

ASCII copy, created on March 10, 2014, is named 44287-722-601_SeqList and is 7,828,964 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Genome engineering can refer to altering the genome by deleting, inserting, mutating, or substituting specific nucleic acid sequences. The altering can be gene or location specific. Genome engineering can use nucleases to cut a nucleic acid thereby generating a site for the alteration. Engineering of non-genomic nucleic acid is also contemplated. A protein containing a nuclease domain can bind and cleave a target nucleic acid by forming a complex with a nucleic acid-targeting nucleic acid. In one example, the cleavage can introduce double stranded breaks in the target nucleic acid. A nucleic acid can be repaired e.g. by endogenous non-homologous end joining (NHEJ) machinery. In a further example, a piece of nucleic acid can be inserted. Modifications of nucleic acid-targeting nucleic acids and site-directed polypeptides can introduce new functions to be used for genome engineering.

SUMMARY OF THE INVENTION

[0004] ~~In one aspect,~~ The disclosure provides for an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a P-domain of the nucleic acid-targeting nucleic acid. In some embodiments, the P-domain starts downstream of a last paired nucleotide of a duplex between a CRISPR repeat and a tracrRNA sequence of the nucleic acid-targeting nucleic acid. In some embodiments, the engineered nucleic acid-targeting nucleic acid further comprises a linker sequence. In some embodiments, the linker sequence links the CRISPR repeat and the tracrRNA sequence. In some embodiments, the engineered nucleic acid-targeting nucleic acid is an isolated engineered nucleic acid-targeting nucleic acid. In some embodiments, the engineered nucleic acid-targeting nucleic acid is a recombinant engineered nucleic acid-targeting nucleic acid. In some embodiments, the engineered nucleic acid-targeting nucleic acid is adapted to hybridize to a target nucleic acid. In some embodiments, the P-domain comprises 2 adjacent nucleotides. In some embodiments, the P-domain comprises 3 adjacent nucleotides. In some embodiments, the P-domain comprises 4 adjacent nucleotides. In some embodiments, the P-domain comprises 5 adjacent nucleotides. In some embodiments, the P-domain comprises 6 or more adjacent nucleotides. In some embodiments, the P-domain starts 1 nucleotide downstream of the last paired nucleotide of

wherein the second polynucleotide comprises a target nucleic acid, and hybridize specifically to the first or second polynucleotide. In some embodiments, the first polynucleotide comprises at least 50% identity to a tracrRNA over 6 contiguous nucleotides. In some embodiments, the first polynucleotide is located downstream of a duplex between a polynucleotide comprising at least 50% identity to a CRISPR repeat over 6 contiguous nucleotides, and a polynucleotide comprising at least 50% identity to a tracrRNA sequence over 6 contiguous nucleotides. In some embodiments, the second polynucleotide comprises a protospacer adjacent motif. In some embodiments, the engineered nucleic acid-targeting nucleic acid is adapted to bind to a site-directed polypeptide. In some embodiments, the mutation comprises an insertion of one or more nucleotides into the P-domain. In some embodiments, the mutation comprises deletion one or more nucleotides from the P-domain. In some embodiments, the mutation comprises mutation of one or more nucleotides. In some embodiments, the mutation is configured to allow the nucleic acid-targeting nucleic acid to hybridize to a different protospacer adjacent motif. In some embodiments, the different protospacer adjacent motif comprises a protospacer adjacent motif selected from the group consisting of: 5'-NGGNG-3', 5'-NNAAAW-3', 5'-NNNGATT-3', 5'-GNNNCNNA-3', and 5'-NNNACA-3', or any combination thereof. In some embodiments, the mutation is configured to allow the engineered nucleic acid-targeting nucleic acid to bind with a lower dissociation constant than an un-engineered nucleic acid-targeting nucleic acid. In some embodiments, the mutation is configured to allow the engineered nucleic acid-targeting nucleic acid to bind with greater specificity than an un-engineered nucleic acid-targeting nucleic acid. In some embodiments, the mutation is configured to reduce binding of the engineered nucleic acid-targeting nucleic acid to a non-specific sequence in a target nucleic acid than an un-engineered nucleic acid-targeting nucleic acid.

[0004a] In one aspect, there is provided a composition comprising: a modified Cas9 protein, wherein the modified Cas9 protein comprises a modification of a region of a wild-type Cas9 protein selected from the group consisting of a Cas9 bridge helix region; a Cas9 highly basic patch within the Cas9 N-terminal region; a Cas9 HNH domain region; a Cas9 RuvC domain region; a Cas9 P-domain recognition region; an added enzymatic activity; insertion, deletion or substitution of a domain; insertion, deletion or substitution of a structural motif; a fusion; and combinations thereof. In some embodiments of the compositions described herein, a vector comprising a nucleic acid sequence encoding the modified Cas9 protein.

[0004b] In another aspect, there is provided an engineered nucleic acid targeting nucleic acid comprising: an engineered CRISPR RNA (crRNA), an engineered trans-activating CRISPR

RNA (tracrRNA), or a combination thereof, wherein (i) the engineered nucleic acid targeting nucleic acid can associate with a Cas9 protein to form a nucleic acid targeting nucleic acid-Cas9 complex capable of binding a target DNA, and (ii) the engineered crRNA, the engineered tracrRNA, or the combination thereof is altered relative to a corresponding wild-type crRNA, tracrRNA, or both; and the engineered nucleic acid targeting nucleic acid comprises at least one of the following: a tracr 3' hybridizing extension sequence, a spacer extension sequence, a tracr extension sequence, a modified bulge region, a modified P-domain, a Csy4 binding sequence to which a Csy4 protein binds with a Kd ~50pM, a Cas5 binding sequence to which a Cas5 protein binds, a Cas6 binding sequence to which a Cas6 protein binds, a nexus/hairpin variant, a modified stem loop structure comprising a functional moiety, or combinations thereof.

[0004b] In another aspect, there is provided a composition comprising a modified Cas9 protein composition as defined herein and an engineered nucleic acid targeting nucleic acid as defined herein.

[0005] In one aspect, ~~the disclosure provides for a method for modifying a target nucleic acid comprising contacting a target nucleic acid with an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a P-domain of the nucleic acid-targeting nucleic acid, and modifying the target nucleic acid. In some embodiments, the method further comprises inserting a donor polynucleotide into the target nucleic acid. In some embodiments, the modifying comprises cleaving the target nucleic acid. In some embodiments, the modifying comprises modifying transcription of the target nucleic acid.~~

[0006] In one aspect, ~~the disclosure provides for a vector comprising a polynucleotide sequence encoding an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a P-domain of the nucleic acid-targeting nucleic acid.~~ In another aspect, there is provided a vector comprising a nucleic acid sequence encoding the modified Cas9 protein as defined herein. In another aspect, there is provided a vector comprising a nucleic acid sequence encoding the engineered nucleic acid targeting nucleic acid as defined herein.

[0007] ~~In one aspect,~~ The disclosure provides for a kit comprising: an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a P-domain of the nucleic acid-targeting nucleic acid; and a buffer. In some embodiments, the kit further comprises a site-directed polypeptide. In some embodiments, the kit further comprises a donor polynucleotide. In some embodiments, the kit further comprises instructions for use.

[0008] ~~In one aspect,~~ The disclosure provides for an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a bulge region of a nucleic acid-targeting nucleic acid. In some embodiments, the bulge is located within a duplex between a CRISPR repeat and a tracrRNA sequence of the nucleic acid-targeting nucleic acid. In some embodiments, the engineered nucleic acid-targeting nucleic acid further comprises a linker sequence. In some embodiments, the linker sequence links the CRISPR repeat and the tracrRNA sequence. In some embodiments, the engineered nucleic acid-targeting nucleic acid is an isolated engineered nucleic acid-targeting nucleic acid. In some embodiments, the engineered nucleic acid-targeting nucleic acid is a recombinant engineered nucleic acid-targeting nucleic acid. In some embodiments, the bulge comprises at least 1 unpaired nucleotide on the CRISPR repeat, and 1 unpaired nucleotide on a the tracrRNA sequence. In some embodiments, the bulge comprises at least 1 unpaired nucleotide on the CRISPR repeat, and at least 2 unpaired nucleotides on a the tracrRNA sequence. In some embodiments, the bulge comprises at least 1 unpaired nucleotide on the CRISPR repeat, and at least 3 unpaired nucleotides on a the tracrRNA sequence. In some embodiments, the bulge comprises at least 1 unpaired nucleotide on the CRISPR repeat, and at least 4 unpaired nucleotides on a the tracrRNA sequence. In some embodiments, the bulge comprises at least one 1 unpaired nucleotide on the CRISPR repeat, and at least 5 unpaired nucleotides on a the tracrRNA sequence. In some embodiments, the bulge comprises at least 2 unpaired nucleotide on the CRISPR repeat, and 1 unpaired nucleotide on a the tracrRNA sequence. In some embodiments, the bulge comprises at least 3 unpaired nucleotide on the CRISPR repeat, and at least 2 unpaired nucleotides on a the tracrRNA sequence. In some embodiments, the bulge comprises at least 4 unpaired nucleotide on the CRISPR repeat, and at least 3 unpaired nucleotides on a the tracrRNA sequence. In some embodiments, the bulge comprises at least 5 unpaired nucleotide on the CRISPR repeat, and at least 4 unpaired nucleotides on the tracrRNA sequence. In some embodiments, the bulge comprises at least one nucleotide on the CRISPR repeat adapted to form a wobble pair with at least one nucleotide on the tracrRNA sequence. In some embodiments, the mutation comprises one or more mutations. In some embodiments, the one or more mutations are adjacent to each other. In some embodiments, the one or more

targeting nucleic acid to a non-specific sequence in a target nucleic acid than an un-engineered nucleic acid-targeting nucleic acid.

[0009] ~~In one aspect,~~ The disclosure provides for a method for modifying a target nucleic acid comprising: contacting the target nucleic acid with an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a bulge region of a nucleic acid-targeting nucleic acid; and modifying the target nucleic acid. In some embodiments, the method further comprises inserting a donor polynucleotide into the target nucleic acid. In some embodiments, the modifying comprises cleaving the target nucleic acid. In some embodiments, the modifying comprises modifying transcription of the target nucleic acid.

[0010] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a bulge region of a nucleic acid-targeting nucleic acid; and modifying the target nucleic acid.

[0011] ~~In one aspect,~~ The disclosure provides for a kit comprising: an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a bulge region of a nucleic acid-targeting nucleic acid; and modifying the target nucleic acid; and a buffer. In some embodiments, the kit further comprises a site-directed polypeptide. In some embodiments, the kit further comprises a donor polynucleotide. In some embodiments, the kit further comprises instructions for use.

[0012] ~~In one aspect,~~ The disclosure provides for a method for producing a donor polynucleotide-tagged cell comprising: cleaving a target nucleic acid in a cell using a complex comprising a site-directed polypeptide and a nucleic acid-targeting nucleic acid, inserting a donor polynucleotide into a cleaved target nucleic acid, propagating the cell carrying the donor polynucleotide, and determining an origin of the donor-polynucleotide tagged cell. In some embodiments, the method is performed in vivo. . In some embodiments, the method is performed in vitro. . In some embodiments, the method is performed in situ. In some embodiments, the propagating produces a population of cells. In some embodiments, the propagating produces a cell line. In some embodiments, the method further comprises determining a nucleic acid sequence of a nucleic acid in the cell. In some embodiments, the nucleic acid sequence determines an origin of the cell. In some embodiments, the determining comprises determining a genotype of the cell. In some embodiments, the propagating comprises differentiating the cell. In some embodiments, the propagating comprises de-differentiating the cell. In some embodiments, the propagating comprises differentiating the cell and then dedifferentiating the cell. In some embodiments,

the propagating comprises passaging the cell. In some embodiments, the propagating comprises inducing the cell to divide. In some embodiments, the propagating comprises inducing the cell to enter the cell cycle. In some embodiments, the propagating comprises the cell forming a metastasis. In some embodiments, the propagating comprises differentiating a pluripotent cell into a differentiated cell. In some embodiments, the cell is a differentiated cell. In some embodiments, the cell is a de-differentiated cell. In some embodiments, the cell is a stem cell. In some embodiments, the cell is a pluripotent stem cell. In some embodiments, the cell is a eukaryotic cell line. In some embodiments, the cell is a primary cell line. In some embodiments, the cell is a patient-derived cell line. In some embodiments, the method further comprises transplanting the cell into an organism. In some embodiments, the organism is a human. In some embodiments, the organism is a mammal. In some embodiments, the organism is selected from the group consisting of: a human, a dog, a rat, a mouse, a chicken, a fish, a cat, a plant, and a primate. In some embodiments, the method further comprises selecting the cell. In some embodiments, the donor polynucleotide is inserted into a target nucleic acid that is expressed in one cell state. In some embodiments, the donor polynucleotide is inserted into a target nucleic acid that is expressed in a plurality of cell types. In some embodiments, the donor polynucleotide is inserted into a target nucleic acid that is expressed in a pluripotent state. In some embodiments, the donor polynucleotide is inserted into a target nucleic acid that is expressed in a differentiated state.

[0013] ~~In one aspect,~~ The disclosure provides for a method for making a clonally expanded cell line comprising: introducing into a cell a complex comprising: a site-directed polypeptide and a nucleic acid-targeting nucleic acid, contacting the complex to a target nucleic acid, cleaving the target nucleic acid, wherein the cleaving is performed by the complex, thereby producing a cleaved target nucleic acid, inserting a donor polynucleotide into the cleaved target nucleic acid, propagating the cell, wherein the propagating produces the clonally expanded cell line. In some embodiments, the cell is selected from the group consisting of: HeLa cell, Chinese Hamster Ovary cell, 293-T cell, a pheochromocytoma, a neuroblastomas fibroblast, a rhabdomyosarcoma, a dorsal root ganglion cell, a NSO cell, CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO- K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C 1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), L-cells, HEK-293 (ATCC CRL 573) and PC 12 (ATCC CRL- 1721), HEK293T (ATCC CRL-11268), RBL (ATCC CRL-1378), SH-SY5Y (ATCC CRL-2266), MDCK (ATCC CCL-34), SJ-RH30 (ATCC CRL-2061), HepG2 (ATCC HB-8065), ND7/23 (ECACC 92090903), CHO (ECACC 85050302),

Vera (ATCC CCL 81), Caco-2 (ATCC HTB 37), K562 (ATCC CCL 243), Jurkat (ATCC TIB- 152), Per.C6, Huvec (ATCC Human Primary PCS 100-010, Mouse CRL 2514, CRL 2515, CRL 2516), HuH-7D12 (ECACC 01042712), 293 (ATCC CRL 10852), A549 (ATCC CCL 185), IMR-90 (ATCC CCL 186), MCF-7 (ATC HTB-22), U-2 OS (ATCC HTB-96), and T84 (ATCC CCL 248), or any combination thereof. In some embodiments, the cell is stem cell. In some embodiments, the cell is a differentiated cell. In some embodiments, the cell is a pluripotent cell.

[0014] ~~In one aspect,~~ The disclosure provides for a method for multiplex cell type analysis comprising: cleaving at least one target nucleic acid in two or more cells using a complex comprising a site-directed polypeptide and a nucleic acid-targeting nucleic acid, to create two cleaved target nucleic acids, inserting a different a donor polynucleotide into each of the cleaved target nucleic acids, and analyzing the two or more cells. In some embodiments, the analyzing comprises simultaneously analyzing the two or more cells. In some embodiments, the analyzing comprises determining a sequence of the target nucleic acid. In some embodiments, the analyzing comprises comparing the two or more cells. In some embodiments, the analyzing comprises determining a genotype of the two or more cells. In some embodiments, the cell is a differentiated cell. In some embodiments, the cell is a de-differentiated cell. In some embodiments, the cell is a stem cell. In some embodiments, the cell is a pluripotent stem cell. In some embodiments, the cell is a eukaryotic cell line. In some embodiments, the cell is a primary cell line. In some embodiments, the cell is a patient-derived cell line. In some embodiments, a plurality of donor polynucleotides are inserted into a plurality of cleaved target nucleic acids in the cell.

[0015] ~~In one aspect,~~ The disclosure provides for a composition comprising: an engineered nucleic acid-targeting nucleic acid comprising a 3' hybridizing extension, and a donor polynucleotide, wherein the donor polynucleotide is hybridized to the 3' hybridizing extension. In some embodiments, the 3' hybridizing extension is adapted to hybridize to at least 5 nucleotides from the 3' of the donor polynucleotide. In some embodiments, the 3' hybridizing extension is adapted to hybridize to at least 5 nucleotides from the 5' of the donor polynucleotide. In some embodiments, the 3' hybridizing extension is adapted to hybridize to at least 5 adjacent nucleotides in the donor polynucleotide. In some embodiments, the 3' hybridizing extension is adapted to hybridize to all of the donor polynucleotide. In some embodiments, the 3' hybridizing extension comprises a reverse transcription template. In some embodiments, the reverse transcription template is adapted to be reverse transcribed by a reverse transcriptase.

In some embodiments, the composition further comprises a reverse transcribed DNA polynucleotide. In some embodiments, the reverse transcribed DNA polynucleotide is adapted to hybridize to the reverse transcription template. In some embodiments, the donor polynucleotide is DNA. In some embodiments, the 3' hybridizing extension is RNA. In some embodiments, the engineered nucleic acid-targeting nucleic acid is an isolated engineered nucleic acid-targeting nucleic acid. In some embodiments, the engineered nucleic acid-targeting nucleic acid is a recombinant engineered nucleic acid-targeting nucleic acid.

[0016] ~~In one aspect,~~ The disclosure provides for a method for introducing a donor polynucleotide into a target nucleic acid comprising: contacting the target nucleic acid with a composition comprising: an engineered nucleic acid-targeting nucleic acid comprising a 3' hybridizing extension, and a donor polynucleotide, wherein the donor polynucleotide is hybridized to the 3' hybridizing extension. In some embodiments, the method further comprises cleaving the target nucleic acid to produce a cleaved target nucleic acid. In some embodiments, the cleaving is performed by a site-directed polypeptide. In some embodiments, the method further comprises inserting the donor polynucleotide into the cleaved target nucleic acid.

[0017] ~~In one aspect,~~ The disclosure provides for a composition comprising: an effector protein, and a nucleic acid, wherein the nucleic acid comprises at least 50% sequence identity to a crRNA over 6 contiguous nucleotides, at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides; and a non-native sequence, wherein the nucleic acid is adapted to bind to the effector protein. In some embodiments, the composition further comprises a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9, wherein the nucleic acid binds to the polypeptide. In some embodiments, the polypeptide comprises at least 60% amino acid sequence identity in a nuclease domain to a nuclease domain of Cas9. In some embodiments, the polypeptide is Cas9. In some embodiments, the nucleic acid further comprises a linker sequence, wherein the linker sequence links the sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and the sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides. In some embodiments, the non-native sequence is located at a position of the nucleic acid selected from the group consisting of: a 5' end and a 3' end, or any combination thereof. In some embodiments, the nucleic acid comprises two nucleic acid molecules. In some embodiments, the nucleic acid comprises a single continuous nucleic acid molecule. In some embodiments, the non-native sequence comprises a CRISPR

protein. In some embodiments, the nucleic acid is an isolated nucleic acid. In some embodiments, the nucleic acid is a recombinant nucleic acid.

[0018] ~~In one aspect,~~ The disclosure provides for a method for introducing a donor polynucleotide into a target nucleic acid comprising: contacting a target nucleic acid with a complex comprising a site-directed polypeptide and a composition comprising: an effector protein, and a nucleic acid, wherein the nucleic acid comprises at least 50% sequence identity to a crRNA over 6 contiguous nucleotides, at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides; and a non-native sequence, wherein the nucleic acid is adapted to bind to the effector protein. In some embodiments, the method further comprises cleaving the target nucleic acid. In some embodiments, the cleaving is performed by the site-directed polypeptide. In some embodiments, the method further comprises inserting the donor polynucleotide into the target nucleic acid.

[0019] ~~In one aspect,~~ The disclosure provides for a method for modulating a target nucleic acid comprising: contacting a target nucleic acid with one or more complexes, each complex comprising a site-directed polypeptide and a composition comprising: an effector protein, and a nucleic acid, wherein the nucleic acid comprises at least 50% sequence identity to a crRNA over 6 contiguous nucleotides, at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides; and a non-native sequence, wherein the nucleic acid is adapted to bind to the effector protein, and modulating the target nucleic acid. In some embodiments, the site-directed polypeptide comprises at least 10% amino acid sequence identity to a nuclease domain of Cas9. In some embodiments, the modulating is performed by the effector protein. In some embodiments, the modulating comprises an activity selected from the group consisting of: methyltransferase activity, demethylase activity, acetylation activity, deacetylation activity, ubiquitination activity, deubiquitination activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase 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, remodelling activity, protease activity, oxidoreductase activity, transferase activity, hydrolase activity, lyase activity, isomerase activity, synthase activity, synthetase activity, and demyristoylation activity, or any combination thereof. In some embodiments, the effector protein comprises one or more effector proteins.

[0020] ~~In one aspect,~~ The disclosure provides for a method for detecting if two complexes are in proximity to one another comprising: contacting a first target nucleic acid with a first complex, wherein the first complex comprises a first site-directed polypeptide, a first modified nucleic acid-targeting nucleic acid, and a first effector protein, wherein the effector protein is adapted to bind to the modified nucleic acid-targeting nucleic acid, and wherein the first effector protein comprises a non-native sequence that comprises a first portion of a split system, and contacting a second target nucleic acid with a second complex, wherein the second complex comprises a second site-directed polypeptide, a second modified nucleic acid-targeting nucleic acid, and a second effector protein, wherein the effector protein is adapted to bind to the modified nucleic acid-targeting nucleic acid, and wherein the second effector protein comprises a non-native sequence that comprises a second portion of a split system. In another aspect, there is provided a method for detecting if two complexes are in proximity to one another comprising: contacting a first target nucleic acid with a first complex, wherein the first complex comprises a first site-directed polypeptide, a first modified nucleic acid-targeting nucleic acid comprising a first nucleic acid sequence complementary to the first target nucleic acid, and a first effector protein, wherein the first effector protein is adapted to bind to the first modified nucleic acid-targeting nucleic acid, and wherein the first effector protein comprises a first non-native sequence that comprises a first portion of a split system; contacting a second target nucleic acid with a second complex, wherein the second complex comprises a second site-directed polypeptide, a second modified nucleic acid-targeting nucleic acid comprising a second nucleic acid sequence complementary to the second target nucleic acid, and a second effector protein, wherein the second effector protein is adapted to bind to the second modified nucleic acid-targeting nucleic acid, and wherein the second effector protein comprises a second non-native sequence that comprises a second portion of a split system; and detecting an interaction between the first portion and the second portion wherein the detecting indicates the first complex and the second complex are in proximity to one another. In some embodiments, the first target nucleic acid and the second target nucleic acid are on the same polynucleotide polymer. In some embodiments, the split system comprises two or more protein fragments that individually are not active, but, when formed into a complex, result in an active protein complex. In some embodiments, the method further comprises detecting an interaction between the first portion and the second portion. In some embodiments, the detecting indicates the first and second complex are in proximity to one another. In some embodiments, the site-directed polypeptide is adapted to be unable to cleave the target nucleic acid. In some

embodiments, the detecting comprises determining the occurrence of a genetic mobility event. In some embodiments, the genetic mobility event comprises a translocation. In some embodiments, prior to the genetic mobility event the two portions of the split system do not interact. In some embodiments, after the genetic mobility event the two portions of the split system do interact. In some embodiments, the genetic mobility event is a translocation between a BCR and an Abl gene. In some embodiments, the interaction activates the split system. In some embodiments, the interaction indicates the target nucleic acids bound by the complexes are close together. In some embodiments, the split system is selected from the group consisting of: split GFP system, a split ubiquitin system, a split transcription factor system, and a split affinity tag system, or any combination thereof. In some embodiments, the split system comprises a split GFP system. In some embodiments, the detecting indicates a genotype. In some embodiments, the method further comprises: determining a course of treatment for a disease based on the genotype. In some embodiments, the method further comprises treating the disease. In some embodiments, the treating comprises administering a drug. In some embodiments, the treating comprises administering a complex comprising a nucleic acid-targeting nucleic acid and a site-directed

targeting nucleic acids. In some embodiments, the one or more nucleic acid-targeting nucleic acids target a plurality of target nucleic acids. In some embodiments, the detecting comprises generating genetic data. In some embodiments, the method further comprises communicating the genetic data from a storage memory system to a remote computer. In some embodiments, the genetic data indicates a genotype. In some embodiments, the genetic data indicates the occurrence of a genetic mobility event. In some embodiments, the genetic data indicates a spatial location of genes.

[0020a] In another aspect, there is provided a method for identifying off-target binding sites relative to a target DNA sequence, comprising: contacting a mixture of DNA fragments comprising the target DNA sequence with an enzymatically inactive Cas9 protein in a complex with a nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein (i) the Cas9 protein comprises an affinity tag, the nucleic acid targeting nucleic acid comprises an affinity tag, or a combination thereof, and (ii) the Cas9 in the complex with the nucleic acid targeting nucleic acid binds to the target DNA sequence; isolating the Cas9 protein in the complex with the nucleic acid targeting nucleic acid and bound DNA fragments using a capture agent that binds to the affinity tag; purifying the DNA fragments that were bound by the Cas9 in the complex with the nucleic acid targeting nucleic acid; sequencing the DNA fragments that were bound by the Cas9 in the complex with the nucleic acid targeting nucleic acid; and identifying the off-target binding sites from the sequences of the DNA fragments that were bound by the Cas9 in the complex with the nucleic acid targeting nucleic acid that did not contain the target DNA binding sequence.

[0020b] In another aspect, there is provided a method for generating sticky ends or blunt cuts in a double-stranded DNA comprising a target site having a first strand and a second strand, the method comprising: contacting the double-stranded DNA with a Cas9 nickase in a complex with a first nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein the Cas9 nickase in the complex with the first nucleic acid targeting nucleic acid cuts the first strand of the target site and creates a first strand cut site; and contacting the double-stranded DNA with a Cas9 nickase in a complex with a second nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein the Cas9 nickase in the complex with the second nucleic acid targeting nucleic acid cuts the second strand of the target site and creates a second strand cut site; wherein either (i) the first strand cut site and the second strand cut site are at the same location in the target site to generate a blunt cut; or (ii) the first strand cut site and the second strand cut site are at different locations in the target site to generate sticky ends.

[0020c] In another aspect, there is provided a method for enrichment of a target nucleic acid, comprising: contacting a mixture of nucleic acids comprising the target nucleic acid with an enzymatically inactive Cas9 protein in a complex with a nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein (i) the Cas9 protein, the nucleic acid targeting nucleic acid, or combinations thereof further comprises an affinity tag, and (ii) the Cas9 in the complex with the nucleic acid targeting nucleic acid binds to the target nucleic acid; and isolating the target nucleic acid bound to the Cas9 protein in the complex with the nucleic acid targeting nucleic acid using a capture agent that binds to the affinity tag to provide enrichment of the target nucleic acid.

[0020d] In another aspect, there is provided a multiplexed genetic targeting agent, comprising: multiple nucleic acid targeting nucleic acids, each nucleic acid targeting nucleic acid comprising a single guide RNA (sgRNA), the sgRNA comprising a crRNA and a tracrRNA, wherein the sgRNA can associate with a Cas9 protein to form a sgRNA-Cas9 complex; and multiple non-native sequences, each non-native sequence comprising a moiety, an endoribonuclease binding sequence, or a ribozyme; wherein a nucleic acid module comprises a nucleic acid targeting nucleic acid adjacent a non-native sequence, and multiple nucleic acid modules are arranged in tandem.

[0020e] In another aspect, there is provided a stoichiometric delivery complex, comprising: an engineered tandem fusion polypeptide comprising multiple nucleic acid binding proteins, wherein each nucleic acid binding protein can bind a nucleic acid comprising a cognate nucleic acid-binding protein binding site that can be bound by the nucleic acid-binding protein.

[0020f] In another aspect, there is provided a method of modifying genomic DNA of a cell wherein the genomic DNA comprises a target nucleic acid, the method comprising: introducing a donor polynucleotide into the target nucleic acid of the genomic DNA, wherein (i) a Cas9 protein in a complex with a first nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA cleaves the target nucleic acid, (ii) the donor polynucleotide is integrated into the cleaved target nucleic acid, and (iii) the donor polynucleotide comprises a genetic element of interest and a reporter element, wherein the reporter element is flanked by a 5' target site and a 3' target site; selecting cells expressing the reporter element; and excising the reporter element, wherein (i) a Cas9 protein in a complex with a second nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA cleaves the 5' target site to create a first end, and a Cas9 protein in a complex with a third nucleic acid targeting nucleic acid

comprising a crRNA and a tracrRNA cleaves the 3' target site to create a second end, and (ii) the first end and the second end are rejoined.

[0020g] In another aspect, there is provided a method for tracking cell lineage development in a population of cells, comprising: tagging genomic DNA, comprising a target site, in cells of the population of cells by contacting genomic DNA of the cells with a Cas9 protein in a complex with a nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA that cuts the target site to create a first end and a second end, wherein a cellular repair mechanism rejoins the first end and the second end and generates a number of signature sequences in a number of the cells; propagating the population of cells comprising the signature sequences; analyzing the cells of the population to identify cells associated with each signature sequence; and using the signature sequences to determine cell lineage of cells in the population of cells.

[0020h] In another aspect, there is provided a method for quantifying genome-editing events of genomic DNA in a population of cells wherein the genomic DNA comprises a target nucleic acid, the method comprising: contacting the genomic DNA of cells in the population with a Cas9 protein in a complex with a nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA wherein (i) the Cas9 protein in the complex with the nucleic acid targeting nucleic acid can cut the target site to create a first end and a second end, (ii) when the Cas9 protein in the complex with the nucleic acid targeting nucleic acid cuts the target site, a cellular repair mechanism rejoins the first end and the second end destroying the target site, and (iii) the contacting produces a number of cells comprising genomic DNA having the target site and a number of cells comprising genomic DNA wherein the target site was destroyed; isolating the genomic DNA from the population of cells; performing polymerase chain reaction (PCR) with the isolated genomic DNA using primers that flank the target site to produce an amplified PCR product; contacting the amplified PCR product with the Cas9 protein in the complex with the nucleic acid targeting nucleic acid; and quantifying genome-editing events by comparing the amount of amplified PCR product derived from genomic DNA that comprises the target site in the amplified PCR product to the amount of amplified PCR product derived from genomic DNA wherein the target site was destroyed.

[0020i] In another aspect, there is provided a composition comprising: an engineered nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein (i) the engineered nucleic acid targeting nucleic acid can associate with a Cas9 protein to form a crRNA-tracrRNA-Cas9 complex, (ii) the tracrRNA comprises a 3' end, (iii) the tracrRNA further comprises a hybridizing sequence at the 3' end, and (iv) the hybridizing sequence comprises a nucleic acid sequence that can hybridize to a donor polynucleotide.

[0020] In another aspect, there is provided a method for delivering a donor polynucleotide to a double-stranded break at a target site, comprising: contacting a DNA sequence comprising the target site with an engineered nucleic acid targeting nucleic acid comprising a crRNA and tracrRNA wherein (i) the engineered nucleic acid targeting nucleic acid is associated in a complex with a Cas9 protein to form a crRNA-tracrRNA-Cas9 complex, (ii) the tracrRNA comprises a 3' end, (iii) the tracrRNA further comprises a hybridizing sequence at the 3' end, (iv) the hybridizing sequence comprises a nucleic acid sequence that hybridizes to the donor polynucleotide, and (v) the crRNA-tracrRNA-Cas9 complex binds to the DNA sequence comprising the target site and cuts the target site to generate a double-strand break and the binding of the crRNA-tracrRNA-Cas9 complex delivers the donor polynucleotide to the double-stranded break at the target site.

[0021] ~~In one aspect,~~ The disclosure provides for a kit comprising: a site-directed polypeptide, a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence, an effector protein that is adapted to bind to the non-native sequence, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0022] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence. In some embodiments, the polynucleotide sequence is operably linked to a promoter. In some embodiments, the promoter is an inducible promoter.

[0023] ~~In one aspect,~~ The disclosure provides for a vector comprising: a polynucleotide sequence encoding: a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a sequence configured to bind to an effector protein, and a site-directed polypeptide. In some embodiments, the polynucleotide sequence is operably linked to a promoter. In some embodiments, the promoter is an inducible promoter.

[0024] ~~In one aspect,~~ The disclosure provides for a vector comprising: a polynucleotide sequence encoding: a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence, a site-directed polypeptide, and an effector protein. In some embodiments, the polynucleotide sequence is operably linked to a promoter. In some embodiments, the promoter is an inducible promoter.

[0025] ~~In one aspect,~~ The disclosure provides for a genetically modified cell comprising a composition comprising: an effector protein, and a nucleic acid, wherein the nucleic acid comprises at least 50% sequence identity to a crRNA over 6 contiguous nucleotides, at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides; and a non-native sequence, wherein the nucleic acid is adapted to bind to the effector protein.

[0026] ~~In one aspect,~~ The disclosure provides for a genetically modified cell comprising a vector comprising a polynucleotide sequence encoding a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence.

[0027] ~~In one aspect,~~ The disclosure provides for a genetically modified cell comprising a vector comprising: a polynucleotide sequence encoding: a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a sequence configured to bind to an effector protein, and a site-directed polypeptide.

[0028] ~~In one aspect,~~ The disclosure provides for a genetically modified cell comprising a vector comprising: a polynucleotide sequence encoding: a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence, a site-directed polypeptide, and an effector protein.

[0029] ~~In one aspect,~~ The disclosure provides for a kit comprising: a vector comprising a polynucleotide sequence encoding a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0030] ~~In one aspect,~~ The disclosure provides for a kit comprising: a vector comprising: a polynucleotide sequence encoding: a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a sequence configured to bind to an effector protein, and a site-directed polypeptide, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0031] ~~In one aspect,~~ The disclosure provides for a kit comprising: a vector comprising: a polynucleotide sequence encoding: a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence, a site-directed polypeptide, and an effector protein, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0032] ~~In one aspect,~~ The disclosure provides for a composition comprising: a multiplexed genetic targeting agent, wherein the multiplexed genetic targeting agent comprises one or more nucleic acid modules, wherein the nucleic acid module comprises a non-native sequence, and wherein the nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein the nucleic acid module is configured to hybridize to a target nucleic acid. In some embodiments, the nucleic acid module comprises a first sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides, and a second sequence

5'-GUUCCAAUUAUUCUUAACCCUAUUAGGGAUUGAAAC-3',
 5'-GUUGCAAGGGAUUGAGCCCCGUAAGGGGAUUGCGAC-3';
 5'-GUUGCAAACCUCGUUAGCCUCGUAGAGGAUUGAAAC-3';
 5'-GGAUCGAUACCCACCCCGAAGAAAAGGGGACGAGAAC-3';
 5'-GUCGUCAGACCCAAAACCCCGAGAGGGGACGGAAAC-3';
 5'-GAUAUAAACCUAAUUAACCUCGAGAGGGGACGGAAAC-3';
 5'-CCCCAGUCACCUCGGGAGGGGACGGAAAC-3';
 5'-GUUCCAAUUAUUCUUAACCCUAUUAGGGAUUGAAAC-3',
 5'-GUCGCCCCCAGCGGGGGCGUGGAUUGAAAC-3';
 5'-CCAGCCGCCUUCGGGCGGCUGUGUGUUGAAAC-3';
 5'-GUCGCACUCUACAUGAGUGCGUGGAUUGAAAU-3';
 5'-UGUCGCACCUUAUUAUAGGUGCGUGGAUUGAAAU-3'; and
 5'-GUCGCGCCCCGCAUGGGGCGCGUGGAUUGAAA-3', or any

combination thereof. In some embodiments, the one or more nucleic acid modules are adapted to be bound by different endoribonucleases. In some embodiments, the multiplexed genetic target agent is an isolated multiplexed genetic targeting agent. In some embodiments, the multiplexed genetic target agent is a recombinant multiplexed genetic target agent.

[0033] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding a multiplexed genetic targeting agent, wherein the multiplexed genetic targeting agent comprises one or more nucleic acid modules, wherein the nucleic acid module comprises a non-native sequence, and wherein the nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein the nucleic acid module is configured to hybridize to a target nucleic acid. In some embodiments, the polynucleotide sequence is operably linked to a promoter. In some embodiments, the promoter is an inducible promoter.

[0034] ~~In one aspect,~~ The disclosure provides for a genetically modified cell comprising a multiplexed genetic targeting agent, wherein the multiplexed genetic targeting agent comprises one or more nucleic acid modules, wherein the nucleic acid module comprises a non-native sequence, and wherein the nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein the nucleic acid module is configured to hybridize to a target nucleic acid.

[0035] ~~In one aspect,~~ The disclosure provides for a genetically modified cell comprising a vector comprising a polynucleotide sequence encoding a multiplexed genetic targeting agent, wherein the multiplexed genetic targeting agent comprises one or more nucleic acid modules,

wherein the nucleic acid module comprises a non-native sequence, and wherein the nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein the nucleic acid module is configured to hybridize to a target nucleic acid.

[0036] ~~In one aspect,~~ The disclosure provides for a kit comprising a multiplexed genetic targeting agent, wherein the multiplexed genetic targeting agent comprises one or more nucleic acid modules, wherein the nucleic acid module comprises a non-native sequence, and wherein the nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein the nucleic acid module is configured to hybridize to a target nucleic acid, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0037] ~~In one aspect,~~ The disclosure provides for a kit comprising: a vector comprising a polynucleotide sequence encoding a multiplexed genetic targeting agent, wherein the multiplexed genetic targeting agent comprises one or more nucleic acid modules, wherein the nucleic acid module comprises a non-native sequence, and wherein the nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein the nucleic acid module is configured to hybridize to a target nucleic acid, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0038] ~~In one aspect,~~ The disclosure provides for a method for generating a nucleic acid, wherein the nucleic acid binds to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and hybridizes to a target nucleic acid comprising: introducing the a multiplexed genetic targeting agent, wherein the multiplexed genetic targeting agent comprises one or more nucleic acid modules, wherein the nucleic acid module comprises a non-native sequence, and wherein the nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein the nucleic acid module is configured to hybridize to a target nucleic acid into a host cell, processing the multiplexed genetic targeting agent into the one or more nucleic acid modules, and contacting the processed one or more nucleic acid modules to one or more target nucleic acids in the cell. In some embodiments, the method further comprises cleaving the target nucleic acid. In some embodiments, the method further comprises modifying the target nucleic acid. In some embodiments, the modifying comprises altering transcription of the target nucleic acid. In some embodiments, the modifying comprises inserting a donor polynucleotide into the target nucleic acid.

[0039] ~~In one aspect,~~ The disclosure provides for a modified site-directed polypeptide comprising: a first nuclease domain, a second nuclease domain, and an inserted nuclease domain. In some embodiments, the site-directed polypeptide comprises at least 15% identity to a nuclease domain of Cas9. In some embodiments, the first nuclease domain comprises a nuclease domain selected from the group consisting of: a HNH domain, and a RuvC domain, or any combination thereof. In some embodiments, the second nuclease domain comprises a nuclease domain selected from the group consisting of: a HNH domain, and a RuvC domain, or any combination thereof. In some embodiments, the inserted nuclease domain comprises a HNH domain. In some embodiments, the inserted nuclease domain comprises a RuvC domain. In some embodiments, the inserted nuclease domain is N-terminal to the first nuclease domain. In some embodiments, the inserted nuclease domain is N-terminal to the second nuclease domain. In some embodiments, the inserted nuclease domain is C-terminal to the first nuclease domain. In some embodiments, the inserted nuclease domain is C-terminal to the second nuclease domain. In some embodiments, the inserted nuclease domain is in tandem to the first nuclease domain. In some embodiments, the inserted nuclease domain is in tandem to the second nuclease domain. In some embodiments, the inserted nuclease domain is adapted to cleave a target nucleic acid at a site different than the first or second nuclease domains. In some embodiments, the inserted nuclease domain is adapted to cleave an RNA in a DNA-RNA hybrid. In some embodiments, the inserted nuclease domain is adapted to cleave a DNA in a DNA-RNA hybrid. In some embodiments, the inserted nuclease domain is adapted to increase specificity of binding of the modified site-directed polypeptide to a target nucleic acid. In some embodiments, the inserted nuclease domain is adapted to increase strength of binding of the modified site-directed polypeptide to a target nucleic acid.

[0040] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding a modified site-directed polypeptide comprising: a first nuclease domain, a second nuclease domain, and an inserted nuclease domain.

[0041] ~~In one aspect,~~ the disclosure provides for a kit comprising: a modified site-directed polypeptide comprising: a first nuclease domain, a second nuclease domain, and an inserted nuclease domain, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0042] ~~In one aspect,~~ The disclosure provides for a composition comprising: a modified site-directed polypeptide, wherein the polypeptide is modified such that it is adapted to target a second protospacer adjacent motif compared to a wild-type site-directed polypeptide. In some

embodiments, the site-directed polypeptide is modified by a modification selected from the group consisting of: an amino acid addition, an amino acid substitution, an amino acid replacement, and an amino acid deletion, or any combination thereof. In some embodiments, the modified site-directed polypeptide comprises a non-native sequence. In some embodiments, the modified site-directed polypeptide is adapted to target the second protospacer adjacent motif with greater specificity than the wild-type site-directed polypeptide. In some embodiments, the modified site-directed polypeptide is adapted to target the second protospacer adjacent motif with a lower dissociation constant compared to the wild-type site-directed polypeptide. In some embodiments, the modified site-directed polypeptide is adapted to target the second protospacer adjacent motif with a higher dissociation constant compared to the wild-type site-directed polypeptide. In some embodiments, the second protospacer adjacent motif comprises a protospacer adjacent motif selected from the group consisting of: 5'-NGGNG-3', 5'-NNAAAW-3', 5'-NNNNGATT-3', 5'-GNNNCNNA-3', and 5'-NNNACA-3', or any combination thereof.

[0043] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding a modified site-directed polypeptide, wherein the polypeptide is modified such that it is adapted to target a second protospacer adjacent motif compared to a wild-type site-directed polypeptide.

[0044] ~~In one aspect,~~ The disclosure provides for a kit comprising: a modified site-directed polypeptide, wherein the polypeptide is modified such that it is adapted to target a second protospacer adjacent motif compared to a wild-type site-directed polypeptide, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0045] ~~In one aspect,~~ The disclosure provides for a composition comprising: a modified site-directed polypeptide, wherein the polypeptide is modified such that it is adapted to target a second nucleic acid-targeting nucleic acid compared to a wild-type site-directed polypeptide. In some embodiments, the site-directed polypeptide is modified by a modification selected from the group consisting of: an amino acid addition, an amino acid substitution, an amino acid replacement, and an amino acid deletion, or any combination thereof. In some embodiments, the modified site-directed polypeptide comprises a non-native sequence. In some embodiments, the modified site-directed polypeptide is adapted to target the second nucleic acid-targeting nucleic acid with greater specificity than the wild-type site-directed polypeptide. In some embodiments, the modified site-directed polypeptide is adapted to target the second nucleic acid-targeting nucleic acid with a lower dissociation constant compared to the wild-type site-directed polypeptide. In some embodiments, the modified

site-directed polypeptide is adapted to target the second nucleic acid-targeting nucleic acid with a higher dissociation constant compared to the wild-type site-directed polypeptide. In some embodiments, the site-directed polypeptide targets a tracrRNA portion of the second nucleic acid target nucleic acid.

[0046] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding a modified site-directed polypeptide, wherein the polypeptide is modified such that it is adapted to target a second nucleic acid-targeting nucleic acid compared to a wild-type site-directed polypeptide.

[0047] ~~In one aspect,~~ The disclosure provides for a kit comprising: a modified site-directed polypeptide, wherein the polypeptide is modified such that it is adapted to target a second nucleic acid-targeting nucleic acid compared to a wild-type site-directed polypeptide, and a buffer. In some embodiments, the kit further comprises instructions for use.

[0048] ~~In one aspect,~~ The disclosure provides for a composition comprising: a modified site-directed polypeptide comprising a modification in a bridge helix as compared to SEQ ID: 8. In some embodiments, the composition is configured to cleave a target nucleic acid.

[0049] ~~In one aspect,~~ The disclosure provides for a composition comprising: a modified site-directed polypeptide comprising a modification in a highly basic patch as compared to SEQ ID: 8. In some embodiments, the composition is configured to cleave a target nucleic acid.

[0050] ~~In one aspect,~~ The disclosure provides for a composition comprising: a modified site-directed polypeptide comprising a modification in a polymerase-like domain as compared to SEQ ID: 8. In some embodiments, the composition is configured to cleave a target nucleic acid.

[0051] ~~In one aspect,~~ The disclosure provides for a composition comprising: a modified site-directed polypeptide comprising a modification in a bridge helix, highly basic patch, nuclease domain, and polymerase domain as compared to SEQ ID: 8, or any combination thereof.

[0052] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding a modified site-directed polypeptide comprising a modification in a bridge helix, highly basic patch, nuclease domain, and polymerase domain as compared to SEQ ID: 8, or any combination thereof.

[0053] ~~In one aspect,~~ The disclosure provides for a kit comprising: a modified site-directed polypeptide comprising a modification in a bridge helix, highly basic patch, nuclease domain, and polymerase domain as compared to SEQ ID: 8, or any combination thereof, and a buffer. In some embodiments, the kit further comprises instructions for use. In some embodiments, the kit further comprises a nucleic acid-targeting nucleic acid.

[0054] ~~In one aspect,~~ The disclosure provides for a genetically modified cell a modified site-directed polypeptide comprising a modification in a bridge helix, highly basic patch, nuclease domain, and polymerase domain as compared to SEQ ID: 8, or any combination thereof.

[0055] ~~In one aspect,~~ The disclosure provides for a method for genome engineering comprising: contacting a target nucleic acid with a complex, wherein the complex comprises a modified site-directed polypeptide comprising a modification in a bridge helix, highly basic patch, nuclease domain, and polymerase domain as compared to SEQ ID: 8, or any combination thereof and a nucleic acid-targeting nucleic acid, and modifying the target nucleic acid. In some embodiments, the contacting comprises contacting the complex to a protospacer adjacent motif in the target nucleic acid. In some embodiments, the contacting comprises contacting the complex to a longer target nucleic acid sequence compared to an unmodified site-directed polypeptide. In some embodiments, the modifying comprises cleaving the target nucleic acid. In some embodiments, the target nucleic acid comprises RNA. In some embodiments, the target nucleic acid comprises DNA. In some embodiments, the modifying comprises cleaving the RNA strand of a hybridized RNA and DNA. In some embodiments, the modifying comprises cleaving the DNA strand of a hybridized RNA and DNA. In some embodiments, the modifying comprises inserting into the target nucleic acid a donor polynucleotide, a portion of a donor polynucleotide, a copy of a donor polynucleotide, or a portion of a copy of a donor polynucleotide, or any combination thereof. In some embodiments, the modifying comprises modifying transcriptional activity of the target nucleic acid. In some embodiments, the modifying comprises a deleting of one or more nucleotides of the target nucleic acid.

[0056] ~~In one aspect,~~ The disclosure provides for a composition comprising: a modified site-directed polypeptide comprising a modified nuclease domain as compared to SEQ ID: 8. In some embodiments, the composition is configured to cleave a target nucleic acid. In some embodiments, the modified nuclease domain comprises a RuvC domain nuclease domain. In some embodiments, the modified nuclease domain comprises an HNH nuclease domain. In some embodiments, the modified nuclease domain comprises duplication of an HNH nuclease domain. In some embodiments, the modified nuclease domain is adapted to increase specificity of the amino acid sequence for a target nucleic acid compared to an unmodified site-directed polypeptide. In some embodiments, the modified nuclease domain is adapted to increase specificity of the amino acid sequence for a nucleic acid-targeting nucleic acid compared to an unmodified site-directed polypeptide. In some embodiments, the modified nuclease domain comprises a modification selected from the group consisting of: an amino

[0057] ~~In one aspect,~~ The disclosure provides for a method for enriching a target nucleic acid for sequencing comprising: contacting a target nucleic acid with a complex comprising a nucleic acid-targeting nucleic acid and a site-directed polypeptide, enriching the target nucleic acid using the complex, and determining a sequence of the target nucleic acid. In some embodiments, the method does not comprise an amplification step. In some embodiments, the method further comprises analyzing the sequence of the target nucleic acid. In some embodiments, the method further comprises fragmenting the target nucleic acid prior to the enriching. In some embodiments, the nucleic acid-targeting nucleic acid comprises RNA. In some embodiments, the method the nucleic acid-targeting nucleic acid comprises two RNA molecules. In some embodiments, the method a portion of each of the two RNA molecules hybridize together. In some embodiments, the method one of the two RNA molecules comprises a CRISPR repeat sequence. In some embodiments, the CRISPR repeat sequence is homologous to a crRNA over 6 contiguous nucleotides. In some embodiments, the CRISPR repeat sequence comprises at least 60% identity to a crRNA over 6 contiguous nucleotides. In some embodiments, the one of the two RNA molecules comprises a tracrRNA sequence. In some embodiments, the tracrRNA sequence is homologous to a tracrRNA over 6 contiguous nucleotides. In some embodiments, the tracrRNA sequence comprises at least 60% identity to a tracrRNA over 6 contiguous nucleotides. In some embodiments, the nucleic acid-targeting nucleic acid is a double guide nucleic acid. In some embodiments, the nucleic acid-targeting nucleic acid comprises one continuous RNA molecule wherein the continuous RNA molecule further comprises two domains and a linker. In some embodiments, a portion of each of the two domains of the continuous RNA molecule hybridize together. In some embodiments, the continuous RNA molecule comprises a CRISPR repeat sequence. In some embodiments, the CRISPR repeat sequence is homologous to a crRNA over 6 contiguous nucleotides. In some embodiments, the CRISPR repeat sequence comprises at least 60% identity to a crRNA over 6 contiguous nucleotides. In some embodiments, the continuous RNA molecule comprises a tracrRNA sequence. In some embodiments, the tracrRNA sequence is homologous to a tracrRNA over 6 contiguous nucleotides. In some embodiments, the tracrRNA sequence comprises at least 60% identity to a tracrRNA over 6 contiguous nucleotides. In some embodiments, the nucleic acid-targeting nucleic acid is a single guide nucleic acid. In some embodiments, the contacting comprises hybridizing a portion of the nucleic acid-targeting nucleic acid with a portion of the target nucleic acid. In some embodiments, the nucleic acid-targeting nucleic acid hybridizes with the target nucleic acid over a region comprising 6-20 nucleotides. In some embodiments, the site-directed

capture agent comprises an activatable enzymatic domain, wherein the activatable enzymatic domain is activated by coming in contact with imidazole. In some embodiments, the capture agent is a Cas6 family member. In some embodiments, the capture agent comprises an affinity tag. In some embodiments, the capture agent comprises a conditionally enzymatically inactive endoribonuclease comprising a mutation in a nuclease domain. In some embodiments, the mutation of a conserved histidine. In some embodiments, the mutation comprises a H29A mutation. In some embodiments, the target nucleic acid is bound to the complex. In some embodiments, the target nucleic acid is an excised nucleic acid that is not bound to the complex. In some embodiments, a plurality of complexes are contacted to a plurality of target nucleic acids. In some embodiments, the plurality of target nucleic acids differ by at least one nucleotide. In some embodiments, the plurality of complexes comprise a plurality of nucleic acid-targeting nucleic acids that differ by at least one nucleotide.

[0058] ~~In one aspect,~~ The disclosure provides for a method for excising a nucleic acid comprising: contacting a target nucleic acid with two or more complexes, wherein each complex comprises a site-directed polypeptide and a nucleic acid-targeting nucleic acid, and cleaving the target nucleic acid, wherein the cleaving produces an excised target nucleic acid. In some embodiments, the cleaving is performed by a nuclease domain of the site-directed polypeptide. In some embodiments, the method does not comprise amplification. In some embodiments, the method further comprises enriching the excised target nucleic acid. In some embodiments, the method further comprises sequencing the excised target nucleic acid. In some embodiments, the nucleic acid-targeting nucleic acid is RNA. In some embodiments, the nucleic acid-targeting nucleic acid comprises two RNA molecules. In some embodiments, a portion of each of the two RNA molecules hybridize together. In some embodiments, one of the two RNA molecules comprises a CRISPR repeat sequence. In some embodiments, the CRISPR repeat sequence comprises a sequence that is homologous to a crRNA over 6 contiguous nucleotides. In some embodiments, the CRISPR repeat sequence comprises a sequence that has at least 60% identity to a crRNA over 6 contiguous nucleotides. In some embodiments, one of the two RNA molecules comprises a tracrRNA sequence. In some embodiments, the tracrRNA sequence is homologous to a crRNA over 6 contiguous nucleotides. In some embodiments, the tracrRNA sequence comprises at least 60% identity to a crRNA over 6 contiguous nucleotides. In some embodiments, the nucleic acid-targeting nucleic acid is a double guide nucleic acid. In some embodiments, the nucleic acid-targeting nucleic acid comprises one continuous RNA molecule wherein the continuous RNA molecule further comprises two domains and a linker. In some embodiments, a portion of each of the

[0059] ~~In one aspect,~~ The disclosure provides for a method for generating a library of target nucleic acids comprising: contacting a plurality of target nucleic acids with a complex comprising a site-directed polypeptide and a nucleic acid-targeting nucleic acid, cleaving the plurality of target nucleic acids, and purifying the plurality of target nucleic acids to create the library of target nucleic acids. In some embodiments, the method further comprises screening the library of target nucleic acids.

[0060] ~~In one aspect,~~ The disclosure provides for a composition comprising: a first complex comprising: a first site-directed polypeptide and a first nucleic acid-targeting nucleic acid, a second complex comprising: a second site-directed polypeptide and a second nucleic acid-targeting nucleic acid, wherein, the first and second nucleic acid-targeting nucleic acids are different. In some embodiments, the composition further comprises a target nucleic acid, which is bound by the first or the second complex. In some embodiments, the first site-directed polypeptide and the second site-directed polypeptide are the same. In some embodiments, the first site-directed polypeptide and the second site-directed polypeptide are different.

[0061] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding: two or more nucleic acid-targeting nucleic acids that differ by at least one nucleotide, and a site-directed polypeptide.

[0062] ~~In one aspect,~~ The disclosure provides a genetically modified host cell comprising: a vector comprising a polynucleotide sequence encoding: two or more nucleic acid-targeting nucleic acids that differ by at least one nucleotide, and a site-directed polypeptide.

[0063] ~~In one aspect,~~ The disclosure provides a kit comprising: a vector comprising a polynucleotide sequence encoding: two or more nucleic acid-targeting nucleic acids that differ by at least one nucleotide, a site-directed polypeptide, and a suitable buffer. In some embodiments, the kit further comprises: a capture agent, a solid support, sequencing adaptors, and a positive control, or any combination thereof. In some embodiments, the kit further comprises instructions for use.

[0064] ~~In one aspect,~~ The disclosure provides for a kit comprising: a site-directed polypeptide comprising reduced enzymatic activity compared to a wild-type site-directed polypeptide, a nucleic acid-targeting nucleic acid, and a capture agent. In some embodiments, the kit further comprises: instructions for use. In some embodiments, the kit further comprises a buffer selected from the group comprising: a wash buffer, a stabilization buffer, a reconstituting buffer, or a diluting buffer.

[0065] ~~In one aspect,~~ The disclosure provides for a method for cleaving a target nucleic acid using two or more nickases comprising: contacting a target nucleic acid with a first complex and a second complex, wherein the first complex comprises a first nickase and a first nucleic acid-targeting nucleic acid, and wherein the second complex comprises a second nickase and a second nucleic acid-targeting nucleic acid, wherein the target nucleic acid comprises a first protospacer adjacent motif on a first strand and a second protospacer adjacent motif on a second strand, wherein the first nucleic acid-targeting nucleic acid is adapted to hybridize to the first protospacer adjacent motif, and wherein the second nucleic acid-targeting nucleic acid is adapted to hybridize to the second protospacer adjacent motif, and nicking the first and second strands of the target nucleic acid, wherein the nicking generates a cleaved target nucleic acid. In some embodiments, the first and second nickase are the same. In some embodiments, the first and second nickase are different. In some embodiments, the first and second nucleic acid-targeting nucleic acid are different. In some embodiments, there are less than 125 nucleotides between the first protospacer adjacent motif and the second protospacer adjacent motif. In some embodiments, the first and second protospacer adjacent motifs comprise of the sequence NGG, where N is any nucleotide. In some embodiments, the first or second nickase comprises at least one substantially inactive nuclease domain. In some embodiments, the first or second nickase comprises a mutation of a conserved aspartic acid. In some embodiments, the mutation is a D10A mutation. In some embodiments, the first or second nickase comprises a mutation of a conserved histidine. In some embodiments, the mutation is a H840A mutation. In some embodiments, there are less than 15 nucleotides between the first and second protospacer adjacent motifs. In some embodiments, there are less than 10 nucleotides between the first and second protospacer adjacent motifs. In some embodiments, there are less than 5 nucleotides between the first and second protospacer adjacent motifs. In some embodiments, the first and second protospacer adjacent motifs are adjacent to one another. In some embodiments, the nicking comprises the first nickase nicking the first strand and the second nickase nicking the second strand. In some embodiments, the nicking generates a sticky end cut. In some embodiments, the nicking generates a blunt end cut. In some embodiments, the method further comprises inserting a donor polynucleotide into the cleaved target nucleic acid.

[0066] ~~In one aspect,~~ The disclosure provides for a composition comprising: a plurality of nucleic acid molecules, wherein each nucleic acid molecule comprises a nucleic acid-binding protein binding site, wherein at least one of the plurality of nucleic acid molecules encodes for a nucleic acid-targeting nucleic acid and one of the plurality of nucleic acid molecules

[0067] ~~In one aspect,~~ The disclosure provides for a method for delivery of nucleic acids to a subcellular location in a cell comprising: introducing into a cell a composition comprising: a plurality of nucleic acid molecules, wherein each nucleic acid molecule comprises a nucleic acid-binding protein binding site, wherein at least one of the plurality of nucleic acid molecules encodes for a nucleic acid-targeting nucleic acid and one of the plurality of nucleic acid molecules encodes for a site-directed polypeptide, and a fusion polypeptide, wherein the fusion polypeptide comprises a plurality of the nucleic acid-binding proteins, wherein the plurality of nucleic acid-binding proteins are adapted to bind to their cognate nucleic acid-binding protein binding site stoichiometrically delivering the composition to the subcellular location, forming a unit comprising a site-directed polypeptide translated from the nucleic acid molecule encoding for a site-directed polypeptide and the nucleic acid-targeting nucleic acid, and cleaving a target nucleic acid, wherein the site-directed polypeptide of the unit cleaves the target nucleic acid. In some embodiments, the plurality of nucleic acid-binding proteins bind to their cognate nucleic acid-binding protein binding site. In some embodiments, an endoribonuclease cleaves one of the one or more nucleic acid-binding protein binding sites. In some embodiments, an endoribonuclease cleaves the nucleic acid-binding protein binding sites of the nucleic acid encoding the nucleic acid-targeting nucleic acid, thereby liberating the nucleic acid-targeting nucleic acid. In some embodiments, the subcellular location is selected from the group consisting of: the nucleus, the ER, the golgi, the mitochondria, the cell wall, the lysosome, and the nucleus. In some embodiments, the subcellular location is the nucleus.

[0068] ~~In one aspect,~~ The disclosure provides for a vector comprising: a polynucleotide sequence encoding a composition comprising: a plurality of nucleic acid molecules, wherein each nucleic acid molecule comprises a nucleic acid-binding protein binding site, wherein at least one of the plurality of nucleic acid molecules encodes for a nucleic acid-targeting nucleic acid and one of the plurality of nucleic acid molecules encodes for a site-directed polypeptide; and a fusion polypeptide, wherein the fusion polypeptide comprises a plurality of the nucleic acid-binding proteins, wherein the plurality of nucleic acid-binding proteins are adapted to bind to their cognate nucleic acid-binding protein binding site stoichiometrically delivering the composition to the subcellular location. In some embodiments, the vector further comprises a polynucleotide encoding a promoter. In some embodiments, the promoter is operably linked to the polynucleotide. In some embodiments, the promoter is an inducible promoter.

[0069] ~~In one aspect,~~ The disclosure provides for a genetically modified organism comprising a vector comprising: a polynucleotide sequence encoding for a plurality of nucleic acid molecules, wherein each nucleic acid molecule comprises a nucleic acid-binding protein binding site, wherein at least one of the plurality of nucleic acid molecules encodes for a nucleic acid-targeting nucleic acid and one of the plurality of nucleic acid molecules encodes for a site-directed polypeptide, and a fusion polypeptide, wherein the fusion polypeptide comprises a plurality of the nucleic acid-binding proteins, wherein the plurality of nucleic acid-binding proteins are adapted to bind to their cognate nucleic acid-binding protein binding site stoichiometrically delivering the composition to the subcellular location.

[0070] ~~In one aspect,~~ The disclosure provides for a genetically modified organism comprising: a composition comprising: a plurality of nucleic acid molecules, wherein each nucleic acid molecule comprises a nucleic acid-binding protein binding site, wherein at least one of the plurality of nucleic acid molecules encodes for a nucleic acid-targeting nucleic acid and one of the plurality of nucleic acid molecules encodes for a site-directed polypeptide; and a fusion polypeptide, wherein the fusion polypeptide comprises a plurality of the nucleic acid-binding proteins, wherein the plurality of nucleic acid-binding proteins are adapted to bind to their cognate nucleic acid-binding protein binding site.

[0071] ~~In one aspect,~~ The disclosure provides for a kit comprising: a composition comprising: a plurality of nucleic acid molecules, wherein each nucleic acid molecule comprises a nucleic acid-binding protein binding site, wherein at least one of the plurality of nucleic acid molecules encodes for a nucleic acid-targeting nucleic acid and one of the plurality of nucleic acid molecules encodes for a site-directed polypeptide, and a fusion polypeptide, wherein the fusion polypeptide comprises a plurality of the nucleic acid-binding proteins, wherein the plurality of nucleic acid-binding proteins are adapted to bind to their cognate nucleic acid-binding protein binding site, and a buffer.

[0072] ~~In one aspect,~~ The disclosure provides for a kit comprising: a vector comprising: a polynucleotide sequence encoding for a plurality of nucleic acid molecules, wherein each nucleic acid molecule comprises a nucleic acid-binding protein binding site, wherein at least one of the plurality of nucleic acid molecules encodes for a nucleic acid-targeting nucleic acid and one of the plurality of nucleic acid molecules encodes for a site-directed polypeptide; and a fusion polypeptide, wherein the fusion polypeptide comprises a plurality of the nucleic acid-binding proteins, wherein the plurality of nucleic acid-binding proteins are adapted to bind to their cognate nucleic acid-binding protein binding site stoichiometrically delivering the composition to the subcellular location, and a buffer. In some embodiments,

the kit further comprises instructions for use. In some embodiments, the buffer is selected from the group comprising: a dilution buffer, a reconstitution buffer, and a stabilization buffer, or any combination thereof.

[0073] ~~In one aspect,~~ The disclosure provides for a donor polynucleotide comprising: a genetic element of interest, and a reporter element, wherein the reporter element comprises a polynucleotide sequence encoding a site-directed polypeptide, and one or more a nucleic acids, wherein the one or more nucleic acids comprises a sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and a sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides. In some embodiments, the genetic element of interest comprises a gene. In some embodiments, the genetic element of interest comprises a non-coding nucleic acid selected from the group consisting of: a microRNA, a siRNA, and a long non-coding RNA, or any combination thereof. In some embodiments, the genetic element of interest comprises a non-coding gene. In some embodiments, the genetic element of interest comprises a non-coding nucleic acid selected from the group consisting of: a microRNA, a siRNA, and a long non-coding RNA, or any combination thereof. In some embodiments, the reporter element comprises a gene selected from the group consisting of: a gene encoding a fluorescent protein, a gene encoding a chemiluminescent protein, and an antibiotic resistance gene, or any combination thereof. In some embodiments, the reporter element comprises a gene encoding a fluorescent protein. In some embodiments, the fluorescent protein comprises green fluorescent protein. In some embodiments, the reporter element is operably linked to a promoter. In some embodiments, the promoter comprises an inducible promoter. In some embodiments, the promoter comprises a tissue-specific promoter. In some embodiments, the site-directed polypeptide comprises at least 15% amino acid sequence identity to a nuclease domain of Cas9. In some embodiments, the site-directed polypeptide comprises at least 95% amino acid sequence identity over 10 amino acids to Cas9. In some embodiments, the nuclease domain is selected from the group consisting of: an HNH domain, an HNH-like domain, a RuvC domain, and a RuvC-like domain, or any combination thereof.

[0074] ~~In one aspect,~~ The disclosure provides for an expression vector comprising a polynucleotide sequence encoding for a genetic element of interest; and a reporter element, wherein the reporter element comprises a polynucleotide sequence encoding a site-directed polypeptide, and one or more a nucleic acids, wherein the one or more nucleic acids comprises a sequence comprising at least 50% sequence identity to a crRNA over 6

contiguous nucleotides and a sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides.

[0075] ~~In one aspect,~~ The disclosure provides for a genetically modified cell comprising a donor polynucleotide comprising: a genetic element of interest; and a reporter element, wherein the reporter element comprises a polynucleotide sequence encoding a site-directed polypeptide, and one or more a nucleic acids, wherein the one or more nucleic acids comprises a sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and a sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides.

[0076] ~~In one aspect,~~ The disclosure provides for a kit comprising: a donor polynucleotide comprising: a genetic element of interest; and a reporter element, wherein the reporter element comprises a polynucleotide sequence encoding a site-directed polypeptide, and one or more a nucleic acids, wherein the one or more nucleic acids comprises a sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and a sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides; and a buffer. In some embodiments, the kit further comprises: a polypeptide comprising at least 10% amino acid sequence identity to Cas9; and a nucleic acid, wherein the nucleic acid binds to the polypeptide and hybridizes to a target nucleic acid. In some embodiments, the kit further comprises instructions for use. In some embodiments, the kit further comprises a polynucleotide encoding a polypeptide, wherein the polypeptide comprises at last 15% amino acid sequence identity to Cas9. In some embodiments, the kit further comprises a polynucleotide encoding a nucleic acid, wherein the nucleic acid comprises a sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and a sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides.

[0077] ~~In one aspect,~~ The disclosure provides for a method for selecting a cell using a reporter element and excising the reporter element from the cell comprising: contacting a target nucleic acid with a complex comprising a site-directed polypeptide and a nucleic acid-targeting nucleic acid; cleaving the target nucleic acid with the site-directed polypeptide, to generate a cleaved target nucleic acid; inserting the donor polynucleotide comprising a genetic element of interest; and a reporter element, wherein the reporter element comprises a polynucleotide sequence encoding a site-directed polypeptide, and one or more a nucleic acids, wherein the one or more nucleic acids comprises a sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and a sequence comprising at

least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides into the cleaved target nucleic acid; and selecting the cell based on the donor polynucleotide to generate a selected cell. In some embodiments, selecting comprises selecting the cell from a subject being treated for a disease. In some embodiments, selecting comprises selecting the cell from a subject being diagnosed for a disease. In some embodiments, after the selecting, the cell comprises the donor polynucleotide. In some embodiments, the method further comprises excising all, some or none of the reporter element, thereby generating a second selected cell. In some embodiments, excising comprises contacting the 5' end of the reporter element with a complex comprising a site-directed polypeptide and a nucleic acid-targeting nucleic acid, wherein the complex cleaves the 5' end. In some embodiments, excising comprises contacting the 3' end of the reporter element with a complex comprising a site-directed polypeptide and a nucleic acid-targeting nucleic acid, wherein the complex cleaves the 3' end. In some embodiments, excising comprises contacting the 5' and 3' end of the reporter element with one or more complexes comprising a site-directed polypeptide and a nucleic acid-targeting nucleic acid, wherein the complex cleaves the 5' and 3' end. In some embodiments, the method further comprises screening the second selected cell. In some embodiments, screening comprises observing an absence of all or some of the reporter element.

[0078] ~~In one aspect,~~ The disclosure provides for a composition comprising: a nucleic acid comprising: a spacer, wherein the spacer is between 12-30 nucleotides, inclusive, and wherein the spacer is adapted to hybridize to a sequence that is 5' to a PAM; a first duplex, wherein the first duplex is 3' to the spacer; a bulge, wherein the bulge comprises at least 3 unpaired nucleotides on a first strand of the first duplex and at least 1 unpaired nucleotide on a second strand of the first duplex; a linker, wherein the linker links the first strand and the second strand of the duplex and is at least 3 nucleotides in length; a P-domain; and a second duplex, wherein the second duplex is 3' of the P-domain and is adapted to bind to a site directed polypeptide. In some embodiments, the sequence that is 5' to a PAM is at least 18 nucleotides in length. In some embodiments, the sequence that is 5' to a PAM is adjacent to the PAM. In some embodiments, the PAM comprises 5'-NGG-3'. In some embodiments, the first duplex is adjacent to the spacer. In some embodiments, the P-domain starts from 1-5 nucleotides downstream of the duplex, comprises at least 4 nucleotides, and is adapted to hybridize to sequence selected from the group consisting of: a 5'-NGG-3' protospacer adjacent motif sequence, a sequence comprising at least 50% identity to amino acids 1096-1225 of Cas9 from *S. pyogenes*, or any combination thereof. In some embodiments, the site-directed polypeptide comprises at least 15% identity to a nuclease domain of Cas9 from *S.*

pyogenes. In some embodiments, the nucleic acid is RNA. In some embodiments, the nucleic acid is an A-form RNA. In some embodiments, the first duplex is at least 6 nucleotides in length. In some embodiments, the 3 unpaired nucleotides of the bulge comprise 5'-AAG-3'. In some embodiments, adjacent to the 3 unpaired nucleotides is a nucleotide that forms a wobble pair with a nucleotide on the second strand of the first duplex. In some embodiments, the polypeptide binds to a region of the nucleic acid selected from the group consisting of: the first duplex, the second duplex, and the P-domain, or any combination thereof.

[0079] ~~In one aspect,~~ The disclosure provides for a method of modifying a target nucleic acid comprising: contacting a target nucleic acid with a composition comprising: a nucleic acid comprising: a spacer, wherein the spacer is between 12-30 nucleotides, inclusive, and wherein the spacer is adapted to hybridize to a sequence that is 5' to a PAM; a first duplex, wherein the first duplex is 3' to the spacer; a bulge, wherein the bulge comprises at least 3 unpaired nucleotides on a first strand of the first duplex and at least 1 unpaired nucleotide on a second strand of the first duplex; a linker, wherein the linker links the first strand and the second strand of the duplex and is at least 3 nucleotides in length; a P-domain; and a second duplex, wherein the second duplex is 3' of the P-domain and is adapted to bind to a site directed polypeptide; and modifying the target nucleic acid. In some embodiments, the method further comprises contacting with a site-directed polypeptide. In some embodiments, the contacting comprises contacting the spacer to the target nucleic acid. In some embodiments, the modifying comprises cleaving the target nucleic acid to produce a cleaved target nucleic acid. In some embodiments, the cleaving is performed by the site-directed polypeptide. In some embodiments, the method further comprises inserting a donor polynucleotide into the cleaved target nucleic acid. In some embodiments, the modifying comprises modifying transcription of the target nucleic acid.

[0080] ~~In one aspect,~~ The disclosure provides for a vector comprising a polynucleotide sequence encoding a nucleic acid comprising: a spacer, wherein the spacer is between 12-30 nucleotides, inclusive, and wherein the spacer is adapted to hybridize to a sequence that is 5' to a PAM; a first duplex, wherein the first duplex is 3' to the spacer; a bulge, wherein the bulge comprises at least 3 unpaired nucleotides on a first strand of the first duplex and at least 1 unpaired nucleotide on a second strand of the first duplex; a linker, wherein the linker links the first strand and the second strand of the duplex and is at least 3 nucleotides in length; a P-domain; and a second duplex, wherein the second duplex is 3' of the P-domain and is adapted to bind to a site directed polypeptide.

[0081] ~~In one aspect,~~ The disclosure provides for a kit comprising: a composition comprising: a nucleic acid comprising: a spacer, wherein the spacer is between 12-30 nucleotides, inclusive, and wherein the spacer is adapted to hybridize to a sequence that is 5' to a PAM; a first duplex, wherein the first duplex is 3' to the spacer; a bulge, wherein the bulge comprises at least 3 unpaired nucleotides on a first strand of the first duplex and at least 1 unpaired nucleotide on a second strand of the first duplex; a linker, wherein the linker links the first strand and the second strand of the duplex and is at least 3 nucleotides in length; a P-domain; and a second duplex, wherein the second duplex is 3' of the P-domain and is adapted to bind to a site directed polypeptide; and a buffer. In some embodiments, the kit further comprises a site-directed polypeptide. In some embodiments, the kit further comprises a donor polynucleotide. In some embodiments, the kit further comprises instructions for use.

[0082] ~~In one aspect,~~ The disclosure provides for a method of creating a synthetically designed nucleic acid-targeting nucleic acid comprising: designing a composition comprising: a nucleic acid comprising: a spacer, wherein the spacer is between 12-30 nucleotides, inclusive, and wherein the spacer is adapted to hybridize to a sequence that is 5' to a PAM; a first duplex, wherein the first duplex is 3' to the spacer; a bulge, wherein the bulge comprises at least 3 unpaired nucleotides on a first strand of the first duplex and at least 1 unpaired nucleotide on a second strand of the first duplex; a linker, wherein the linker links the first strand and the second strand of the duplex and is at least 3 nucleotides in length; a P-domain; and a second duplex, wherein the second duplex is 3' of the P-domain and is adapted to bind to a site directed polypeptide;.

[0083] ~~In one aspect,~~ The disclosure provides for a pharmaceutical composition comprising an engineered nucleic acid-targeting nucleic acid selected from the group consisting of: an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a P-domain of said nucleic acid-targeting nucleic acid; an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a bulge region of a nucleic acid-targeting nucleic acid

[0084] ~~In one aspect,~~ The disclosure provides for a pharmaceutical composition comprising a composition selected from the group consisting of: A composition comprising: an engineered nucleic acid-targeting nucleic acid comprising a 3' hybridizing extension, and a donor polynucleotide, wherein said donor polynucleotide is hybridized to said 3' hybridizing extension; a composition comprising: an effector protein, and a nucleic acid, wherein said nucleic acid comprises: at least 50% sequence identity to a crRNA over 6 contiguous nucleotides, at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides, and a non-native sequence, wherein said nucleic acid is adapted to bind to said effector protein; a

strand of said first duplex, a linker, wherein said linker links said first strand and said second strand of said duplex and is at least 3 nucleotides in length, a P-domain, and a second duplex, wherein said second duplex is 3' of said P-domain and is adapted to bind to a site directed polypeptide; or any combination thereof.

[0085] ~~In one aspect,~~ The disclosure provides for a pharmaceutical composition comprising a modified site-directed polypeptide comprising: a first nuclease domain, a second nuclease domain, and an inserted nuclease domain.

[0086] ~~In one aspect,~~ The disclosure provides for a pharmaceutical composition comprising a donor polynucleotide comprising: a genetic element of interest, and a reporter element, wherein said reporter element comprises a polynucleotide sequence encoding a site-directed polypeptide, and one or more nucleic acids, wherein said one or more nucleic acids comprises a sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and a sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides.

[0087] ~~In one aspect,~~ The disclosure provides for a pharmaceutical composition a vector selected from the group consisting of: a vector comprising a polynucleotide sequence encoding An engineered nucleic acid-targeting nucleic acid comprising: a mutation in a P-domain of said nucleic acid-targeting nucleic acid; a vector comprising a polynucleotide sequence encoding an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a bulge region of a nucleic acid-targeting nucleic acid; and modifying the target nucleic acid; a vector comprising a polynucleotide sequence encoding a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence; a vector comprising: a polynucleotide sequence encoding: a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a sequence configured to bind to an effector protein, and a site-directed polypeptide; a vector comprising: a polynucleotide sequence encoding: a modified nucleic acid-targeting nucleic acid, wherein the modified nucleic acid-targeting nucleic acid comprises a non-native sequence, a site-directed polypeptide, and an effector protein; a vector comprising a polynucleotide sequence encoding a multiplexed genetic targeting agent, wherein the multiplexed genetic targeting agent comprises one or more nucleic acid modules, wherein the nucleic acid module comprises a non-native sequence, and wherein the nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein the nucleic acid module is configured to hybridize to a target nucleic acid; a vector comprising a polynucleotide

sequence encoding a modified site-directed polypeptide comprising a modification in a bridge helix, highly basic patch, nuclease domain, and polymerase domain as compared to SEQ ID: 8, or any combination thereof; a vector comprising a polynucleotide sequence encoding: two or more nucleic acid-targeting nucleic acids that differ by at least one nucleotide; and a site-directed polypeptide; a vector comprising: a polynucleotide sequence encoding a composition comprising: a plurality of nucleic acid molecules, wherein each nucleic acid molecule comprises a nucleic acid-binding protein binding site, wherein at least one of the plurality of nucleic acid molecules encodes for a nucleic acid-targeting nucleic acid and one of the plurality of nucleic acid molecules encodes for a site-directed polypeptide; and a fusion polypeptide, wherein the fusion polypeptide comprises a plurality of the nucleic acid-binding proteins, wherein the plurality of nucleic acid-binding proteins are adapted to bind to their cognate nucleic acid-binding protein binding site stoichiometrically delivering the composition to the subcellular location; an expression vector comprising a polynucleotide sequence encoding for a genetic element of interest; and a reporter element, wherein the reporter element comprises a polynucleotide sequence encoding a site-directed polypeptide, and one or more nucleic acids, wherein the one or more nucleic acids comprises a sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and a sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides; and a vector comprising a polynucleotide sequence encoding a nucleic acid comprising: a spacer, wherein the spacer is between 12-30 nucleotides, inclusive, and wherein the spacer is adapted to hybridize to a sequence that is 5' to a PAM; a first duplex, wherein the first duplex is 3' to the spacer; a bulge, wherein the bulge comprises at least 3 unpaired nucleotides on a first strand of the first duplex and at least 1 unpaired nucleotide on a second strand of the first duplex; a linker, wherein the linker links the first strand and the second strand of the duplex and is at least 3 nucleotides in length; a P-domain; and a second duplex, wherein the second duplex is 3' of the P-domain and is adapted to bind to a site directed polypeptide; or any combination thereof.

[0088] ~~In one aspect,~~ The disclosure provides for a method of treating a disease comprising administering to a subject: an engineered nucleic acid-targeting comprising: a mutation in a P-domain of said nucleic acid-targeting nucleic acid; an engineered nucleic acid-targeting nucleic acid comprising: a mutation in a bulge region of a nucleic acid-targeting nucleic acid; a composition comprising: an engineered nucleic acid-targeting nucleic acid comprising a 3' hybridizing extension, and a donor polynucleotide, wherein said donor polynucleotide is hybridized to said 3' hybridizing extension; a composition comprising: an effector protein,

CLAIMS

WHAT IS CLAIMED IS:

1. A method for detecting if two complexes are in proximity to one another comprising:
 - (a) contacting a first target nucleic acid with a first complex, wherein said first complex comprises a first site-directed polypeptide, a first modified nucleic acid-targeting nucleic acid, and a first effector protein, wherein said effector protein is adapted to bind to said modified nucleic acid-targeting nucleic acid, and wherein said first effector protein comprises a non-native sequence that comprises a first portion of a split system; and
 - (b) contacting a second target nucleic acid with a second complex, wherein said second complex comprises a second site-directed polypeptide, a second modified nucleic acid-targeting nucleic acid, and a second effector protein, wherein said effector protein is adapted to bind to said modified nucleic acid-targeting nucleic acid, and wherein said second effector protein comprises a non-native sequence that comprises a second portion of a split system.
2. The method of claim 1, wherein said split system comprises two or more protein fragments that individually are not active, but, when formed into a complex, result in an active protein complex.
3. The method of claim 1, further comprising detecting an interaction between said first portion and said second portion.
4. The method of claim 3, wherein said detecting indicates said first and second complex are in proximity to one another.
5. The method of claim 3, wherein said detecting comprises determining the occurrence of a genetic mobility event.
6. The method of claim 5, wherein said genetic mobility event comprises a translocation.
7. The method of claim 5, wherein prior to said genetic mobility event said two portions of said split system do not interact.
8. The method of claim 5, wherein after said genetic mobility event said two portions of said split system do interact.
9. The method of claim 5, wherein said genetic mobility event is a translocation between a BCR and an Abl gene.

10. The method of claim 1, wherein said split system is selected from the group consisting of: split GFP system, a split ubiquitin system, a split transcription factor system, and a split affinity tag system, or any combination thereof.
11. The method of claim 3, wherein said detecting indicates a genotype.
12. The method of claim 11, further comprising: determining a course of treatment for a disease based on said genotype.
13. The method of claim 12, further comprising, treating said disease.
14. The method of claim 13, wherein said treating comprises administering a drug.
15. The method of claim 13, wherein said treating comprises administering a complex comprising a nucleic acid-targeting nucleic acid and a site-directed polypeptide, wherein said complex can modify a genetic element involved in said disease.
16. An engineered nucleic acid-targeting nucleic acid comprising: a mutation in a P-domain of said nucleic acid-targeting nucleic acid.
17. An engineered nucleic acid-targeting nucleic acid comprising: a mutation in a bulge region of a nucleic acid-targeting nucleic acid.
18. A composition comprising: a multiplexed genetic targeting agent, wherein said multiplexed genetic targeting agent comprises one or more nucleic acid modules, wherein said nucleic acid module comprises a non-native sequence, and wherein said nucleic acid module is configured to bind to a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9 and wherein said nucleic acid module is configured to hybridize to a target nucleic acid.
19. A composition comprising: a modified site-directed polypeptide, wherein said polypeptide is modified such that it is adapted to target a second protospacer adjacent motif compared to a wild-type site-directed polypeptide.
20. A method for producing a donor polynucleotide-tagged cell comprising:
 - (a) cleaving a target nucleic acid in a cell using a complex comprising a site-directed polypeptide and a nucleic acid-targeting nucleic acid;
 - (b) inserting a donor polynucleotide into a cleaved target nucleic acid;
 - (c) propagating said cell carrying said donor polynucleotide; and
 - (d) determining an origin of said donor-polynucleotide tagged cell.
21. A composition comprising:
 - (a) an effector protein; and
 - (b) a nucleic acid, wherein said nucleic acid comprises:

- (i) at least 50% sequence identity to a crRNA over 6 contiguous nucleotides;
 - (ii) at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides; and
 - (iii) a non-native sequence,
- wherein said nucleic acid is adapted to bind to said effector protein.

22. The composition of claim 21, further comprising a polypeptide comprising at least 10% amino acid sequence identity to a nuclease domain of Cas9, wherein said nucleic acid binds to said polypeptide.

23. The composition of claim 21, wherein said polypeptide comprises at least 60% amino acid sequence identity in a nuclease domain to a nuclease domain of Cas9.

24. The composition of claim 21, wherein said polypeptide is Cas9.

25. The composition of claim 21, wherein said nucleic acid further comprises a linker sequence, wherein said linker sequence links said sequence comprising at least 50% sequence identity to a crRNA over 6 contiguous nucleotides and said sequence comprising at least 50% sequence identity to a tracrRNA over 6 contiguous nucleotides.

26. The composition of claim 21, wherein said non-native sequence is located at a position of said nucleic acid selected from the group consisting of: a 5' end and a 3' end, or any combination thereof.

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising:
a modified Cas9 protein, wherein the modified Cas9 protein comprises a modification of a region of a wild-type Cas9 protein selected from the group consisting of a Cas9 bridge helix region; a Cas9 highly basic patch within the Cas9 N-terminal region; a Cas9 HNH domain region; a Cas9 RuvC domain region; a Cas9 P-domain recognition region; an added enzymatic activity; insertion, deletion or substitution of a domain; insertion, deletion or substitution of a structural motif; a fusion; and combinations thereof.
2. The composition of claim 1, wherein the wild-type Cas9 protein is a *Streptococcus pyogenes* Cas9 protein.
3. The compositions of claims 1 or 2, wherein the modification is in the region of the wild-type Cas9 protein corresponding to amino acids 1-270 of a *Streptococcus pyogenes* Cas9.
4. The compositions of claim 3, wherein the modification is in the region corresponding to amino acids 50-100 of a *Streptococcus pyogenes* Cas9.
5. The compositions of claim 4, wherein the modification is in the region corresponding to amino acids 65-95 of a *Streptococcus pyogenes* Cas9.
6. The compositions of claim 3, wherein the modification is in the region corresponding to amino acids 22-49 of a *Streptococcus pyogenes* Cas9.
7. The compositions of claims 1 or 2, wherein the modification is in the region corresponding to amino acids 551-556 of a *Streptococcus pyogenes* Cas9.
8. The compositions of claims 1 or 2, wherein the modification is in the region corresponding to amino acids 445-507 of a *Streptococcus pyogenes* Cas9.
9. The compositions of claims 1 or 2, wherein the modification is in the region corresponding to amino acids 860-1100 of a *Streptococcus pyogenes* Cas9.
10. The compositions of claims 1 or 2, wherein the modification is in the region corresponding to amino acids 1096-1225 of a *Streptococcus pyogenes* Cas9.
11. The compositions of claim 10, wherein the modification is in the region corresponding to amino acids 1105-1138 of a *Streptococcus pyogenes* Cas9.
12. The compositions of claims 1 or 2, wherein the modification is in the region corresponding to amino acids 718-757 of a *Streptococcus pyogenes* Cas9.

13. The compositions of claims 1 or 2, wherein the modification is an inserted nuclease domain or an inserted RNA binding domain.

14. The compositions of claim 13, wherein the inserted RNA binding domain is Csy4.

15. A vector comprising a nucleic acid sequence encoding the modified Cas9 protein of any one of claims 1 to 14.

16. An engineered nucleic acid targeting nucleic acid comprising: an engineered CRISPR RNA (crRNA), an engineered trans-activating CRISPR RNA (tracrRNA), or a combination thereof, wherein (i) the engineered nucleic acid targeting nucleic acid can associate with a Cas9 protein to form a nucleic acid targeting nucleic acid-Cas9 complex capable of binding a target DNA, and (ii) the engineered crRNA, the engineered tracrRNA, or the combination thereof is altered relative to a corresponding wild-type crRNA, tracrRNA, or both; and

the engineered nucleic acid targeting nucleic acid comprises at least one of the following: a tracr 3' hybridizing extension sequence, a spacer extension sequence, a tracr extension sequence, a modified bulge region, a modified P-domain, a Csy4 binding sequence to which a Csy4 protein binds with a $K_d \sim 50\text{pM}$, a Cas5 binding sequence to which a Cas5 protein binds, a Cas6 binding sequence to which a Cas6 protein binds, a nexus/hairpin variant, a modified stem loop structure comprising a functional moiety, or combinations thereof.

17. The engineered nucleic acid targeting nucleic acid of claim 16, wherein the engineered nucleic acid targeting nucleic acid is a double guide nucleic acid structure.

18. The engineered nucleic acid targeting nucleic acid of claim 16, wherein the engineered nucleic acid targeting nucleic acid is a single guide nucleic acid structure.

19. The engineered nucleic acid targeting nucleic acid of claim 16, wherein the nexus/hairpin variant comprises a modified nexus stem length or a modified nexus loop size relative to the corresponding wild-type tracrRNA.

20. The engineered nucleic acid targeting nucleic acid of claim 16, wherein the P-domain starts 2, 3, 4, or 5 nucleotides downstream of the last paired nucleotide of the duplex formed between the crRNA and the tracrRNA, and the P-domain comprises 2, 3, 4, or 5 adjacent nucleotides.

21. The engineered nucleic acid targeting nucleic acid of claim 20, wherein the modified P-domain comprises one or more mutation to allow the engineered nucleic acid-targeting nucleic acid to hybridize to a different protospacer adjacent motif relative to the corresponding wild-type tracrRNA.

22. The engineered nucleic acid targeting nucleic acid of claim 16, wherein the Csy4 binding sequence is one of the Csy4-tags selected from the group consisting of

AGTAATAATACGACTCACTATAGGGGGCCACTAGGGACAGGATGTTTT
AGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTA
SGR-v42 TACTGCCGTATAGGCAGAGATTTTTT
AGTAATAATACGACTCACTATAGGGGGCCACTAGGGACAGGATGTTTT
AGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTA
TACTGCCGTATAGGCAGAGAAATGGACTCGGAATACTGCCGTATAGGC
SGR-v43 AGAGATTTTTT
AGTAATAATACGACTCACTATAGGGGGCCACTAGGGACAGGATGTTTT
AGAGCTATCACTGCCGTATAGGCAGTGATAGCAAGTTAAAATAAGGCT
SGR-v44 AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT
AGTAATAATACGACTCACTATAGGGGGCCACTAGGGACAGGATGTTTT
AGAGCTATCACTGCCGTATAGGCAGTGATAGCAAGTTAAAATAAGGCT
AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTAATGGAC
SGR-v45 TCGATACTGCCGTATAGGCAGAGATTTTTT

23. The engineered nucleic acid targeting nucleic acid of claim 16, wherein the tracer extension sequence further comprises a functional moiety.

24. The engineered nucleic acid targeting nucleic acid of claim 16, wherein the spacer extension sequence further comprises a functional moiety.

25. The engineered nucleic acid targeting nucleic acid of claim 16, wherein the engineered nucleic acid targeting nucleic acid comprises DNA.

26. A vector comprising a nucleic acid sequence encoding the engineered nucleic acid targeting nucleic acid of any one of claims 16 to 24.

27. A composition comprising a modified Cas9 protein composition of any one of claims 1 to 14 and an engineered nucleic acid targeting nucleic acid of any one of claims 16 to 25.

28. A method for detecting if two complexes are in proximity to one another comprising:

contacting a first target nucleic acid with a first complex, wherein the first complex comprises a first site-directed polypeptide, a first modified nucleic acid-targeting

nucleic acid comprising a first nucleic acid sequence complementary to the first target nucleic acid, and a first effector protein, wherein the first effector protein is adapted to bind to the first modified nucleic acid-targeting nucleic acid, and wherein the first effector protein comprises a first non-native sequence that comprises a first portion of a split system;

contacting a second target nucleic acid with a second complex, wherein the second complex comprises a second site-directed polypeptide, a second modified nucleic acid-targeting nucleic acid comprising a second nucleic acid sequence complementary to the second target nucleic acid, and a second effector protein, wherein the second effector protein is adapted to bind to the second modified nucleic acid-targeting nucleic acid, and wherein the second effector protein comprises a second non-native sequence that comprises a second portion of a split system; and

detecting an interaction between the first portion and the second portion wherein the detecting indicates the first complex and the second complex are in proximity to one another.

29. The method of claim 28, wherein the split system comprises two or more protein fragments wherein individually the protein fragments are not active and a complex formed between the protein fragments is active.

30. The method of claim 28, wherein the detecting comprises determining the occurrence of a genetic mobility event.

31. A method for identifying off-target binding sites relative to a target DNA sequence, comprising:

contacting a mixture of DNA fragments comprising the target DNA sequence with an enzymatically inactive Cas9 protein in a complex with a nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein (i) the Cas9 protein comprises an affinity tag, the nucleic acid targeting nucleic acid comprises an affinity tag, or a combination thereof, and (ii) the Cas9 in the complex with the nucleic acid targeting nucleic acid binds to the target DNA sequence;

isolating the Cas9 protein in the complex with the nucleic acid targeting nucleic acid and bound DNA fragments using a capture agent that binds to the affinity tag;

purifying the DNA fragments that were bound by the Cas9 in the complex with the nucleic acid targeting nucleic acid;

sequencing the DNA fragments that were bound by the Cas9 in the complex with the nucleic acid targeting nucleic acid; and

identifying the off-target binding sites from the sequences of the DNA fragments that were bound by the Cas9 in the complex with the nucleic acid targeting nucleic acid that did not contain the target DNA binding sequence.

32. A method for generating sticky ends or blunt cuts in a double-stranded DNA comprising a target site having a first strand and a second strand, the method comprising:

contacting the double-stranded DNA with a Cas9 nickase in a complex with a first nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein the Cas9 nickase in the complex with the first nucleic acid targeting nucleic acid cuts the first strand of the target site and creates a first strand cut site; and

contacting the double-stranded DNA with a Cas9 nickase in a complex with a second nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein the Cas9 nickase in the complex with the second nucleic acid targeting nucleic acid cuts the second strand of the target site and creates a second strand cut site;

wherein either (i) the first strand cut site and the second strand cut site are at the same location in the target site to generate a blunt cut; or (ii) the first strand cut site and the second strand cut site are at different locations in the target site to generate sticky ends.

33. A method for enrichment of a target nucleic acid, comprising:

contacting a mixture of nucleic acids comprising the target nucleic acid with an enzymatically inactive Cas9 protein in a complex with a nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein (i) the Cas9 protein, the nucleic acid targeting nucleic acid, or combinations thereof further comprises an affinity tag, and (ii) the Cas9 in the complex with the nucleic acid targeting nucleic acid binds to the target nucleic acid; and

purifying the target nucleic acid bound to the Cas9 protein in the complex with the nucleic acid targeting nucleic acid using a capture agent that binds to the affinity tag to provide enrichment of the target nucleic acid.

34. A multiplexed genetic targeting agent, comprising:

multiple nucleic acid targeting nucleic acids, each nucleic acid targeting nucleic acid comprising a single guide RNA (sgRNA), the sgRNA comprising a crRNA and a tracrRNA, wherein the sgRNA can associate with a Cas9 protein to form a sgRNA-Cas9 complex; and

multiple non-native sequences, each non-native sequence comprising a moiety, an endoribonuclease binding sequence, or a ribozyme;

wherein a nucleic acid module comprises a nucleic acid targeting nucleic acid adjacent a non-native sequence, and multiple nucleic acid modules are arranged in tandem.

35. The multiplexed genetic targeting agent of claim 34, wherein each non-native sequence comprises an endonuclease binding sequence and the endonuclease binding sequence is selected from the group consisting of a Csy4 protein binding site, a Cas5 protein binding site, and a Cas6 protein binding site.

36. A stoichiometric delivery complex, comprising:

an engineered tandem fusion polypeptide comprising multiple nucleic acid binding proteins, wherein each nucleic acid binding protein can bind a nucleic acid comprising a cognate nucleic acid-binding protein binding site that can be bound by the nucleic acid-binding protein.

37. The stoichiometric delivery complex of claim 36, wherein each nucleic acid binding protein is an RNA binding protein or a DNA binding protein.

38. The stoichiometric delivery complex of claim 36, wherein a nucleic acid binding protein is bound to a nucleic acid comprising a cognate nucleic acid-binding protein binding site.

39. A method of modifying genomic DNA of a cell wherein the genomic DNA comprises a target nucleic acid, the method comprising:

introducing a donor polynucleotide into the target nucleic acid of the genomic DNA, wherein (i) a Cas9 protein in a complex with a first nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA cleaves the target nucleic acid, (ii) the donor polynucleotide is integrated into the cleaved target nucleic acid, and (iii) the donor polynucleotide comprises a genetic element of interest and a reporter element, wherein the reporter element is flanked by a 5' target site and a 3' target site;

selecting cells expressing the reporter element; and

excising the reporter element, wherein (i) a Cas9 protein in a complex with a second nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA cleaves the 5' target site to create a first end, and a Cas9 protein in a complex with a third nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA cleaves the 3' target site to create a second end, and (ii) the first end and the second end are rejoined.

40. A method for tracking cell lineage development in a population of cells, comprising:

tagging genomic DNA, comprising a target site, in cells of the population of cells by contacting genomic DNA of the cells with a Cas9 protein in a complex with a nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA that cuts the target site to create a first end and a second end, wherein a cellular repair mechanism rejoins the first end and the second end and generates a number of signature sequences in a number of the cells;

propagating the population of cells comprising the signature sequences;

analyzing the cells of the population to identify cells associated with each signature sequence; and

using the signature sequences to determine cell lineage of cells in the population of cells.

41. The method of claim 40 for tracking cell lineage development in a population of cells, the method further comprising contacting the genomic DNA with (i) the Cas9 protein in the complex with the nucleic acid targeting nucleic acid, and (ii) a donor polynucleotide.

42. A method for quantifying genome-editing events of genomic DNA in a population of cells wherein the genomic DNA comprises a target nucleic acid, the method comprising:

contacting the genomic DNA of cells in the population with a Cas9 protein in a complex with a nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA wherein (i) the Cas9 protein in the complex with the nucleic acid targeting nucleic acid can cut the target site to create a first end and a second end, (ii) when the Cas9 protein in the complex with the nucleic acid targeting nucleic acid cuts the target site, a cellular repair mechanism rejoins the first end and the second end destroying the target site, and (iii) the contacting produces a number of cells comprising genomic DNA having the target site and a number of cells comprising genomic DNA wherein the target site was destroyed;

isolating the genomic DNA from the population of cells;

performing polymerase chain reaction (PCR) with the isolated genomic DNA using primers that flank the target site to produce an amplified PCR product;

contacting the amplified PCR product with the Cas9 protein in the complex with the nucleic acid targeting nucleic acid; and

quantifying genome-editing events by comparing the amount of amplified PCR product derived from genomic DNA that comprises the target site in the amplified PCR product to the amount of amplified PCR product derived from genomic DNA wherein the target site was destroyed.

43. The method of claim 42 for quantifying genome-editing events of genomic DNA, wherein the quantifying is carried out using gel electrophoresis.

44. A composition comprising:

an engineered nucleic acid targeting nucleic acid comprising a crRNA and a tracrRNA, wherein (i) the engineered nucleic acid targeting nucleic acid can associate with a Cas9 protein to form a crRNA-tracrRNA-Cas9 complex, (ii) the tracrRNA comprises a 3' end, (iii) the tracrRNA further comprises a hybridizing sequence at the 3' end, and (iv) the hybridizing sequence comprises a nucleic acid sequence that can hybridize to a donor polynucleotide.

45. The composition of claim 44, further comprising the Cas9 protein.

46. A method for delivering a donor polynucleotide to a double-stranded break at a target site, comprising:

contacting a DNA sequence comprising the target site with an engineered nucleic acid targeting nucleic acid comprising a crRNA and tracrRNA wherein (i) the engineered nucleic acid targeting nucleic acid is associated in a complex with a Cas9 protein to form a crRNA-tracrRNA-Cas9 complex, (ii) the tracrRNA comprises a 3' end, (iii) the tracrRNA further comprises a hybridizing sequence at the 3' end, (iv) the hybridizing sequence comprises a nucleic acid sequence that hybridizes to the donor polynucleotide, and (v) the crRNA-tracrRNA-Cas9 complex binds to the DNA sequence comprising the target site and cuts the target site to generate a double-strand break and the binding of the crRNA-tracrRNA-Cas9 complex delivers the donor polynucleotide to the double-stranded break at the target site.