

OMNI 16



1	2	3	4	5	6	7	8	Ratio
		A	A	A	C			0.175
			A	A	C	A		0.205
		T	A	A	C			0.218
G	A	A	A					0.240
			A	A	C	T		0.258
		C	A	A	C			0.271
	G	A	A	A				0.288
	A	A	A	A				0.312
			A	A	C	G		0.319
		A	A	A	T			0.360
		A	G	A	C			0.374
		A	A	T	C			0.382
			A	A	C	C		0.387
	A	A	A	T				0.397
	A	A	A	C				0.403
C	A	A	A					0.418
			G	A	C	A		0.435
T	A	A	A					0.442
			A	G	C	A		0.469
G	G	A	A					0.472
	A	C	A	A				0.479

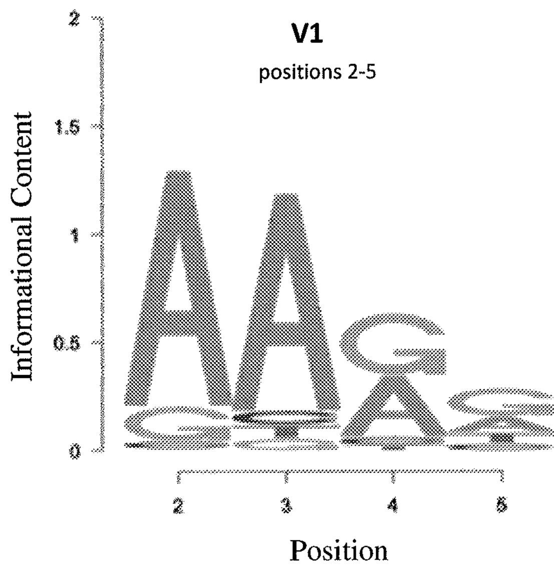
Fig. 26A



1	2	3	4	5	6	7	8	Ratio
		A	A	A	C			0.107
G	A	A	A					0.133
			A	A	C	A		0.146
		T	A	A	C			0.158
	A	A	A	A				0.178
			A	A	C	T		0.198
	G	A	A	A				0.234
	A	A	A	C				0.252
		C	A	A	C			0.254
C	A	A	A					0.257
			A	A	C	C		0.264
			A	A	C	G		0.270
T	A	A	A					0.280
G	G	A	A					0.285
		A	A	T	C			0.297
		A	G	A	C			0.305
	A	A	A	T				0.314
	G	A	A	T				0.341
	A	C	A	A				0.357
		G	A	A	C			0.362
T	G	A	A					0.365

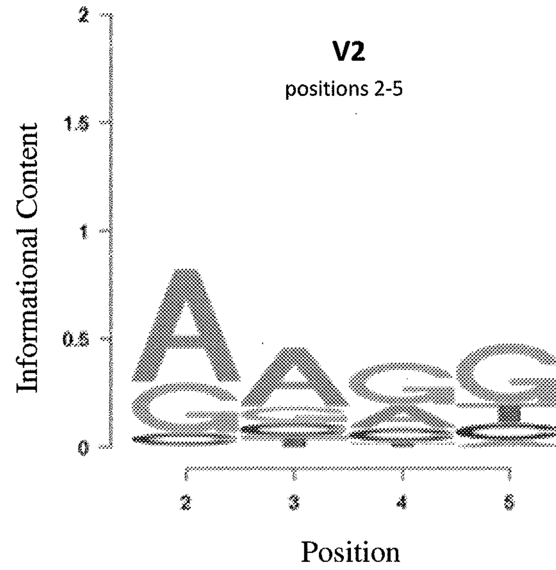
Fig. 26B

OMNI 21



1	2	3	4	5	6	7	8	Ratio
	A	A	G	G				0.227
	A	A	A	G				0.295
C	A	A	G					0.329
G	A	A	G					0.340
G	A	A	A					0.347
T	A	A	G					0.356
T	A	A	A					0.377
	A	A	G	T				0.388
	A	A	G	A				0.389
	G	A	G	G				0.402
C	A	A	A					0.475
	A	A	A	T				0.484
		A	G	G	T			0.535
		A	G	G	A			0.543
		A	A	G	T			0.566
		A	G	G	G			0.569
		A	A	G	A			0.629
		A	A	G	C			0.690
			A	C	C	C		0.739
				A	A	A	A	0.759
			G	A	A	A		0.767

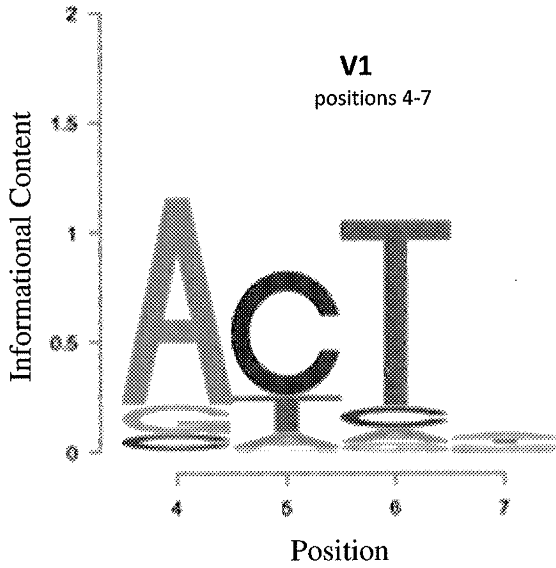
Fig. 27A

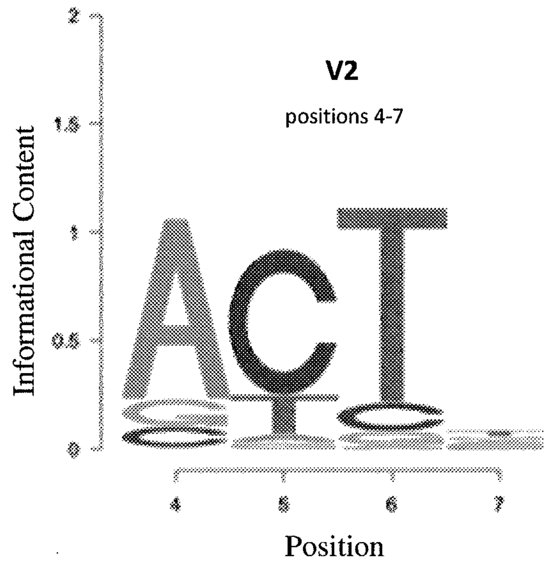


1	2	3	4	5	6	7	8	Ratio
	A	A	G	G				0.334
	A	A	A	G				0.468
C	A	A	G					0.509
T	A	A	G					0.541
	G	A	G	G				0.605
	A	A	G	T				0.627
G	A	A	G					0.628
		A	G	G	A			0.639
T	A	A	A					0.640
G	A	A	A					0.660
	G	A	A	G				0.701
	A	G	G	G				0.704
		A	A	G	T			0.706
		A	G	G	T			0.710
		A	G	G	G			0.719
		C	G	C	C			0.728
				G	C	C	C	0.736
C	A	A	A					0.748
				G	T	G	C	0.783
		A	A	G	A			0.790
			G	C	C	T		0.808

Fig. 27B

OMNI 27





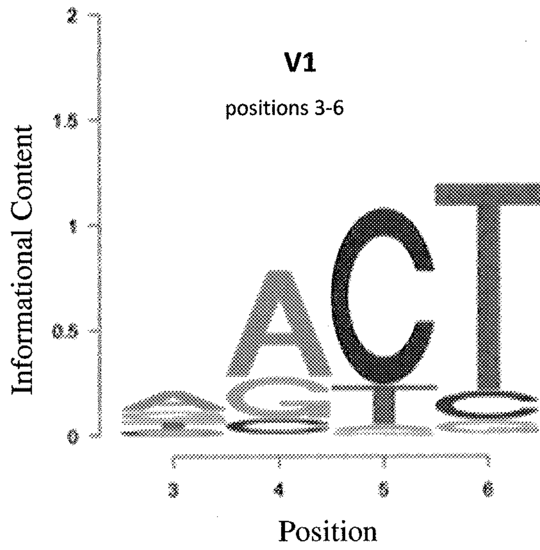
1	2	3	4	5	6	7	8	Ratio
			A	C	T	A		0.051
		A	A	T	T			0.061
		T	A	C	T			0.064
		G	A	C	T			0.066
			A	C	T	G		0.072
		A	A	C	T			0.078
		G	A	T	T			0.083
			A	C	T	T		0.084
		A	G	C	T			0.084
			A	C	T	C		0.095
	C	A	A	C				0.133
	C	G	A	C				0.134
	A	A	C	T				0.154
	G	A	C	T				0.157
			A	T	T	T		0.184
			A	T	T	A		0.215
	A	A	A	C				0.222
	G	A	A	C				0.227
				C	T	T	T	0.381
				C	T	A	A	0.410
				A	C	T	T	0.471

Fig. 28A

1	2	3	4	5	6	7	8	Ratio
		T	A	C	T			0.057
			A	C	T	T		0.060
		A	A	C	T			0.071
			A	C	T	G		0.073
			A	C	T	A		0.074
		C	A	C	T			0.080
		G	A	C	T			0.087
			A	C	T	C		0.091
		G	A	T	T			0.096
		A	A	T	T			0.106
	A	A	C	T				0.120
	C	A	A	C				0.146
	C	G	A	C				0.151
	G	A	C	T				0.173
			A	T	T	T		0.214
	G	A	A	C				0.223
			G	C	T	T		0.229
	A	A	A	C				0.281
				C	T	G	G	0.403
				C	T	C	G	0.438
				C	T	T	T	0.442

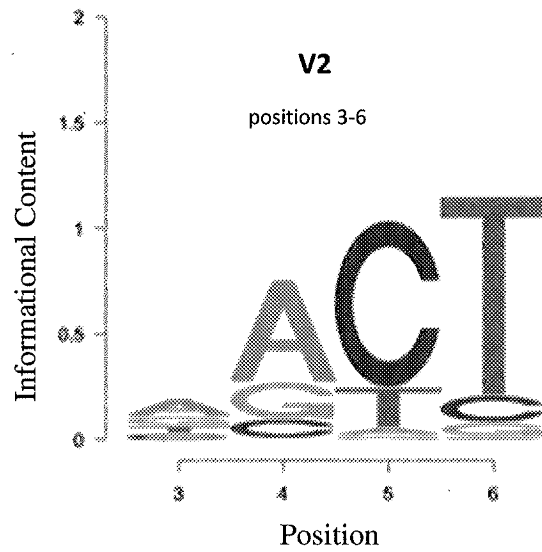
Fig. 28B

OMNI 30



1	2	3	4	5	6	7	8	Ratio
			A	C	T	C		0.039
			A	C	T	A		0.039
		T	A	C	T			0.040
		G	A	C	T			0.046
		A	G	C	T			0.047
		A	A	C	T			0.047
			A	C	T	G		0.047
		C	A	C	T			0.055
			A	C	T	T		0.061
		A	A	C	C			0.075
			G	C	T	T		0.085
	G	A	A	C				0.091
	A	A	A	C				0.111
			A	T	T	T		0.124
	C	A	A	C				0.274
	G	A	G	C				0.298
	T	A	A	C				0.318
				C	T	T	A	0.332
				C	T	C	G	0.337
				C	T	T	T	0.338
				C	T	T	C	0.339
				C	T	T	G	0.356
				C	T	A	G	0.367

Fig. 29A



1	2	3	4	5	6	7	8	Ratio
		T	A	C	T			0.057
			A	C	T	T		0.060
		A	A	C	T			0.071
			A	C	T	G		0.073
			A	C	T	A		0.074
		C	A	C	T			0.080
		G	A	C	T			0.087
			A	C	T	C		0.091
		G	A	T	T			0.096
		A	A	T	T			0.106
	A	A	C	T				0.120
	C	A	A	C				0.146
	C	G	A	C				0.151
	G	A	C	T				0.173
			A	T	T	T		0.214
	G	A	A	C				0.223
			G	C	T	T		0.229
	A	A	A	C				0.281
				C	T	G	G	0.403
				C	T	C	G	0.438
				C	T	T	T	0.442
				C	T	T	G	0.443
				C	T	T	A	0.449
				C	T	G	C	0.453

Fig. 29B

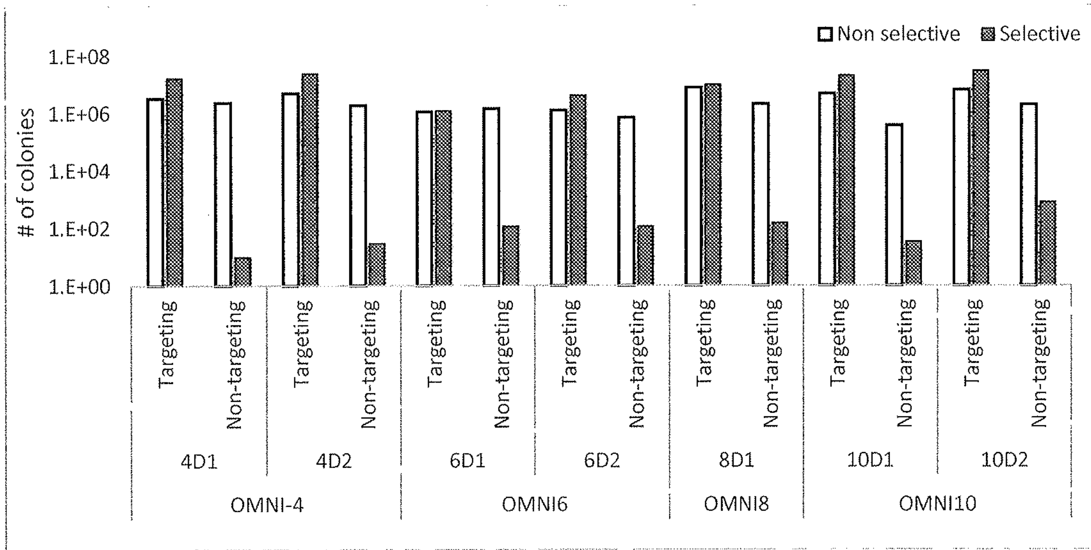


Fig. 30A

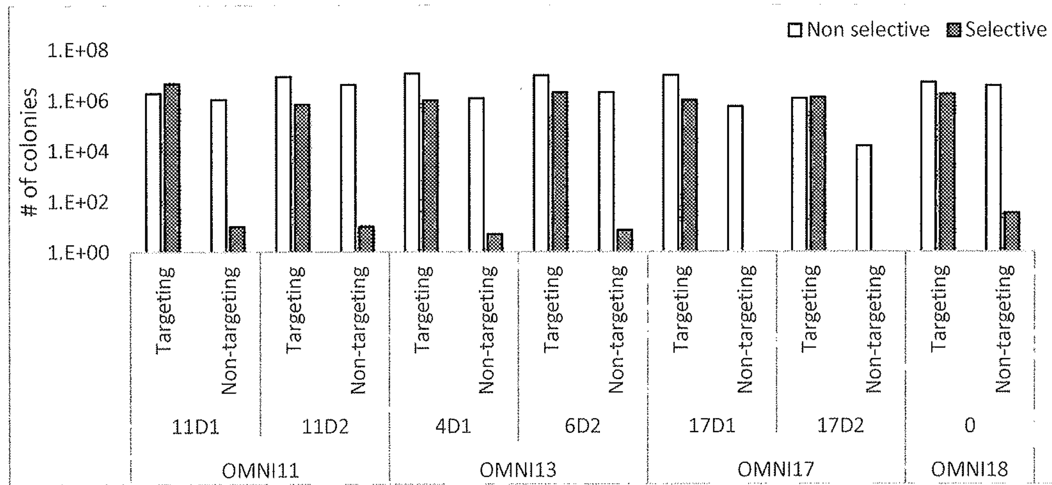


Fig. 30B

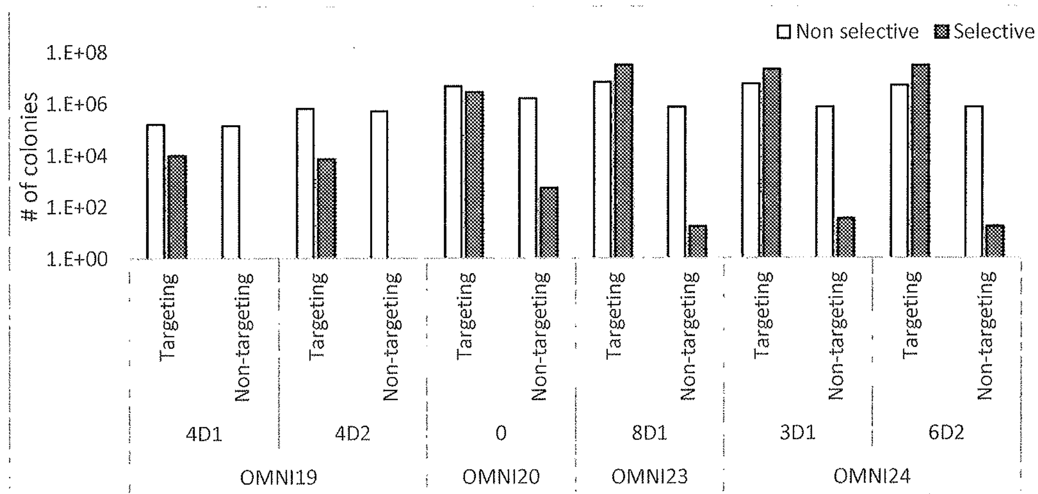


Fig. 30C

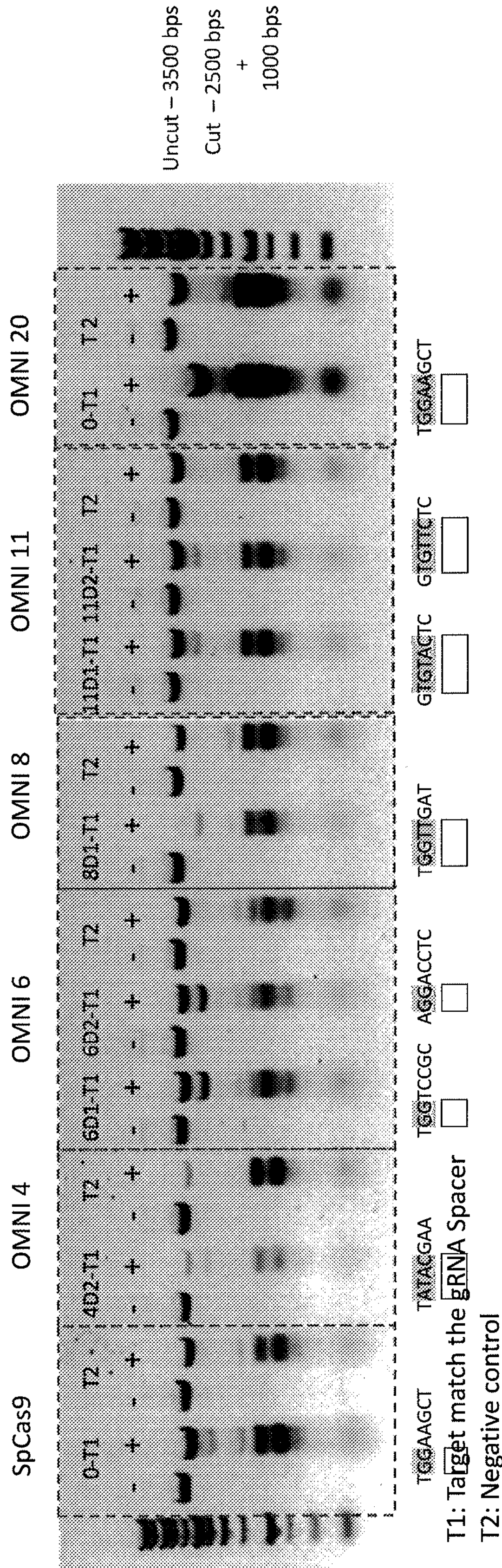


Fig. 31A

PAM	N 1	R 2	R 3	R 4	R 5	N 6	N 7	N 8	
0-T1	T	G	G	A	A	G	C	T	++++
2D4 – T1	C	G	G	G	A	G	A	G	+
3D2-T1	T	A	A	G	G	T	C	C	++++
5-T1	T	T	A	T	T	T	T	C	-
6D1-T1	T	G	G	T	C	C	G	C	-
9D1-T1	C	G	G	A	T	T	T	C	++
10-T1	G	G	A	A	T	G	A	T	+++
0-T2	T	G	G	A	A	G	C	T	-

OMNI 20

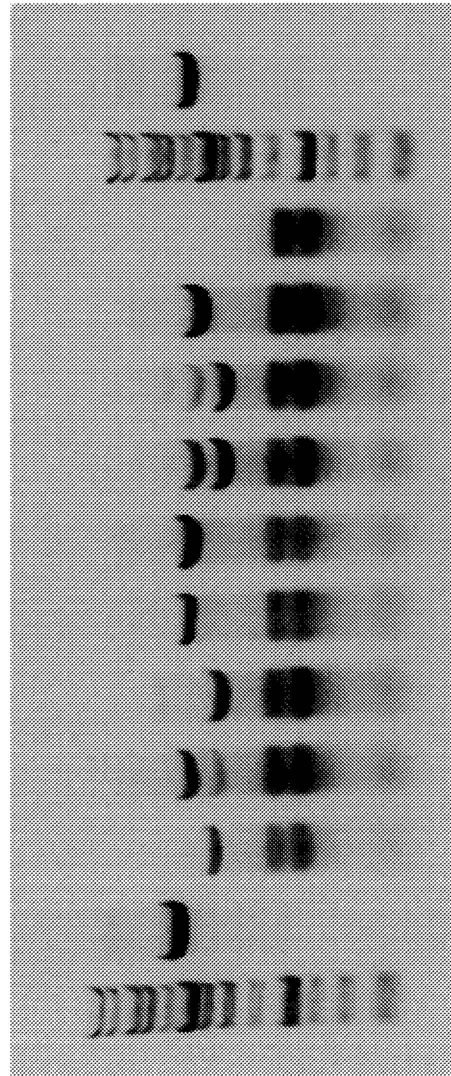


Fig. 31B

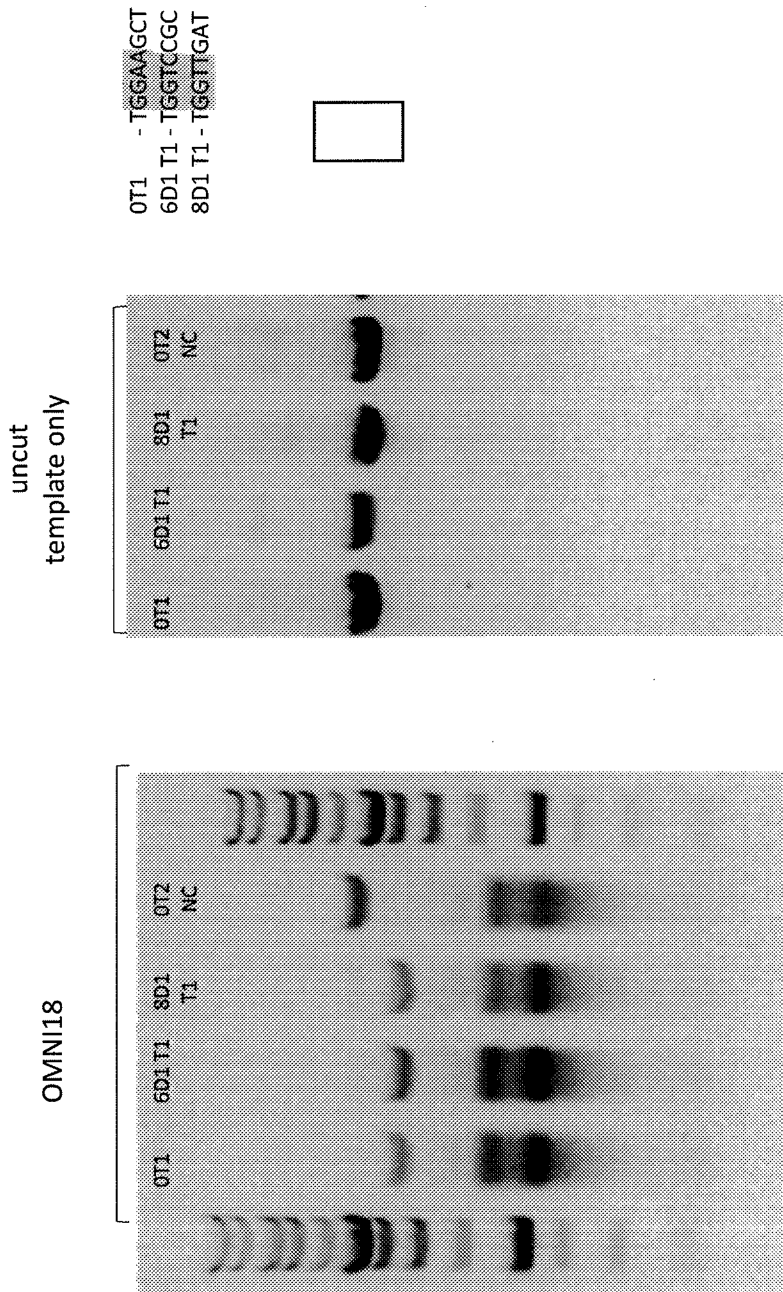


Fig. 31C

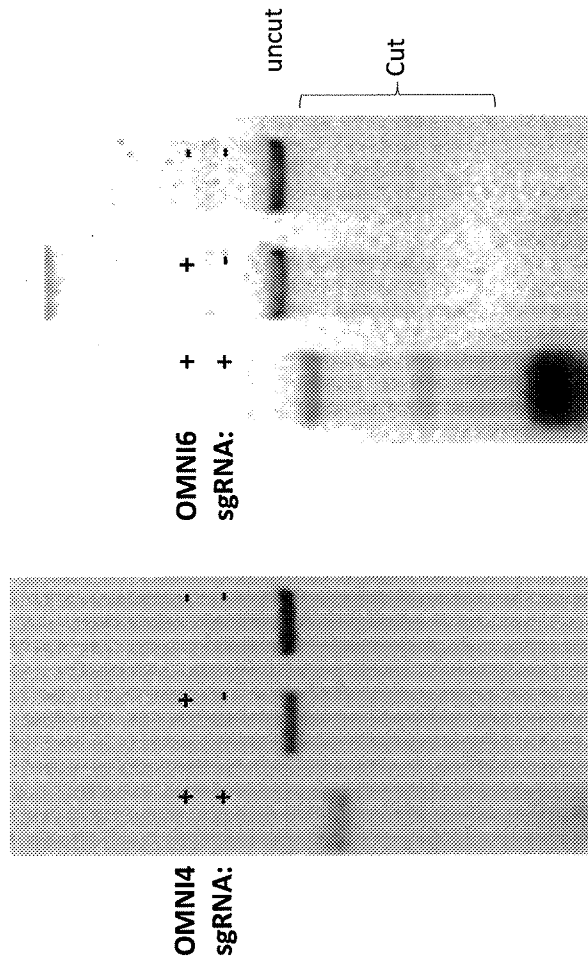


Fig. 32B

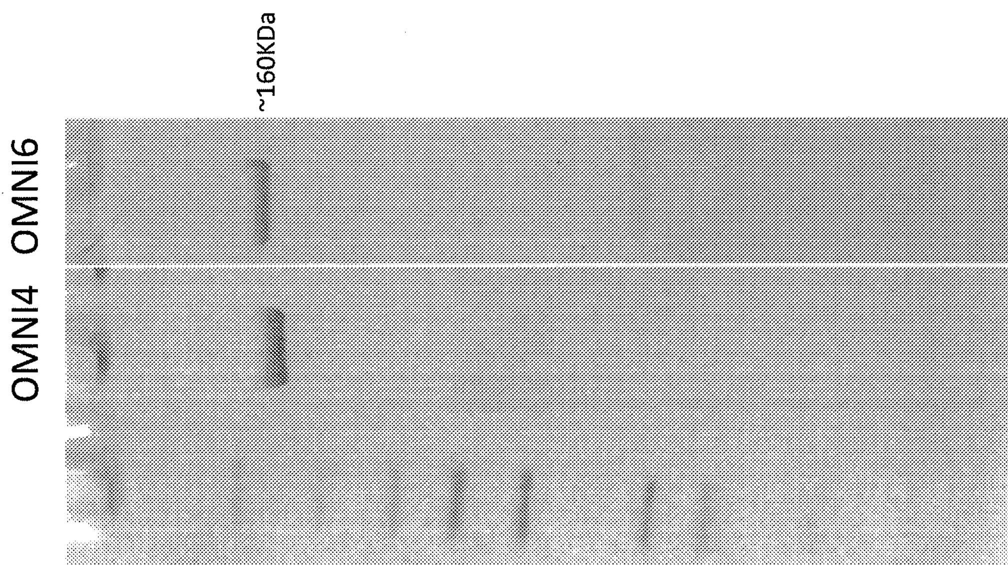


Fig. 32A

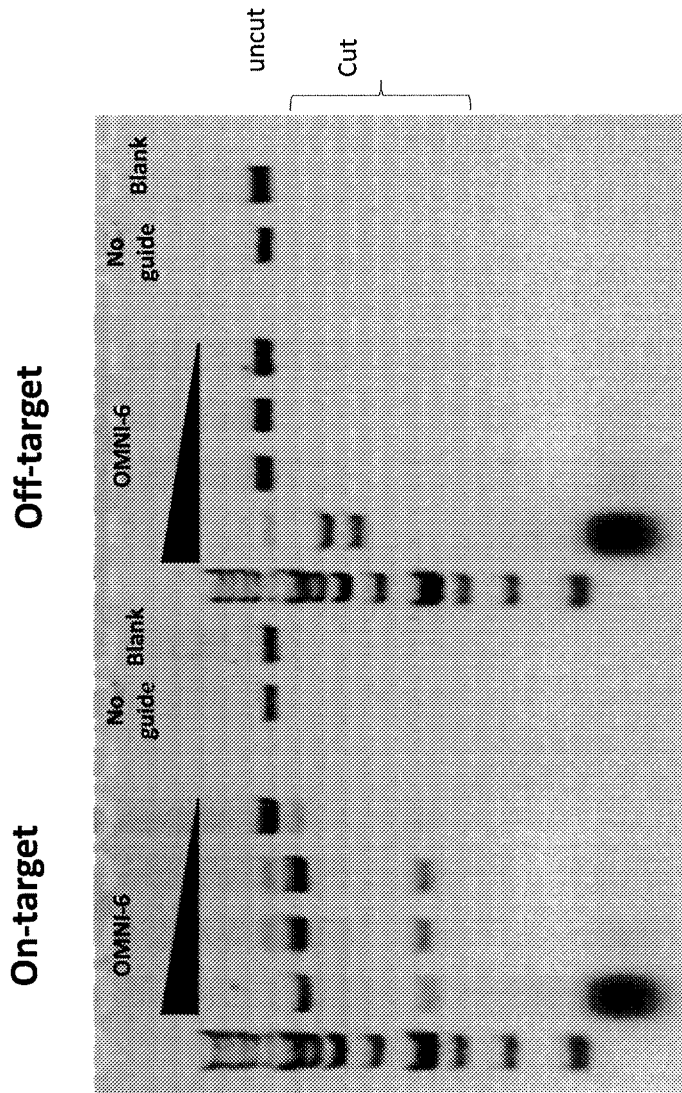


Fig. 32C

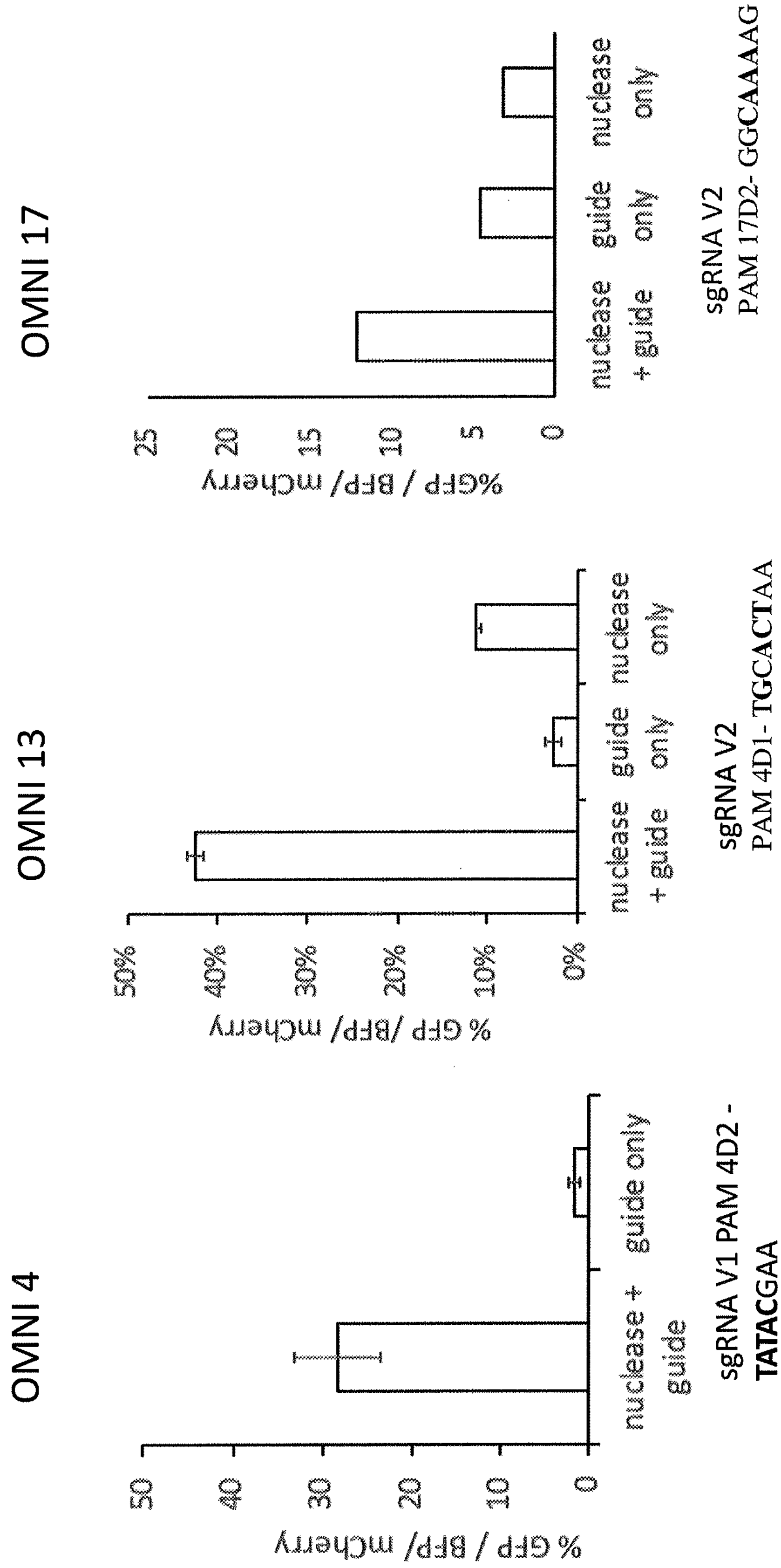


Fig. 33A

Fig. 33B

Fig. 33C

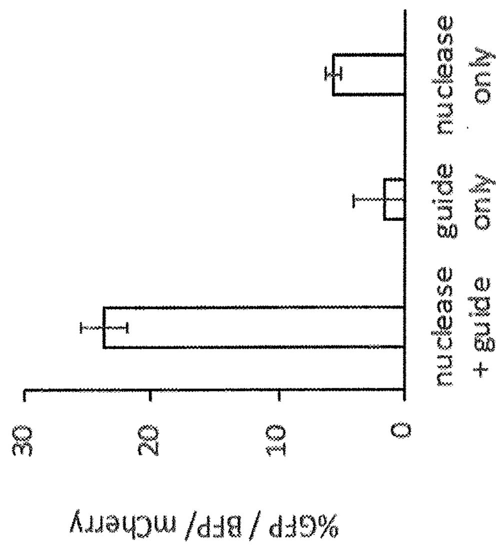


Fig. 33D

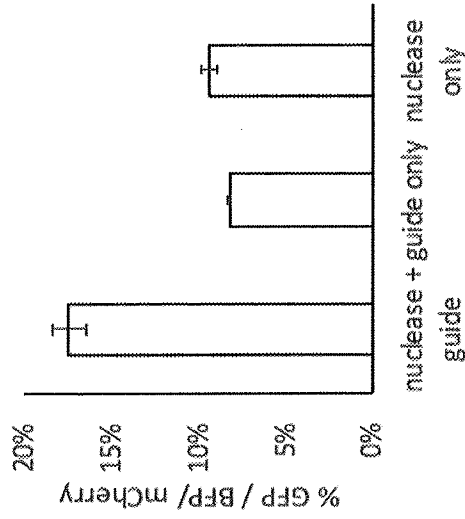


Fig. 33E

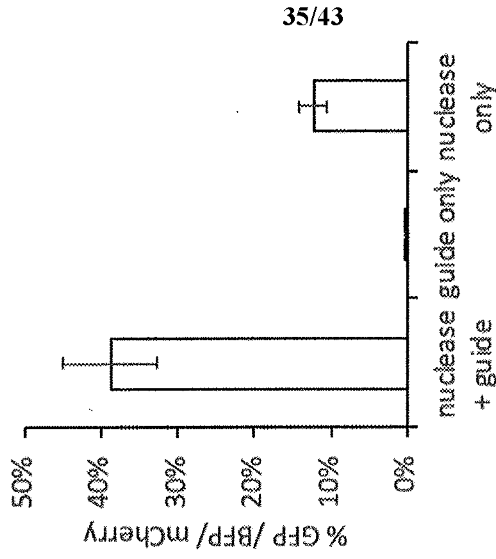


Fig. 33F

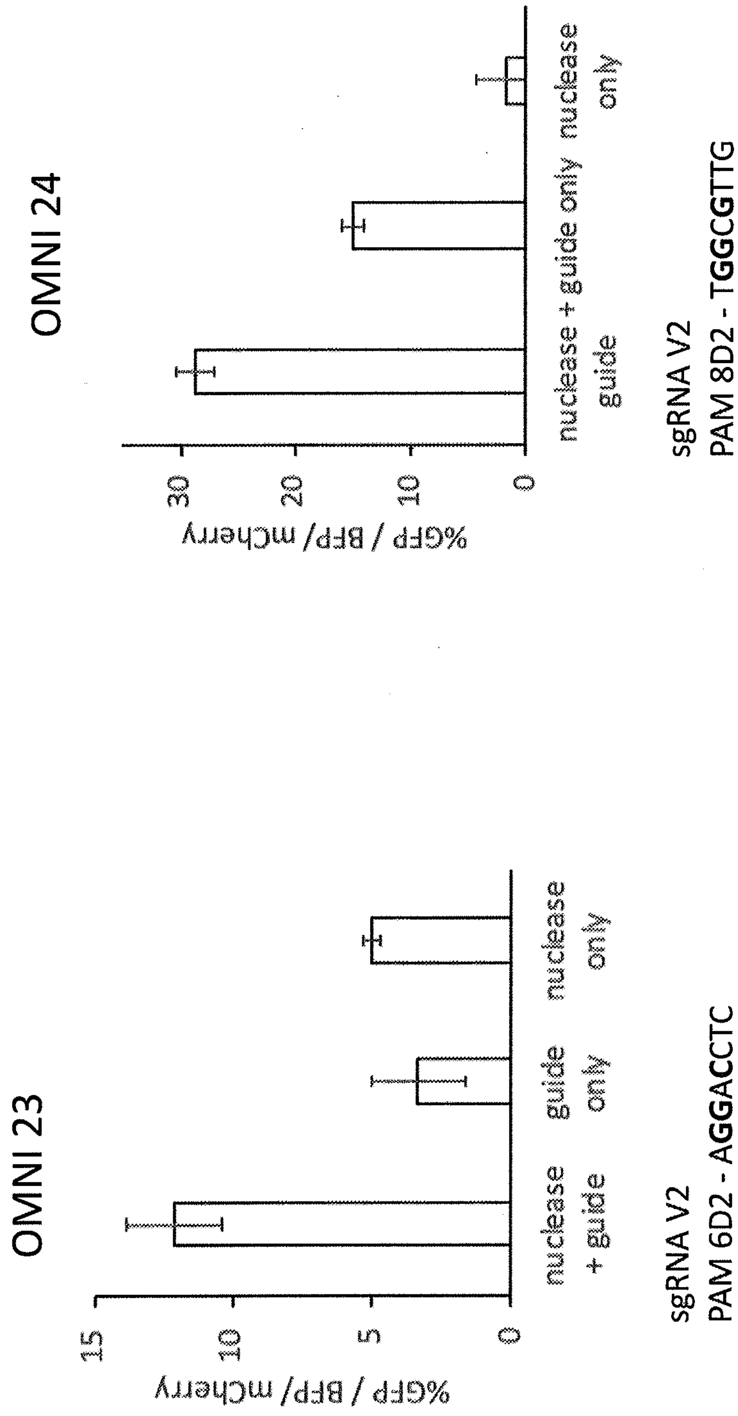


Fig. 33H

Fig. 33G

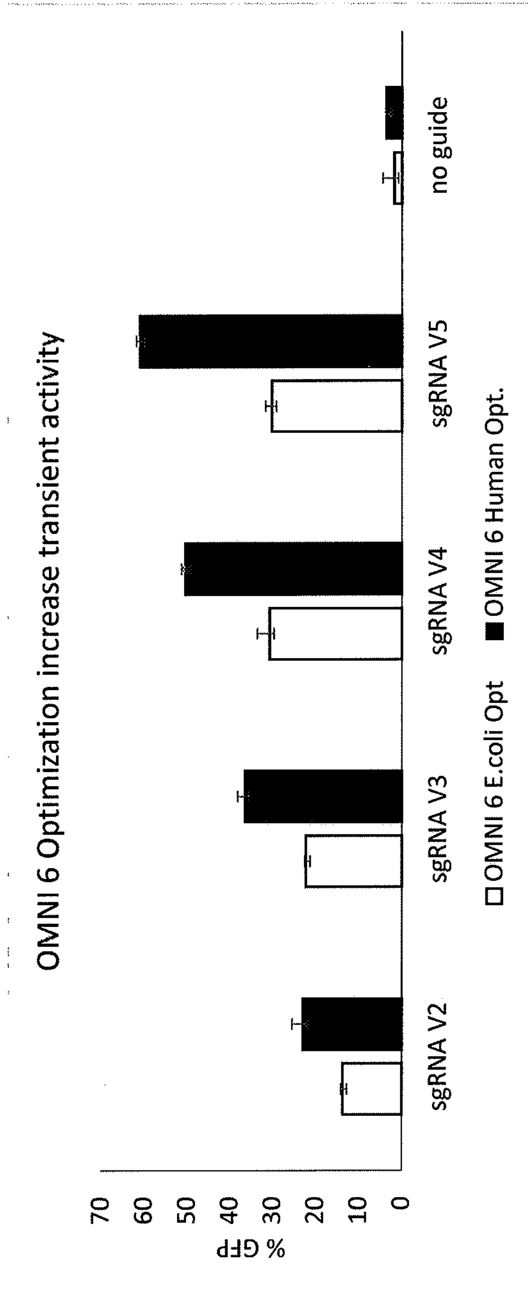


Fig. 34A

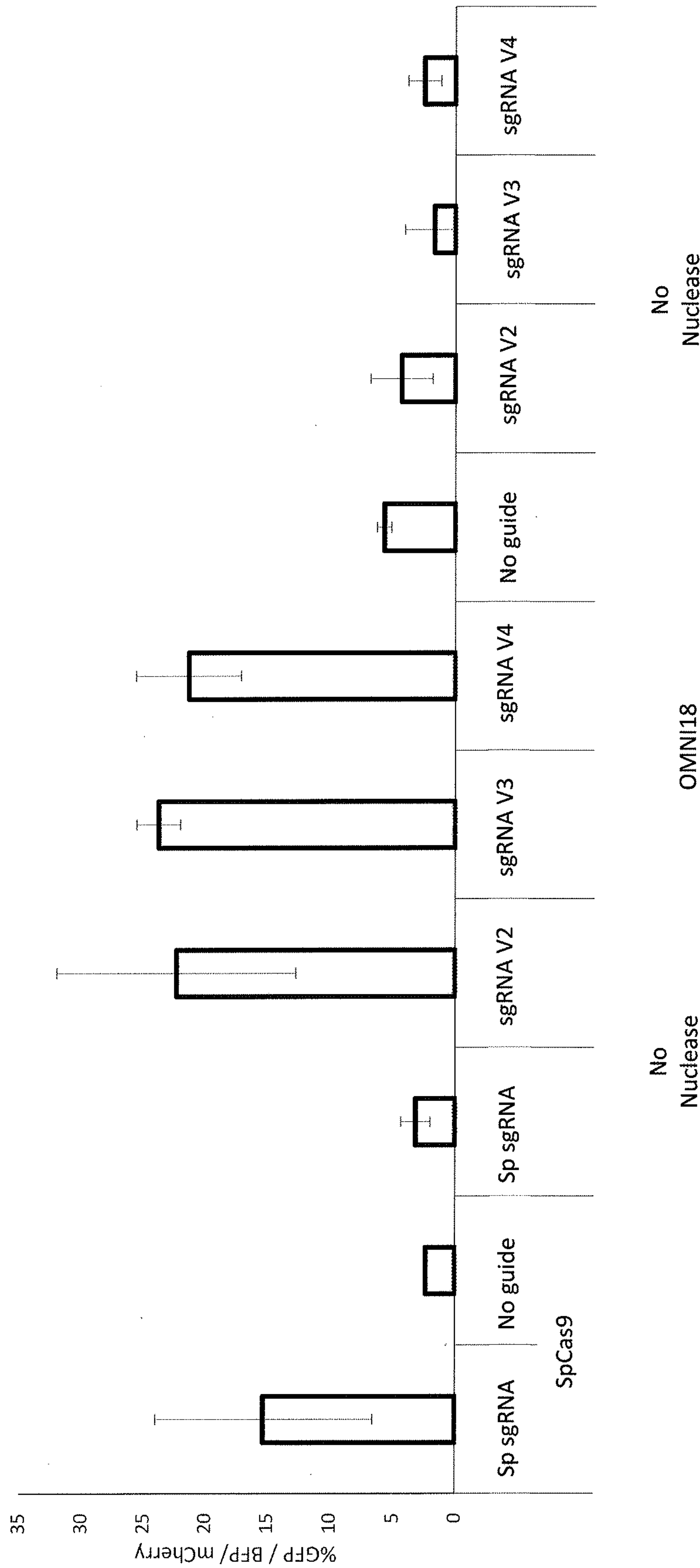


Fig. 34B

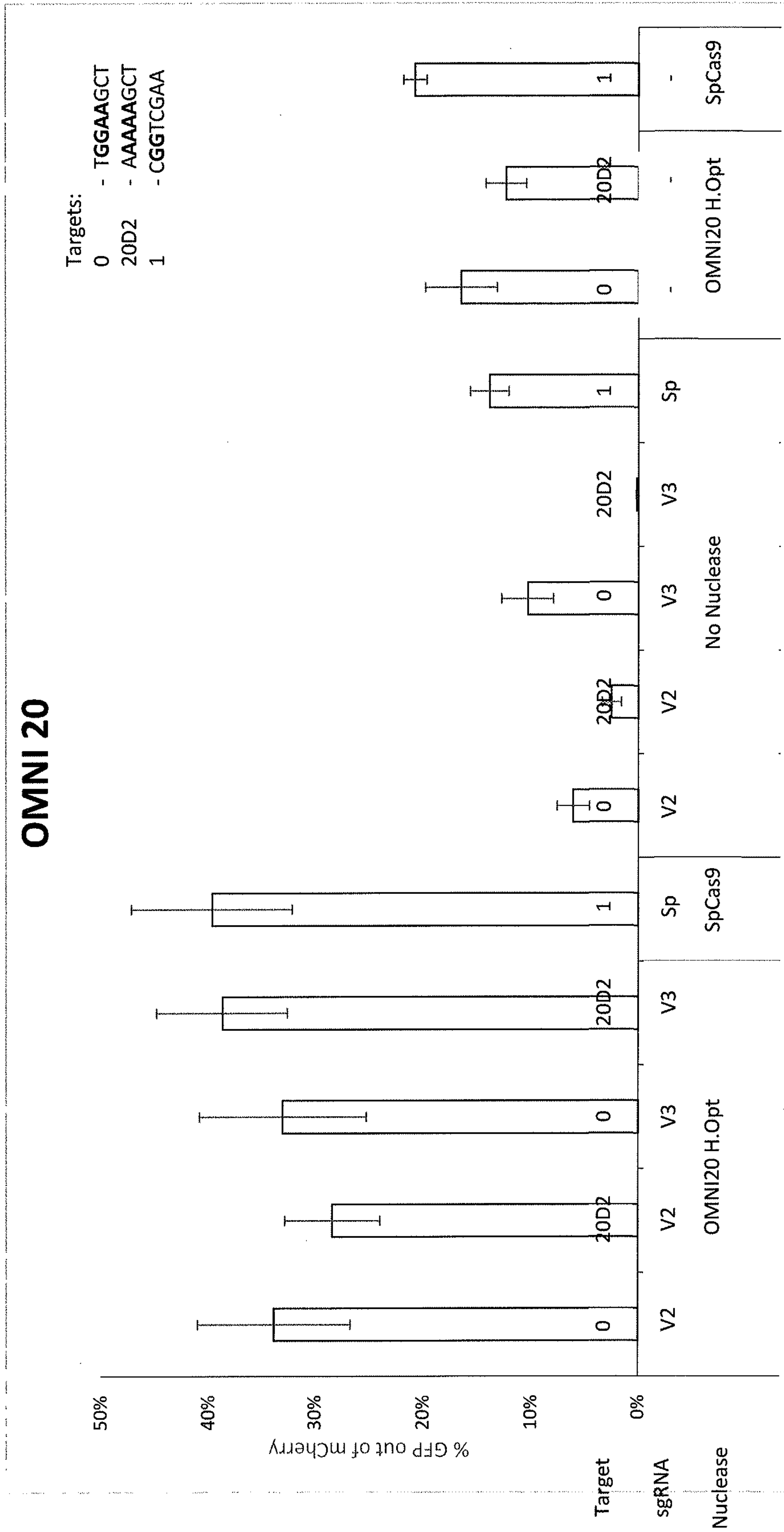


Fig. 34C

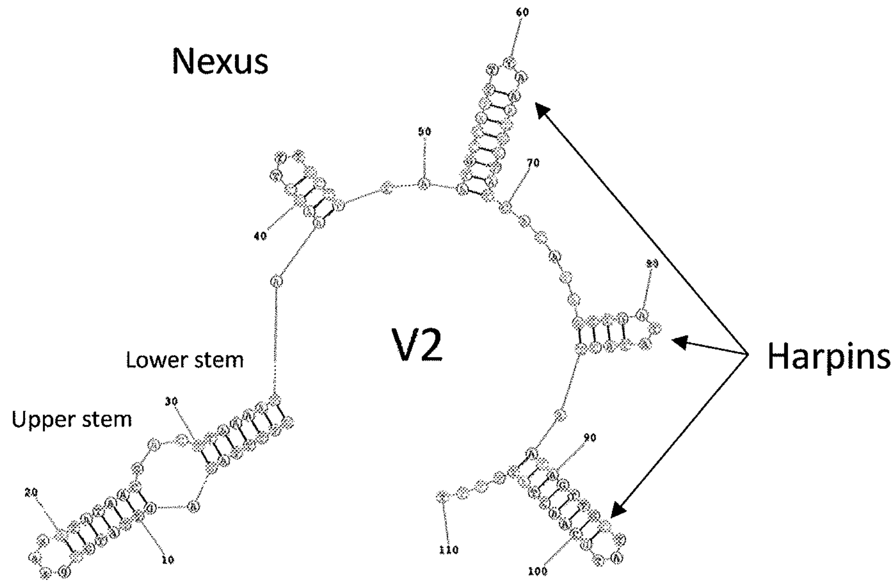


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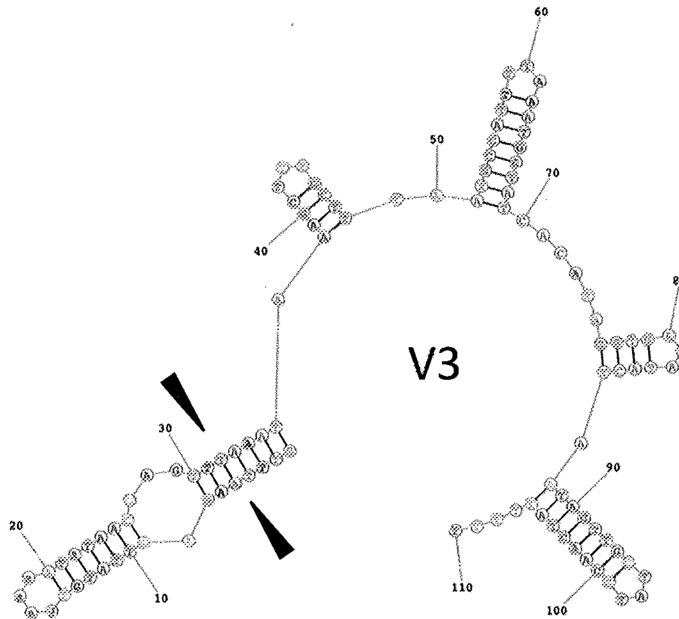
Fig. 35A



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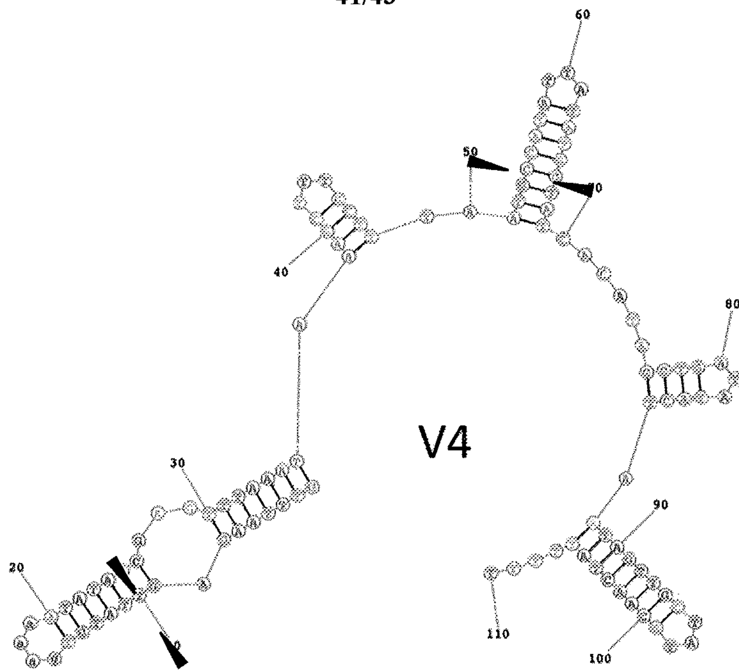
CUUUGCUUUAAUGCCAUUUUAAAUGGUAUCACAUAGGUG
AUAUACUAAUAGUUGCUAUGCAACUAUUUUU

Fig. 35B



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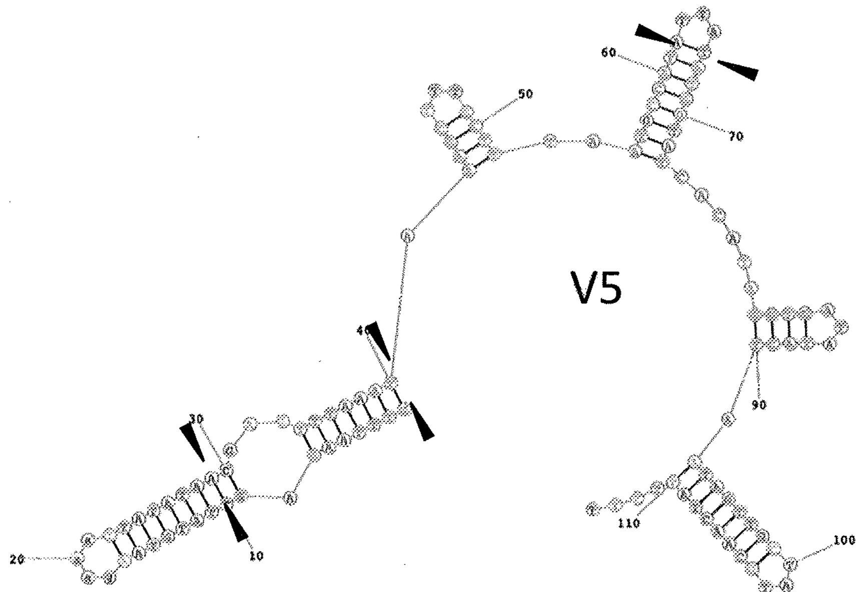
CUUUGCUUUAAUGCCAU**A**UUA**U**AUGGUAUCACAUAGGUG
AUAUACUAAUAGUUGCUAUGCAACUAUUUUU

Fig. 35C



GUUU**A**AGAGUUAUGU**AA**gaaa**UU**AUAUAACGAGUU**U**AAAU
AAAGCUUUGCUUUAAUGCCAUAUUAUAUGGUAUCACAUAG
GUGAUAUACUAAUAGUUGCUAUGCAACUAUUUUU

Fig. 35D

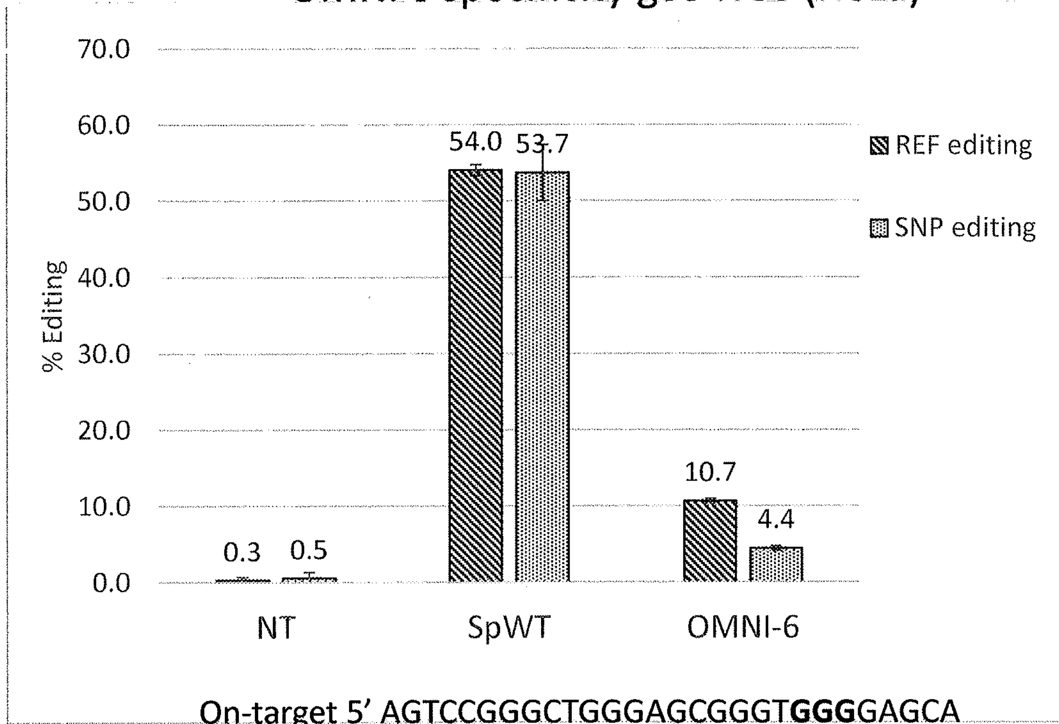


Fig. 36A

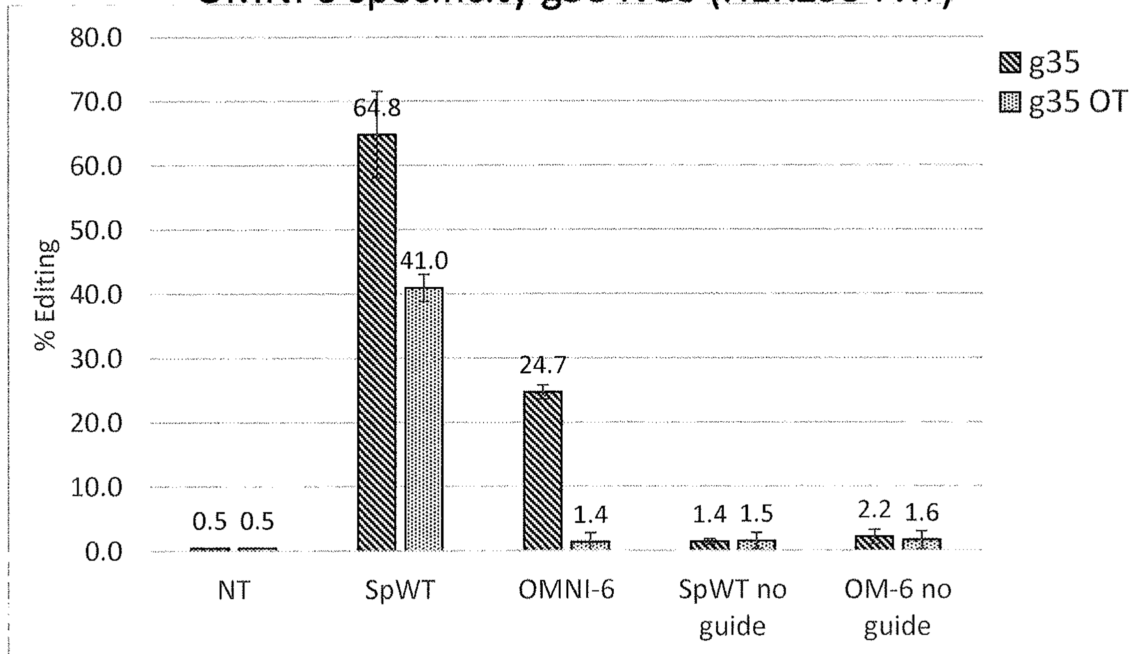
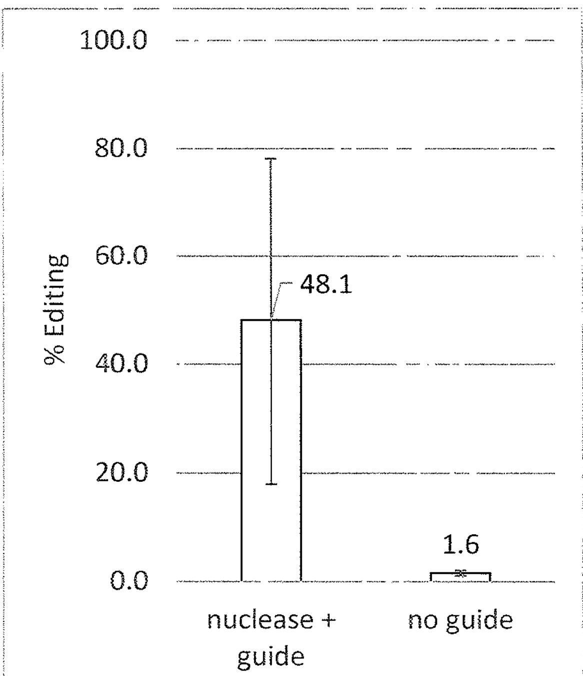
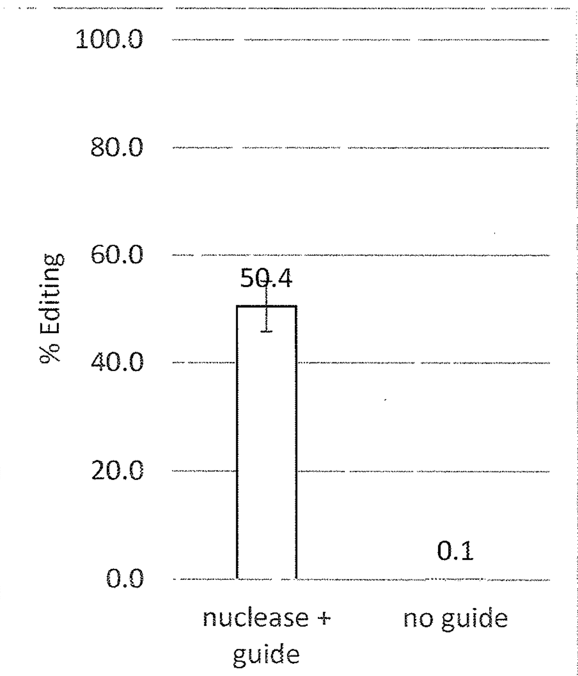


Fig. 36B





OMNI 4 on-target: 5’ GGGCCTGATTCCCACCTCTCAATACGTT

OMNI 6 on-target: 5’ GCTGCGGGAAAGGGATTCCCAGGACCCA

Figure 37

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/053018

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 C12N9/22 C12N15/11
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/186946 A1 (PIONEER HI-BRED INT INC [US]) 24 November 2016 (2016-11-24)	18-22
Y	the whole document	1-8,16, 17, 23-29, 31-43, 67-85
A		9-11,30, 44-54

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2019	10/03/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/053018

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE UniProt [Online] 22 November 2017 (2017-11-22), "RecName: Full=CRISPR-associated endonuclease Cas9 {ECO:0000256¦HAMAP-Rule:MF_01480}; EC=3.1.-.- {ECO:0000256¦HAMAP-Rule:MF_01480};", XP002796576, retrieved from EBI accession no. UNIPROT:A0A1I0EHL5 Database accession no. A0A1I0EHL5 the whole document -----	1-8,16, 17, 23-29, 31-43, 67-85

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/053018

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

8-11, 42-54(completely); 1-7, 16-41, 67-85(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 8-11, 42-54(completely); 1-7, 16-41, 67-85(partially)

   a CRISPR nuclease having at least 95% identity to SEQ ID NO:1, compositions and methods related thereto

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2. claims: 12-15, 55-66(completely); 1-7, 16-41, 67-85(partially)

   a CRISPR nuclease having at least 95% identity to SEQ ID NO:2, compositions and methods related thereto

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3-22. claims: 1-7, 16-41, 67-85(all partially)

   CRISPR nucleases having at least 95% identity to SEQ ID NOs:3-22, compositions and methods related thereto

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/053018

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016186946 A1	24-11-2016	AU 2016263026 A1	09-11-2017
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		WO 2016186953 A1	24-11-2016