[0006] The present disclosure provides guide RNAs (referred to herein as "CasY guide RNAs") that bind to and provide sequence specificity to the CasY proteins; nucleic acids encoding the CasY guide RNAs; and modified host cells comprising the CasY guide RNAs and/or nucleic acids encoding same. CasY guide RNAs are useful in a variety of applications, which are provided.

[0007] The present disclosure provides methods of identifying a CRISPR RNA-guided endonuclease.

[0007a] In one aspect, the present disclosure refers to a composition comprising:

a) a CasY polypeptide, or a nucleic acid molecule encoding the CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a CasY guide RNA, or one or more DNA molecules encoding the CasY guide RNA, wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasY polypeptide is capable of forming a complex with the CasY guide RNA, and wherein the complex is capable of binding the target nucleic acid.

[0007b] In one aspect, the present disclosure refers to a CasY fusion polypeptide comprising:

a) a CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a heterologous polypeptide.

[0007c] In one aspect, the present disclosure refers to a nucleic acid molecule encoding the CasY fusion polypeptide as described above.

[0007d] In one aspect, the present disclosure refers to one or more recombinant expression vectors comprising nucleotide sequences encoding:

(a) a non-naturally occurring CasY guide RNA, wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid; and

(b) a CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain.

[0007e] In one aspect, the present disclosure refers to a eukaryotic cell comprising one or more of:

a) a CasY polypeptide, or a nucleic acid molecule encoding the CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain,

b) a CasY fusion polypeptide, or a nucleic acid molecule encoding the CasY fusion polypeptide, wherein the CasY fusion polypeptide comprises: I) a CasY polypeptide; and II) a heterologous polypeptide, and wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain, and

c) a CasY guide RNA, or a nucleic acid molecule encoding the CasY guide RNA, wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid,

wherein the eukaryotic cell is not a human embryo or human germ cell.

[0007f] In one aspect, the present disclosure refers to a cell comprising a CasY fusion polypeptide as described above, or a nucleic acid molecule encoding the CasY fusion polypeptide as described above.

[0007g] In one aspect, the present disclosure refers to an *in vitro* or *ex vivo* method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a CasY guide RNA comprising a nucleotide sequence that hybridizes to a target sequence of the target nucleic acid,

wherein said contacting results in modification of the target nucleic acid by the CasY polypeptide and wherein said method excludes preparation of a modified nucleic acid from the target nucleic acid for use in germ line modification in humans.

[0007h]In one aspect, the present disclosure refers to an *in vitro* or *ex vivo* method of modulating transcription from a target DNA, modifying a target nucleic acid, or modifying a protein associated with a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a CasY fusion polypeptide as described above; and

b) a CasY guide RNA comprising a nucleotide sequence that hybridizes to a target sequence of the target nucleic acid,

wherein said method excludes preparation of a modified nucleic acid, modulating transcription from the target DNA and modifying the protein associated with the target nucleic acid for use in germ line modification in humans.

[0007i] In one aspect, the present disclosure refers to a transgenic, multicellular, non-human organism whose genome comprises a transgene comprising a nucleotide sequence encoding one or more of:

a) a CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain,

b) a CasY fusion polypeptide as described above, and

c) a CasY guide RNA, wherein the CasY gujde RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid.

[0007j] In one aspect, the present disclosure refers to a kit comprising:

a) a CasY polypeptide and a CasY guide RNA;

b) a CasY polypeptide, a CasY guide RNA, and a DNA donor template;

c) a CasY fusion polypeptide and a CasY guide RNA;

d) a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

e) an mRNA encoding a CasY polypeptide, and a CasY guide RNA;

f) an mRNA encoding a CasY polypeptide; a CasY guide RNA, and a DNA donor template;

g) an mRNA encoding a CasY fusion polypeptide, and a CasY guide RNA;

h) an mRNA encoding a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and a DNA donor template,

wherein the CasY polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasY polypeptide is capable of forming a complex with the CasY guide RNA, and wherein the complex is capable of binding the target nucleic acid; and

wherein the CasY fusion polypeptide is a CasY fusion polypeptide as described above.

[0007k] In one aspect, the present disclosure refers to a sterile container comprising a CasY system comprising:

a) a CasY polypeptide and a CasY guide RNA;

b) a CasY polypeptide, a CasY guide RNA, and a DNA donor template;

c) a CasY fusion polypeptide and a CasY guide RNA;

d) a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

e) an mRNA encoding a CasY polypeptide, and a CasY guide RNA;

f) an mRNA encoding a CasY polypeptide; a CasY guide RNA, and a DNA donor template;

g) an mRNA encoding a CasY fusion polypeptide, and a CasY guide RNA;

h) an mRNA encoding a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and a DNA donor template,

wherein the CasY polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasY polypeptide is capable of forming a complex with the CasY guide RNA, and wherein the complex is capable of binding the target nucleic acid; and

wherein the CasY fusion polypeptide is a CasY fusion polypeptide as described above.

[0007l] In one aspect, the present disclosure refers to an implantable device comprising a CasY system comprising:

a) a CasY polypeptide and a CasY guide RNA;

b) a CasY polypeptide, a CasY guide RNA, and a DNA donor template;

c) a CasY fusion polypeptide and a CasY guide RNA;

d) a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

e) an mRNA encoding a CasY polypeptide, and a CasY guide RNA;

f) an mRNA encoding a CasY polypeptide; a CasY guide RNA, and a DNA donor template;

g) an mRNA encoding a CasY fusion polypeptide, and a CasY guide RNA;

h) an mRNA encoding a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and a DNA donor template,

wherein the CasY polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasY polypeptide is capable of forming a

complex with the CasY guide RNA, and wherein the complex is capable of binding the target nucleic acid; and

wherein the CasY fusion polypeptide is a CasY fusion polypeptide as described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] **Figure 1** depicts examples of naturally occurring CasY protein sequences.

[0009] **Figure 2** depicts an alignment of naturally occurring CasY protein sequences.

[0010] **Figure 3 (panels a-b)** depicts a schematic domain representation for CasY. Also shown are results from various searches attempting to identify homologs of CasY. Also depicted are portions of the CasY-containing CRISPR loci there were identified.

[0011] **Figure 4** depicts a schematic diagram of CasY and C2c3 loci. Interference proteins are shown in green, acquisition proteins in red. Repeats folded using RNA structure are shown to the right revealing a strong hairpin at the 5' end, suggesting self processing of the CRISPR array by CasY.

[0012] **Figure 5 (panels a-d)** depicts experiments performed (PAM dependent plasmid interference by CasY) to determine a PAM sequence for CasY**.**

[0013] **Figure 6 (panels a-b)** presents 'repeat' sequences of naturally occurring CasY guide RNAs, and an example CasY guide RNA hybridizing to target DNA. (Top to bottom, SEQ ID NOs: 11-15, and 20)

[0014] **Figure 7 (panels a-b)** presents novel identified CRISPR-Cas systems from uncultivated organisms. **a,** Ratio of major lineages with and without isolated representatives in all bacteria and archaea, based on data of Hug et al.[32]. The results highlight the massive scale of as yet little investigated biology in these domains. Archaeal Cas9 and the novel CRISPR-CasY were found exclusively in lineages with no isolated representatives. **b,** Locus organization of the newly discovered CRISPR-Cas systems.

[0015] **Figure 8 (panels a-b)** presents ARMAN-1 CRISPR array diversity and identification of the ARMAN-1 Cas9 PAM sequence. **a,** CRISPR arrays reconstructed from 15 different AMD samples. White boxes indicate repeats and colored diamonds indicate spacers (identical spacers are similarly colored; unique spacers are in black). The conserved region of the array is highlighted (on the right). The diversity of recently acquired spacers (on the left) indicates the system is active. An analysis that also includes CRISPR fragments from the read data is presented in Figure 14. **b**, A single putative viral contig reconstructed from AMD metagenomic data contains 56 protospacers (red vertical bars) from the

CLAIMS

What is claimed is:

1. A composition comprising:

a) a CasY polypeptide, or a nucleic acid molecule encoding the CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a CasY guide RNA, or one or more DNA molecules encoding the CasY guide RNA, wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasY polypeptide is capable of forming a complex with the CasY guide RNA, and wherein the complex is capable of binding the target nucleic acid.

2. The composition of claim 1, wherein the CasY polypeptide comprises an amino acid sequence having 70% or 85% or more identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7.

3. The composition of claim 1 or claim 2, wherein the CasY guide RNA comprises a nucleotide sequence having 80% or more identity with the crRNA sequence set forth in any one of SEQ ID NOs: 11-15.

4. The composition of claim 1 or claim 2, wherein the CasY polypeptide is fused to a nuclear localization signal.

5. The composition of any one of claims 1-4, wherein the composition comprises a lipid or one or more of a buffer, a nuclease inhibitor, and a protease inhibitor or wherein a) and b) are within a liposome or wherein a) and b) are within a particle.

6. The composition of any one of claims 1-5, wherein the CasY polypeptide is a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule or wherein the CasY polypeptide is a catalytically inactive CasY polypeptide (dCasY).

7. The composition of claim 6, wherein the CasY polypeptide is a catalytically inactive CasY polypeptide (dCasY) and comprises one or more mutations at a position corresponding to those selected from: D672, E769, and D935 of SEQ ID NO: 1.

8. The composition of any one of claims 1-7, further comprising a DNA donor template.

9. A CasY fusion polypeptide comprising:

a) a CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a heterologous polypeptide.

10. The CasY fusion polypeptide of Claim 9, wherein the CasY polypeptide comprises an amino acid sequence having 70% or 85% or more identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7.

11. The CasY fusion polypeptide of any one of claims 9-10, wherein the CasY polypeptide is a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule or wherein the CasY polypeptide is a catalytically inactive CasY Polypeptide (dCasY).

12. The CasY fusion polypeptide of claim 11, wherein the CasY polypeptide is a catalytically inactive CasY Polypeptide (dCasY)wherein the CasY polypeptide comprises one or more mutations at a position corresponding to those selected from: D672, E769, and D935 of SEQ ID NO: 1.

13. The CasY fusion polypeptide of any one of claims 9-12, wherein the heterologous polypeptide is fused to the N-terminus and/or the C-terminus of the CasY polypeptide.

14. The CasY fusion polypeptide of any one of claims 9-13, comprising a nuclear localization signal.

15. The CasY fusion polypeptide of any one of claims 9-14, wherein the heterologous polypeptide:

a) is a targeting polypeptide that provides for binding to a cell surface moiety on a target cell or target cell type;

b) exhibits an enzymatic activity that modifies target DNA;

c) exhibits one or more enzymatic activities selected from: nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer

forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity and glycosylase activity;

d) exhibits an enzymatic activity that modifies a target polypeptide associated with a target nucleic acid;

e) exhibits an enzymatic activity that modifies a target polypeptide associated with a target nucleic acid, wherein the enzymatic activity comprises histone modification activity;

f) exhibits an enzymatic activity that modifies a target polypeptide associated with a target nucleic acid, wherein the enzymatic activity is selected from: methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, demyristoylation activity, glycosylation activity (e.g., from O-GlcNAc transferase) and deglycosylation activity;

g) is an endosomal escape polypeptide;

h) is an endosomal escape polypeptide comprising an amino acid sequence selected from: GLFXALLXLLXSLWXLLLXA (SEQ ID NO:94), and GLFHALLHLLHSLWHLLLHA (SEQ ID NO:95), wherein each X is independently selected from lysine, histidine, and arginine;

a chloroplast transit peptide;

i) is a chloroplast transit peptide comprising an amino acid sequence selected from

MASMISSSAVTTVSRASRGQSAAMAPFGGLKSMTGFPVRKVNTDITSITSNGGRVKCMQVWPPIGKKKFETLSYLPPLTRDSRA (SEQ ID NO:83);

MASMISSSAVTTVSRASRGQSAAMAPFGGLKSMTGFPVRKVNTDITSITSNGGRVKS (SEQ ID NO:84);

MASSMLSSATMVASPAQATMVAPFNGLKSSAAFPATRKANNDITSITSNGGRVNCMQVWPPIEKKKFETLSYLPDLTDSGGRVNC (SEQ ID NO:85);

MAQVSRICNGVQNPSLISNLSKSSQRKSPLSVSLKTQQHPRAYPISSSWGLKKSGMTLIGSELRPLKVMSSVSTAC (SEQ ID NO:86);

MAQVSRICNGVWNPSLISNLSKSSQRKSPLSVSLKTQQHPRAYPISSSWGLKKSGMTLIGSELRPLKVMSSVSTAC (SEQ ID NO:87);

MAQINNMAQGIQTLNPNSNFHKPQVPKSSSFLVFGSKKLKNSANSMLVLKKDSIFMQLFCSFRISASVATAC (SEQ ID NO:88);

MAALVTSQLATSGTVLSVTDRFRRPGFQGLRPRNPADAALGMRTVGASAAPKQSRKPHRFDRRCLSMVV (SEQ ID NO:89);

MAALTTSQLATSATGFGIADRSAPSSLLRHGFQGLKPRSPAGGDATSLSVTTSARATPKQQRSVQRGSRRFPSVVVC (SEQ ID NO:90);

MASSVLSSAAVATRSNVAQANMVAPFTGLKSAASFPVSRKQNLDITSIASNGGRVQC (SEQ ID NO:91);

MESLAATSVFAPSRVAVPAARALVRAGTVVPTRRTSSTSGTSGVKCSAAVTPQASPVI SRSAAAA (SEQ ID NO:92); and

MGAAATSMQSLKFSNRLVPPSRRLSPVPNNVTCNNLPKSAAPVRTVKCCASSWNSTI NGAAATTNGASAASS (SEQ ID NO:93);

j) is protein that increases or decreases transcription;

k) is a transcriptional repressor domain;

l) is a transcriptional activation domain; or

m) comprises a protein binding domain.

16. A nucleic acid molecule encoding the CasY fusion polypeptide of any one of claims 9-15.

17. The nucleic acid molecule of Claim 16, wherein the nucleotide sequence encoding the CasY fusion polypeptide is operably linked to:

a) a promoter;

b) a promoter that is functional in a eukaryotic cell;

c) a promoter that is functional in a eukaryotic cell and is functional in one or more of: a plant cell, a fungal cell, an animal cell, cell of an invertebrate, a fly cell, a cell of a vertebrate, a mammalian cell, a primate cell, a non-human primate cell, and a human cell; or

d) a promoter as in a), b), or c) and wherein the promoter is one or more of: a constitutive promoter, an inducible promoter, a cell type-specific promoter, and a tissue-specific promoter.

18. The nucleic acid molecule of any one of Claims 16-17, wherein the DNA molecule is a recombinant expression vector or wherein the DNA molecule is a recombinant expression vector that is a recombinant adeno-associated viral vector, a recombinant retroviral vector, or a recombinant lentiviral vector.

19. The nucleic acid molecule of Claim 17, wherein the promoter is functional in a prokaryotic cell.

20. The nucleic acid molecule of Claim 16, wherein the nucleic acid molecule is an mRNA.

21. One or more recombinant expression vectors comprising nucleotide sequences encoding:

(a) a non-naturally occurring CasY guide RNA, wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid; and

(b) a CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain.

22. The one or more recombinant expression vectors of claim 21, wherein the CasY polypeptide comprises an amino acid sequence having 70% or 85% or more identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7.

23. The one or more recombinant expression vectors of any one of claims 21-22, wherein the CasY guide RNA comprises a nucleotide sequence having 80% or more identity with the crRNA sequence set forth in any one of SEQ ID NOs: 11-15.

24. The one or more recombinant expression vectors of any one of claims 21-23, wherein the CasY polypeptide is fused to a nuclear localization signal.

25. The one or more recombinant expression vectors of any one of claims 21-24, wherein said one or more nucleic acid molecules comprises a nucleotide sequence encoding the CasY guide RNA that is operably linked to a promoter and/or wherein said one or more nucleic acid molecules comprises a nucleotide sequence encoding the CasY polypeptide that is operably linked to a promoter.

26. The one or more recombinant expression vectors of Claim 25, wherein the promoter operably linked to the nucleotide sequence encoding the CasY guide RNA, and/or the promoter operably linked to the nucleotide sequence encoding the CasY polypeptide, is functional in a eukaryotic cell or wherein the promoter is functional in one or more of: a plant cell, a fungal cell, an animal cell, cell of an invertebrate, a fly cell, a cell of a vertebrate, a mammalian cell, a primate cell, a non-human primate cell, and a human cell; and/or wherein the promoter is one or more of: a constitutive promoter, an inducible promoter, a cell type-specific promoter, and a tissue-specific promoter.

27. The one or more recombinant expression vectors of any one of Claims 21-26, wherein the one or more recombinant expression vectors are selected from: one or more adenoassociated viral vectors, one or more recombinant retroviral vectors, or one or more recombinant lentiviral vectors.

28. The one or more recombinant expression vectors of Claim 25, wherein the promoter is functional in a prokaryotic cell.

29. A eukaryotic cell comprising one or more of:

a) a CasY polypeptide, or a nucleic acid molecule encoding the CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain,

b) a CasY fusion polypeptide, or a nucleic acid molecule encoding the CasY fusion polypeptide, wherein the CasY fusion polypeptide comprises: I) a CasY polypeptide; and II) a heterologous polypeptide, and wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain, and

c) a CasY guide RNA, or a nucleic acid molecule encoding the CasY guide RNA, wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid,

wherein the eukaryotic cell is not a human embryo or human germ cell.

30. The eukaryotic cell of claim 29, comprising the nucleic acid molecule encoding the CasY polypeptide, wherein said nucleic acid molecule is integrated into the genomic DNA of the cell.

31. The eukaryotic cell of claim 29 or claim 30, wherein the eukaryotic cell is a plant cell, a mammalian cell, an insect cell, an arachnid cell, a fungal cell, a bird cell, a reptile cell, an amphibian cell, an invertebrate cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, or a human cell.

32. A cell comprising a CasY fusion polypeptide of any one of claims 9-15, or a nucleic acid molecule encoding the CasY fusion polypeptide of any one of claims 9-15.

33. The cell of claim 32, wherein the cell is a prokaryotic cell and/or comprises the nucleic acid molecule encoding the CasY fusion polypeptide, wherein said nucleic acid molecule is integrated into the genomic DNA of the cell.

34. An *in vitro* or *ex vivo* method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain; and

b) a CasY guide RNA comprising a nucleotide sequence that hybridizes to a target sequence of the target nucleic acid,
wherein said contacting results in modification of the target nucleic acid by the CasY polypeptide and wherein said method excludes preparation of a modified nucleic acid from the target nucleic acid for use in germ line modification in humans.

35. The method of claim 34, wherein said modification is cleavage of the target nucleic acid, wherein the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA,
wherein said contacting takes place *in vitro* outside of a cell,
wherein said contacting takes place inside of a cell in culture,
wherein said contacting takes place inside of a cell in culture and the cell is a eukaryotic cell,
wherein said contacting takes place inside of a cell in culture and the cell is a eukaryotic cell selected from: a plant cell, a fungal cell, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell,
wherein said contacting takes place inside of a cell in culture and the cell is a prokaryotic cell, or
wherein said contacting results in genome editing.

36. The method of any one of claims 34-35, wherein said contacting comprises: introducing into a cell: (a) the CasY polypeptide, or a nucleic acid molecule encoding the CasY polypeptide, and (b) the CasY guide RNA, or a nucleic acid molecule encoding the CasY guide RNA.

37. The method of claim 36, wherein said contacting further comprises: introducing a DNA donor template into the cell.

38. The method of any one of claims 34-37, wherein the CasY guide RNA comprises a nucleotide sequence having 80% or more identity with the crRNA sequence set forth in any one of SEQ ID NOs: 11-15.

39. The method of any one of claims 34-38, wherein the CasY polypeptide is fused to a nuclear localization signal.

40. An *in vitro* or *ex vivo* method of modulating transcription from a target DNA, modifying a target nucleic acid, or modifying a protein associated with a target nucleic acid, the method comprising contacting the target nucleic acid with:
a) a CasY fusion polypeptide of any one of claims 9-15; and

b) a CasY guide RNA comprising a nucleotide sequence that hybridizes to a target sequence of the target nucleic acid,
wherein said method excludes preparation of a modified nucleic acid, modulating transcription from the target DNA and modifying the protein associated with the target nucleic acid for use in germ line modification in humans.

41. The method of claim 40, wherein the CasY guide RNA comprises a nucleotide sequence having 80% or more identity with the crRNA sequence set forth in any one of SEQ ID NOs: 11-15.

42. The method of claim 40 or claim 41, wherein the CasY fusion polypeptide comprises a nuclear localization signal.

43. The method of any of claims 40-42, wherein said modification is not cleavage of the target nucleic acid.

44. The method of any of claims 40-43, wherein the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA, and/or wherein
said contacting takes place *in vitro* outside of a cell;
said contacting takes place inside of a cell in culture;
the cell is a eukaryotic cell,
the cell is a eukaryotic cell selected from: a plant cell, a fungal cell, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell, or
the cell is a prokaryotic cell.

45. The method of any one of claims 40-44, wherein said contacting comprises: introducing into a cell: (a) the CasY fusion polypeptide, or a nucleic acid molecule encoding the CasY fusion polypeptide, and (b) the CasY guide RNA, or a nucleic acid molecule encoding the CasY guide RNA.

46. The method of any one of claims 40-45, wherein the CasY polypeptide is a catalytically inactive CasY Polypeptide (dCasY) and/or wherein the CasY polypeptide comprises one or more mutations at a position corresponding to those selected from: D672, E769, and D935 of SEQ ID NO: 1.

47. A transgenic, multicellular, non-human organism whose genome comprises a transgene comprising a nucleotide sequence encoding one or more of:

a) a CasY polypeptide, wherein the CasY polypeptide:

i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and

ii) comprises 3 partial RuvC domains that form a single RuvC domain,

b) a CasY fusion polypeptide of any one of claims 9-16, and

c) a CasY guide RNA, wherein the CasY gujde RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid.

48. The transgenic, multicellular, non-human organism of claim 47, wherein the CasY polypeptide comprises an amino acid sequence having 70% or 85% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7.

49. A kit comprising:

a) a CasY polypeptide and a CasY guide RNA;

b) a CasY polypeptide, a CasY guide RNA, and a DNA donor template;

c) a CasY fusion polypeptide and a CasY guide RNA;

d) a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

e) an mRNA encoding a CasY polypeptide, and a CasY guide RNA;

f) an mRNA encoding a CasY polypeptide; a CasY guide RNA, and a DNA donor template;

g) an mRNA encoding a CasY fusion polypeptide, and a CasY guide RNA;

h) an mRNA encoding a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and a DNA donor template,

wherein the CasY polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasY polypeptide is capable of forming a complex with the CasY guide RNA, and wherein the complex is capable of binding the target nucleic acid; and

wherein the CasY fusion polypeptide is a CasY fusion polypeptide of any one of claims 9-15.

50. The kit of claim 49, wherein the CasY polypeptide comprises an amino acid sequence having 70% or 85% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7.

51. A sterile container comprising a CasY system comprising:

a) a CasY polypeptide and a CasY guide RNA;

b) a CasY polypeptide, a CasY guide RNA, and a DNA donor template;

c) a CasY fusion polypeptide and a CasY guide RNA;

d) a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

e) an mRNA encoding a CasY polypeptide, and a CasY guide RNA;

f) an mRNA encoding a CasY polypeptide; a CasY guide RNA, and a DNA donor template;

g) an mRNA encoding a CasY fusion polypeptide, and a CasY guide RNA;

h) an mRNA encoding a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and a DNA donor template,

wherein the CasY polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasY polypeptide is capable of forming a complex with the CasY guide RNA, and wherein the complex is capable of binding the target nucleic acid; and

wherein the CasY fusion polypeptide is a CasY fusion polypeptide of any one of claims 9-15.

52. The sterile container of claim 51, wherein the container is a syringe.

53. An implantable device comprising a CasY system comprising:

a) a CasY polypeptide and a CasY guide RNA;

b) a CasY polypeptide, a CasY guide RNA, and a DNA donor template;

c) a CasY fusion polypeptide and a CasY guide RNA;

d) a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

e) an mRNA encoding a CasY polypeptide, and a CasY guide RNA;

f) an mRNA encoding a CasY polypeptide; a CasY guide RNA, and a DNA donor template;

g) an mRNA encoding a CasY fusion polypeptide, and a CasY guide RNA;

h) an mRNA encoding a CasY fusion polypeptide, a CasY guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; and ii) a nucleotide sequence encoding a CasY guide RNA; or

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a CasY fusion polypeptide; ii) a nucleotide sequence encoding a CasY guide RNA; and a DNA donor template,

wherein the CasY polypeptide: i) comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of SEQ ID NO:1 to SEQ ID NO:7; and ii) comprises 3 partial RuvC domains that form a single RuvC domain;

wherein the CasY guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid, wherein the CasY polypeptide is capable of forming a complex with the CasY guide RNA, and wherein the complex is capable of binding the target nucleic acid; and

wherein the CasY fusion polypeptide is a CasY fusion polypeptide of any one of claims 9-15.