

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'-NNAAAAW-3', 5'-NNNNGATT-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 another aspect, there is provided an engineered single-guide nucleic acid-targeting nucleic acid capable of forming a complex with a Type II CRISPR Cas9 protein, the engineered single-guide nucleic acid-targeting nucleic acid comprising: a spacer sequence; a duplex comprising: a region of hybridization between a minimum CRISPR repeat sequence and a minimum tracr sequence, wherein the region of hybridization forms a first duplex and the spacer sequence is 5' of the first duplex, and a bulge region that is not followed by a first stem, wherein the bulge region comprises a region of unpaired nucleotides; a single guide connector sequence connecting the ends of the first duplex to form a hairpin structure and a 3' tracr sequence.

[0004b] In another aspect, there is provided a polynucleotide encoding the engineered single-guide nucleic acid-targeting nucleic acid as defined herein.

[004c] In another aspect, there is provided a composition comprising: the engineered single-guide nucleic acid-targeting nucleic acid as defined herein; and a Cas9 protein.

[0005] 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] 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.

[0020] 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 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

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 binding a target nucleic acid, comprising: contacting a nucleic acid comprising the target nucleic acid with the composition as defined herein, thereby facilitating binding of the complex to the target nucleic acid, wherein binding of the complex to the target nucleic acid optionally results in cleavage of the target nucleic acid.

[0021] 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 another aspect, there is provided a kit comprising: the engineered single-guide nucleic acid-targeting nucleic acid as defined herein or a polynucleotide encoding the engineered single-guide nucleic-acid targeting nucleic acid; and a buffer. In some embodiments, the kit further comprises instructions for use.

[0022] 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] 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] 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] 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.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An engineered single-guide nucleic acid-targeting nucleic acid capable of forming a complex with a Type II CRISPR Cas9 protein, the engineered single-guide nucleic acid-targeting nucleic acid comprising:
 - a spacer sequence, comprising a sequence that can hybridize to the target nucleic acid sequence;
 - a duplex comprising:
 - a region of hybridization between a minimum CRISPR repeat sequence and a minimum tracr sequence, wherein the region of hybridization forms a first duplex and the spacer sequence is 5' of the first duplex, and
 - a bulge region that is not followed by a first stem, wherein the bulge region comprises a region of unpaired nucleotides;
 - a single guide connector sequence connecting the ends of the first duplex to form a hairpin structure; and
 - a 3' tracr sequence.
2. The engineered single-guide nucleic acid-targeting nucleic acid of claim 1, wherein the region of hybridization between the minimum CRISPR repeat sequence and the minimum tracr sequence comprises no more than 2 mismatches.
3. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, the minimum CRISPR repeat sequence has a length of between 6 and 15 nucleotides.
4. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the minimum CRISPR repeat sequence has a length of 12 nucleotides.
5. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the minimum tracr sequence has a length of 14 nucleotides.
6. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the bulge region comprises an unpaired purine on the

minimum CRISPR repeat sequence side of the bulge region.

7. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the bulge region comprises, on one side of the duplex, an unpaired 5'-XXX_Y-3' wherein X is any purine and Y is a nucleotide that can form a wobble pair with a nucleotide on the opposite strand.
8. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the bulge sequence comprises one unpaired nucleotide.
9. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the bulge sequence comprises four unpaired nucleotides.
10. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the single guide connector sequence has a length of from 4 to 40 nucleotides.
11. The engineered single-guide nucleic acid-targeting nucleic acid of any one of claims 1 to 9, wherein the single guide connector sequence has a length of from 3 to about 10 nucleotides.
12. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the engineered single-guide nucleic acid-targeting nucleic acid comprises DNA.
13. The engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims, wherein the engineered single-guide nucleic acid-targeting nucleic acid comprises RNA.
14. A polynucleotide encoding the engineered single-guide nucleic acid-targeting nucleic acid of any one of the preceding claims.
15. The polynucleotide of claim 14, further comprising a promoter operably linked to the polynucleotide.

16. A composition comprising:
 - the engineered single-guide nucleic acid-targeting nucleic acid of any one of claims 1 to 13; and
 - a Cas9 protein.
17. The composition of claim 16, wherein the engineered single-guide nucleic acid-targeting nucleic acid and the Cas9 protein form a complex.
18. The composition of claim 16 or 17, wherein the Cas9 protein is a *Streptococcus pyogenes* Cas9 protein.
19. A method of binding a target nucleic acid, comprising:
 - contacting a nucleic acid comprising the target nucleic acid with the composition of claim 17, thereby facilitating binding of the complex to the target nucleic acid, wherein binding of the complex to the target nucleic acid optionally results in cleavage of the target nucleic acid.
20. A kit comprising:
 - the engineered single-guide nucleic acid-targeting nucleic acid of any one of claims 1 to 13 or a polynucleotide encoding the engineered single-guide nucleic-acid targeting nucleic acid; and
 - a buffer.
21. The kit of claim 20, further comprising a Cas9 protein or a polynucleotide encoding the Cas9 protein.