CLAIMS

1. A fusion protein comprising at least one nuclear localization signal and a Cas9 protein linked to 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 a sequence set forth in SEQ ID NO:35 or 36, and an optional second linker,

   wherein the nuclear localization signal, the marker protein, the first linker, the optional second linker, if present, and the Cas9 protein are arranged in the following order from 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.

2. The fusion protein of claim 1, 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.

3. The fusion protein of claim 1 or 2, further comprising at least one heterologous domain.

4. The fusion protein of claim 3, 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.

5. The fusion protein of any one of claims 1 to 4, 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.

6. The fusion protein of any one of claims 1 to 5, wherein the Cas9 protein is from *Streptococcus pyogenes, Streptococcus thermophilus*, *Neisseria meningitidis*, *Staphylococcus aureus*, or *Campylobacter jejuni*.

7. The fusion protein of any one of claims 1 to 6, wherein the marker protein has an amino acid sequence having at least 90% sequence identity with SEQ ID NO:19 or 20.

8. The fusion protein of claim 7, wherein the fusion protein has an amino acid sequence having at least 95% sequence identity with SEQ ID NO:48, 49, or 50.

9. The fusion protein of claim 8, wherein the fusion protein has an amino acid sequence having at least 98% sequence identity with SEQ ID NO:48, 49, or 50.

10. The fusion protein of claim 9, wherein the fusion protein has an amino acid sequence having at least 99% sequence identity with SEQ ID NO:48, 49, or 50.

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 fusion protein has an amino acid sequence having at least 90% sequence identity with SEQ ID NO:48, 49, or 50.

13. The fusion protein of any one of claims 1 to 11, wherein the fusion protein has an amino acid sequence as set forth in SEQ ID NO:48, 49, or 50.

14. A system comprising the fusion protein of any one of claims 1 to 13 and an engineered guide RNA.

15. The system of claim 14, wherein the engineered guide RNA is a single molecule.

16. The system of any one of claims 14 or 15, 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.

17. A plurality of nucleic acids encoding the fusion protein of any one of claims 1 to 13.

18. A plurality of nucleic acids encoding the system of any one of claims 14 to 16, 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.

19. The plurality of nucleic acids of claim 18, wherein the at least one nucleic acid encoding the fusion protein is RNA.

20. The plurality of nucleic acids of claim 18, wherein the at least one nucleic acid encoding the fusion protein is DNA.

21. The plurality of nucleic acids of any one of claims 17 to 20, wherein the at least one nucleic acid encoding the fusion protein is codon optimized for expression in a eukaryotic cell.

22. The plurality of nucleic acids of claim 21, 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.

23. The plurality of nucleic acids of any one of claims 18 to 22, wherein the at least one nucleic acid encoding the engineered guide RNA is DNA.

24. The plurality of nucleic acids of any one of claims 18 to 23, 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.

25. The plurality of nucleic acids of any one of claims 18 to 23, 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.

26. At least one vector comprising the plurality of nucleic acids of any one of claims 17 to 25.

27. The at least one vector of claim 26, which is a plasmid vector, a viral vector, or a self-replicating viral RNA replicon.

28. A eukaryotic cell comprising at least one system comprising a fusion protein as defined in claims 1 to 13, a system as defined in claims 14 to 16, a plurality of nucleic acids as defined in claims 17 to 25, or at least one vector as defined in claims 26 to 27.

29. The eukaryotic cell of claim 28, 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.

30. The eukaryotic cell of any one of claims 28 to 29, which is *ex vivo*, or *in vitro*.

31. An in vitro 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 27 into the living or chemically fixed eukaryotic cell and detecting a signal from the marker protein.

32. The in vitro method of claim 31, 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.