CLAIMS

1. A fusion protein comprising a Cas9 protein and at least one marker protein, wherein the at least one marker protein is linked to the Cas9 protein directly or indirectly via a first linker having at least 90% sequence identity with SEQ ID NO:35 or 36.

2. The fusion protein of claim 1, wherein the at least one marker protein is linked to the Cas9 protein at its N-terminus, C-terminus, an internal location, or a combination thereof.

3. The fusion protein of claim 1 or 2, further comprising at least one nuclear localization signal.

4. The fusion protein of claim 3, further comprising one or more optional second linkers, wherein the nuclear localization signal, the marker protein, the first linker, the one or more optional second linker (if present), and the Cas9 protein are arranged in the following order (N-terminus to C-terminus):

   marker protein – first linker – nuclear localization signal – Cas9 protein;

   marker protein – nuclear localization signal – first linker – Cas9 protein;

   nuclear localization signal – first linker – marker protein – Cas9 protein;

   nuclear localization signal – marker protein – first linker – Cas9 protein;

   marker protein – first linker – nuclear localization signal – second linker – Cas9 protein; or

   nuclear localization signal – first linker – marker protein – second linker – Cas9 protein.

5. The fusion protein of claim 4, wherein the nuclear localization signal, the marker protein, the linker, and the Cas9 protein are arranged in the following order (N-terminus to C-terminus):

   marker protein – first linker – nuclear localization signal – Cas9 protein.

6. The fusion protein of any one of claims 1 to 5, further comprising at least one heterologous domain.

7. The fusion protein of claim 6, wherein the at least one heterologous domain is a cell-penetrating domain, a chromatin modulating motif, an epigenetic modification domain, a transcriptional regulation domain, an RNA aptamer binding domain, or combination thereof.

8. The fusion protein of any one of claims 1 to 7, wherein the fusion 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.

9. The fusion protein of any one of claims 1 to 8, wherein the Cas9 protein is from *Streptococcus pyogenes, Streptococcus thermophilus*, *Neisseria meningitidis*, *Staphylococcus aureus*, or *Campylobacter jejuni*.

10. The fusion protein of any one of claims 1 to 9, wherein the marker protein has an amino acid sequence having at least 90% sequence identity with SEQ ID NO:19 or 20.

11. The fusion protein of any one of claims 1 to 10, wherein the marker protein has an amino acid sequence set forth in SEQ ID NO:19 or 20.

12. The fusion protein of any one of claims 1 to 11, wherein the linker has an amino acid sequence set forth in SEQ ID NO:35 or 36.

13. The fusion protein of any one of claims 1 to 12, wherein the fusion protein has an amino acid sequence having at least 90% sequence identity with SEQ ID NO:48, 49, or 50.

14. The fusion protein of any one of claims 1 to 12, wherein the fusion protein has an amino acid sequence as set forth in SEQ ID NO:48, 49, or 50.

15. A system comprising the fusion protein of any one of claims 1 to 14 and an engineered guide RNA.

16. The system of claim 15, wherein the engineered guide RNA is a single molecule.

17. The system of any one of claims 15 or 16, 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.

18. A plurality of nucleic acids encoding the fusion protein of any one of claims 1 to 14.

19. A plurality of nucleic acids encoding the system of any one of claims 15 to 17, the plurality of nucleic acid comprising at least one nucleic acid encoding the fusion protein, and at least one nucleic acid encoding the engineered guide RNA.

20. The plurality of nucleic acids of claim 19, wherein the at least one nucleic acid encoding the fusion protein is RNA.

21. The plurality of nucleic acids of claim 19, wherein the at least one nucleic acid encoding the fusion protein is DNA.

22. The plurality of nucleic acids of any one of claims 19 to 21, wherein the at least one nucleic acid encoding the fusion protein is codon optimized for expression in a eukaryotic cell.

23. The plurality of nucleic acids of claim 22, 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.

24. The plurality of nucleic acids of any one of claims 19 to 23, wherein the at least one nucleic acid encoding the engineered guide RNA is DNA.

25. The plurality of nucleic acids of any one of claims 19 to 24, wherein the at least one nucleic acid encoding the fusion 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.

26. The plurality of nucleic acids of any one of claims 19 to 24, wherein the at least one nucleic acid encoding the fusion 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.

27. At least one vector comprising the plurality of nucleic acids of any one of claims 18 to 26.

28. The at least one vector of claim 27, which is a plasmid vector, a viral vector, or a self-replicating viral RNA replicon.

29. A eukaryotic cell comprising at least one system comprising a fusion protein as defined in claims 1 to 14, a system as defined in claims 15 to 17, a plurality of nucleic acids as defined in claims 18 to 26, or at least one vector as defined in claims 27 to 28.

30. The eukaryotic cell of claim 29, which is 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.

31. The eukaryotic cell of any one of claims 29 to 30, which is *ex vivo*, or *in vitro*.

32. A method for determining chromosome identity and location within a living eukaryotic cell or chemically fixed eukaryotic cell, the method

comprising introducing the fusion protein, system, plurality of nucleic acids, or vector of any one of claims 1 to 28 into the living or chemically fixed eukaryotic cell and detecting a signal from the marker protein.

33. The method of claim 32, wherein the eukaryotic cell is 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.

34. The method of any one of claims 32 to 33, wherein the eukaryotic cell is *ex vivo,* or *in vitro*.