## CLAIMS

1. A system for modifying a chromosomal sequence in a eukaryotic cell, the system comprising: (a) an engineered *Bacillus smithii* Cas9 protein; and (b) an engineered guide RNA, wherein the engineered guide RNA is designed to complex with the engineered Cas9 protein and the engineered guide RNA comprises a 5’ guide sequence designed to hybridize with a target sequence in a double-stranded sequence, wherein the target sequence is 5’ to a protospacer adjacent motif (PAM) and the PAM comprises the sequence 5'-NNNNCAAA-3', wherein N is A, C, G, or T.

2. The system of claim 1, wherein the engineered Cas9 protein comprises at least one modification relative to its wild-type counterpart.

3. The system of claim 2, wherein the at least one modification comprises:

   (a) the addition of at least one heterologous domain; or

   (b) a substitution of one or more amino acids, an insertion of one or more amino acids, a deletion of one or more amino acids, or combination thereof, wherein the at least one modification is within a RuvC domain, HNH domain, REC domain, PAM interacting domain, or combination thereof.

4. The system of claim 3, wherein the at least one heterologous domain is a nuclear localization signal, a cell-penetrating domain, a marker domain, a chromatin modulating motif, an epigenetic modification domain, a transcriptional regulation domain, an RNA aptamer binding domain, or combination thereof.

5. The system of any one of claims 1 to 4, wherein the engineered Cas9 protein is a nuclease and cleaves both strands of a double-stranded sequence, is a nickase and cleaves one strand of a double-stranded sequence, or has no nuclease or nickase activity.

6. The system of any one of claims 1 to 5, wherein the engineered guide RNA is a single molecule.

7. The system of any one of claims 1 to 6, wherein the engineered guide RNA sequence is optimized to facilitate base-paring within the engineered guide RNA, minimize base-paring within the engineered guide RNA, increase stability of the engineered guide RNA, facilitate transcription of the engineered guide RNA in a eukaryotic cell, or a combination thereof.

8. The system of any one of claims 1 to 7, wherein the engineered guide RNA comprises the nucleotide sequence of SEQ ID NO:31.

9. The system of any one of claims 1 to 8, wherein the engineered Cas9 protein has an amino acid sequence having at least about 90% sequence identity to SEQ ID NO:2, 119, or 120.

10. The system of any one of claims 1 to 9, wherein the engineered Cas9 protein has an amino acid sequence as set forth in SEQ ID NO:2, 119, or 120.

11. A plurality of nucleic acids encoding the system of any one of claims 1 to 10, the plurality of nucleic acid comprising at least one nucleic acid encoding the engineered Cas9 protein, and at least one nucleic acid encoding the engineered guide RNA.

12. The plurality of nucleic acids of claim 11, wherein the at least one nucleic acid encoding the engineered Cas9 protein is RNA.

13. The plurality of nucleic acids of claim 11, wherein the at least one nucleic acid encoding the engineered Cas9 protein is DNA.

14. The plurality of nucleic acids of any one of claims 11 to 13, wherein the at least one nucleic acid encoding the engineered Cas9 protein is codon optimized for expression in a eukaryotic cell.

15. The plurality of nucleic acids of claim 14, wherein the eukaryotic cell is a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism.

16. The plurality of nucleic acids of claim 11, wherein the at least one nucleic acid encoding the engineered guide RNA is DNA.

17. The plurality of nucleic acids of any one of claims 11 to 16, wherein the at least one nucleic acid encoding the engineered Cas9 protein is operably linked to a phage promoter sequence for *in vitro* RNA synthesis or protein expression in a bacterial cell, and the at least one nucleic acid encoding the engineered guide RNA is operably linked to a phage promoter sequence for *in vitro* RNA synthesis.

18. The plurality of nucleic acids of any one of claims 11 to 16, wherein the at least one nucleic acid encoding the engineered Cas9 protein is operably linked to a eukaryotic promoter sequence for expression in a eukaryotic cell, and the at least one nucleic acid encoding the engineered guide RNA is operably linked to a eukaryotic promoter sequence for expression in a eukaryotic cell.

19. At least one vector comprising the plurality of nucleic acids of any one of claims 11 to 18, optionally wherein the vector is selected from: a plasmid vector, a viral vector, or a self-replicating viral RNA replicon.

20. A eukaryotic cell comprising at least one system comprising an engineered Cas9 protein and an engineered guide RNA as defined in claims 1 to 10, at least one nucleic acid as defined in claims 11 to 18, or at least one vector as defined in claim 19, optionally wherein the eukaryotic cell is selected from: a human cell, a non-human mammalian cell, a plant cell, a non-mammalian vertebrate cell, an invertebrate cell, or a single cell eukaryotic organism, optionally wherein the eukaryotic cell is *in vivo*, *ex vivo*, or *in vitro*.