Table 1: Summary of SEQ IDs and CRISPR associated systems

RGN ID	SEQ ID NO.	Source	crRNA repeat seq (SEQ ID NO.)	tracrRNA (SEQ ID NO.)	sgRNA (SEQ ID NO)
APG05083.1	1	*Bacillus* sp.	2	3	10
APG07433.1	11	*Bacillus* sp.	12	13	18
APG07513.1	19	*Bacillus* sp.	20	21	26
APG08290.1	27	*Bacillus* sp.	28	29	35
APG05459.1	36	*Enterococcus* sp.	37	38	44
APG04583.1	45	*Enterococcus* sp.	46	47	53
APG01688.1	54	*Empedobacter* sp.	55	56	62

Example 2: Guide RNA identification and sgRNA construction

Cultures of bacteria that natively express the RNA-guided nuclease system under investigation were grown to mid-log phase (OD600 of ~0.600), pelleted, and flash frozen. RNA was isolated from the pellets using a mirVANA miRNA Isolation Kit (Life Technologies, Carlsbad, CA), and sequencing libraries were prepared from the isolated RNA using an NEBNext Small RNA Library Prep kit (NEB,

Beverly, MA). The library prep was fractionated on a 6% polyacrylamide gel into 2 size fractions corresponding to 18-65nt and 90-200nt RNA species to detect crRNAs and tracrRNAs, respectively. Deep sequencing (40 bp paired-end for the smaller fraction and 80bp paired-end for the larger fraction) was performed on a Next Seq 500 (High Output kit) by a service provider (MoGene, St. Louis, MO). Reads were quality trimmed using Cutadapt and mapped to reference genomes using Bowtie2. A custom RNAseq pipeline was written in python to detect the crRNA and tracrRNA transcripts. Processed crRNA boundaries were determined by sequence coverage of the native repeat spacer array. The anti-repeat portion of the tracrRNA was identified using permissive BLASTn parameters. RNA sequencing depth confirmed the boundaries of the processed tracrRNA by identifying the transcript containing the anti-repeat. Manual curation of RNAs was performed using secondary structure prediction by NUPACK, an RNA folding software. sgRNA cassettes were prepared by DNA synthesis and were generally designed as follows (5'->3'): 20-30 bp spacer sequence -- processed repeat portion of the crRNA -- 4 bp noncomplementary linker (AAAG; SEQ ID NO: 63)-- processed tracrRNA. Other 4 bp noncomplementary linkers may also be used, for example GAAA (SEQ ID NO: 64) or ACUU (SEQ ID NO: 65). In some instances, a 6 bp nucleotide linker may be used, for example CAAAGG (SEQ ID NO: 66). For *in vitro* assays, sgRNAs were synthesized by *in vitro* transcription of the sgRNA cassettes with a GeneArt™ Precision gRNA Synthesis Kit (ThermoFisher). Processed crRNA and tracrRNA sequences for each of the RGN polypeptides are identified and are set forth in Table 1. See below for the sgRNAs constructed for PAM libraries 1 and 2.

THAT WHICH IS CLAIMED:

1. A nucleic acid molecule comprising a polynucleotide encoding an RNA-guided nuclease (RGN) polypeptide, wherein said polynucleotide comprises a nucleotide sequence encoding an RGN polypeptide comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NOs: 11, 27, 36, 1, 19, 45, or 54;

wherein said RGN polypeptide binds a target DNA sequence in an RNA-guided sequence specific manner when bound to a guide RNA (gRNA) capable of hybridizing to said target DNA sequence, and

wherein said polynucleotide encoding an RGN polypeptide is operably linked to a promoter heterologous to said polynucleotide.

2. The nucleic acid molecule of claim 1, wherein said RGN polypeptide is nuclease dead or functions as a nickase.

3. The nucleic acid molecule of claim 2, wherein the RGN polypeptide is operably fused to a base-editing polypeptide.

4. A vector comprising the nucleic acid molecule of any one of claims 1-3.

5. The vector of claim 4, wherein said vector further comprises at least one nucleotide sequence encoding said guide RNA, and wherein the guide RNA comprises a CRISPR RNA comprising a CRISPR repeat sequence having at least 95% sequence identity to SEQ ID NOs: 12, 28, 37, 2, 20, 46, or 55.

6. The vector of claims 4 or 5, wherein the guide RNA comprises a tracrRNA having at least 95% sequence identity to SEQ ID NOs: 13, 29, 38, 3, 21, 47, or 56.

7. A cell comprising the nucleic acid molecule of any one of claims 1-3 or the vector of any one of claims 4-6.

8. A system for binding a target DNA sequence, said system comprising:

a) one or more guide RNAs capable of hybridizing to said target DNA sequence or one or more nucleotide sequences encoding the one or more guide RNAs (gRNAs); and

b) an RNA-guided nuclease (RGN) polypeptide comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NOs: 11, 27, 36, 1, 19, 45, or 54 or a nucleotide sequence encoding the RGN polypeptide;

wherein said nucleotide sequences encoding the one or more guide RNAs and encoding the RGN polypeptide are each operably linked to a promoter heterologous to each said nucleotide sequence;

wherein the one or more guide RNAs hybridize to the target DNA sequence, and

wherein the one or more guide RNAs form a complex with the RGN polypeptide, thereby directing said RGN polypeptide to bind to said target DNA sequence.

9. The system of claim 8, wherein the target DNA sequence is within a eukaryotic cell.

10. The system of claim 8 or 9, wherein said RGN polypeptide is nuclease dead or functions as a nickase, and wherein the RGN polypeptide is operably linked to a base-editing polypeptide.

11. The system of any one of claims 8-10, wherein said system further comprises one or more donor polynucleotides or one or more nucleotide sequences encoding the one or more donor polynucleotides, wherein said nucleotide sequences encoding the one or more donor polynucleotides are each operably linked to a promoter heterologous to each said nucleotide sequence.

12. A method for binding a target DNA sequence comprising delivering a system according to any one of embodiments 8-11, to said target DNA sequence or a cell comprising the target DNA sequence.

13. A method for cleaving and/or modifying a target DNA sequence, comprising contacting the target DNA sequence with:

a) an RNA-guided nuclease (RGN) polypeptide, wherein said RGN comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NOs: 11, 27, 36, 1, 19, 45 or 54; and

b) one or more guide RNAs capable of targeting the RGN of (a) to the target DNA sequence;

wherein the one or more guide RNAs hybridize to the target DNA sequence, thereby directing said RGN polypeptide to bind to said target DNA sequence and cleavage and/or modification of said target DNA sequence occurs.

14. The method of claim 13, wherein said modified target DNA sequence comprises insertion of heterologous DNA into the target DNA sequence.

15. The method of claim 13, wherein said modified target DNA sequence comprises deletion of at least one nucleotide from the target DNA sequence.

16. The method of claim 13, wherein said modified target DNA sequence comprises mutation of at least one nucleotide in the target DNA sequence.

17. The method of any one of claims 14-16, wherein the target DNA sequence is within a cell.

18. The method of claim 17, wherein the cell is a eukaryotic cell.

19. The method of claim 17 or 18, further comprising culturing the cell under conditions in which the RGN polypeptide is expressed and cleaves the target DNA sequence to produce a modified DNA sequence; and selecting a cell comprising said modified DNA sequence.

20. A cell comprising a modified target DNA sequence according to the method of claim 19.