CLAIMS

WHAT IS CLAIMED IS:

1. A method for increasing Homology Directed Repair (HDR)-mediated genome modification of a target editing site of a eukaryotic cell genome, comprising:

providing genome-editing molecules and HDR promoting agents to a eukaryotic cell, wherein the genome editing molecules comprise: (i) at least one sequence-specific endonuclease which cleaves a DNA sequence in the target editing site or at least one polynucleotide encoding the sequence-specific endonuclease; and (ii) a donor template DNA molecule having homology to the target editing site; and wherein the HDR promoting agents comprise a single-stranded DNA annealing protein (SSAP), an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and a single stranded DNA binding protein (SSB);

whereby the genome editing molecules and HDR promoting agents provide for modification of the target editing site of the eukaryotic cell genome with the donor template polynucleotide by HDR at a frequency that is increased in comparison to a control.

2. A method for making a eukaryotic cell having a genomic modification, comprising:

(a) providing genome editing molecules and Homology Directed Repair (HDR) promoting agents to a eukaryotic cell, wherein the genome editing molecules comprise: (i) at least one sequence-specific endonuclease which cleaves a DNA sequence in the target editing site or at least one polynucleotide encoding the sequence-specific endonuclease and a donor template DNA molecule having homology to the target editing site; and wherein the HDR promoting agents comprise a single-stranded DNA annealing protein (SSAP), an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and a single stranded DNA binding protein (SSB);whereby the genome editing molecules and HDR promoting agents provide for modification of the target editing site of the eukaryotic cell genome with the donor template polynucleotide by HDR at a frequency that is increased in comparison to a control; and

(b) isolating or propagating a eukaryotic cell comprising the genome modification, thereby making the eukaryotic cell having a genomic modification.

3. The method of claim 1 or 2, wherein the sequence-specific endonuclease comprises an RNA-guided nuclease and the target editing site comprises a PAM sequence and a sequence that is complementary to the guide RNA and located immediately adjacent to the PAM sequence.

4. The method of claim 1 or 2, wherein the sequence-specific endonuclease provides a 5' overhang at the target editing site following cleavage.

5. The method of claim 1 or 2, wherein the SSAP provides for DNA strand exchange and base pairing of complementary DNA strands of homologous DNA molecules.

6. The method of claim 1 or 2, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.

7. The method of claim 6, wherein the RecT/ Redβ- family protein comprises a bacterial prophage RecT protein, a bacteriophage λ beta protein, a bacteriophage SPP1 35 protein, or related protein with equivalent SSAP activity.

8. The method of claim 6, wherein the RecT/ Redβ- family protein comprises a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 1, 2, or 3.

9. The method of claim 6, wherein the ERF-family protein comprises a bacteriophage P22 ERF protein, a functionally related protein, or a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 4.

10. The method of claim 6, wherein the RAD52-family protein comprises a *Saccharomyces cerevisiae* Rad52 protein. a *Schizosaccharomyces pombe* Rad22 protein, *Kluyveromyces lactis* Rad52 protein, a functionally related protein, or a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 5, 6, or 7.

11. The method of claim 1 or 2, wherein the single stranded DNA binding protein (SSB) is a bacterial SSB or optionally an *Enterobacteriaceae* sp. SSB.

12. The method of claim 11, wherein the SSB is an *Escherichia* sp,, a *Shigella* sp., an *Enterobacter* sp., a *Klebsiella* sp., a *Serratia* sp., a *Pantoea* sp., or a *Yersinia* sp. SSB.

13. The method of claim 1 or 2, wherein the SSB comprises a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 31, 34-131, or 132.

14. The method of claim 1 or 2, wherein the frequency of HDR is increased by at least 2-fold in comparison to a control method wherein a control eukaryotic cell is provided with the genome editing molecules but is not exposed to at least one of said HDR promoting agents.

15. The method of claim 1 or 2, wherein the frequency of non-homologous end-joining (NHEJ) is maintained or decreased by at least 2-fold in comparison to a control method wherein a control eukaryotic cell is provided with the genome editing molecules but is not exposed to at least one of said HDR promoting agents.

16. The method of claim 1 or 2, wherein the SSAP, the exonuclease, and/or the single stranded DNA binding protein further comprise an operably linked nuclear localization signal (NLS) and/or a cell-penetrating peptide (CPP).

17. The method of claim 1 or 2, wherein the SSAP, the exonuclease, and/or the SSB are provided to the cell as polyproteins comprising protease recognition sites or self-processing protein sequences inserted between the SSAP, the exonuclease, and/or the SSB.

18. The method of any one of claims 1-17, where the eukaryotic cell is a mammalian cell or a plant cell.

19. The method of claim 18, wherein the plant cell is haploid, diploid, or polyploid.

20. The method of claim 19, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

21. The method of claim 18, wherein the SSAP, the exonuclease, and/or the SSB further comprise an operably linked nuclear localization signal (NLS) selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16.

22. The method of claim 18, further comprising the step of isolating and/or growing a plant cell, propagule, or plant obtained from the plant cell comprising the genome modification, wherein the genome of the plant cell, propagule, or plant comprises the genome modification.

23. The method of any one of claims 1-22, wherein the HDR promoting agents, genome-editing molecules and eukaryotic cell or eukaryotic cell comprising the genome modification, are provided in an array comprising a plurality of containers, compartments, or locations and wherein each container, compartment, or location includes the HDR promoting agents, genome-editing molecules and eukaryotic cell or eukaryotic cell comprising the genome modification.

24. A method of genetic engineering of a eukaryotic cell comprising providing to the eukaryotic cell: i) at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) a single-stranded DNA annealing protein (SSAP), iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) a single stranded DNA binding protein (SSB),
wherein the target editing site of the cell is modified by the donor template DNA molecule.

25. The method of claim 1, 2, or 24, wherein the sequence-specific endonuclease comprise an RNA-guided nuclease or a polynucleotide encoding an RNA-guided nuclease and a guide RNA or a polynucleotide encoding a guide RNA.

26. The method of claim 25, wherein the RNA-guided nuclease comprises an RNA-guided DNA endonuclease, a type II Cas nuclease, a Cas9 nuclease, a type V Cas nuclease, a Cas12a nuclease, a Cas12b nuclease, a Cas12c nuclease, a CasY nuclease, a CasX nuclease, Cas12i, Cas14, or an engineered nuclease.

27. The method of claim 1, 2, or 24, wherein the sequence-specific endonuclease comprises a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TAL-effector nuclease), Argonaute, a meganuclease, or engineered meganuclease.

28. The method of claim 1, 2, or 24, further comprising a guide RNA, wherein the sequence-specific endonucleases and guide RNAs cleave a single DNA strand at two distinct DNA sequences in the target editing site.

29. The method of claim 28, wherein the sequence-specific endonucleases comprise at least one Cas9 nickase, Cas12a nickase, a zinc finger nickase, a TALE nickase, or a combination thereof, wherein the sequence-specific endonuclease is specific for an endonuclease recognition sequence in the target editing site.

30. The method of claim 28, wherein the sequence-specific endonucleases comprise Cas9 and/or Cas12a and the guide RNA molecules have at least one base mismatch to DNA sequences in the target editing site.

31. The method of claim 1, 2, or 24, wherein the donor DNA molecule is provided in a plasmid or a geminivirus genome.

32. The method of claim 1, 2, or 24, wherein the donor DNA molecule is flanked by an endonuclease recognition sequence.

33. The method of claim 1, 2, or 24, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.

34. The method of claim 33, wherein the RecT/ Redβ- family protein comprises a Rac bacterial prophage RecT protein, a bacteriophage λ beta protein, a bacteriophage SPP1 35 protein, or related protein with equivalent SSAP activity.

35. The method of claim 1, 2, or 24, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.

36. The method of claim 1, 2, or 24, wherein a linear dsDNA molecule comprising a phosphorylated 5’ terminus is a preferred substrate of the exonuclease.

37. The method of claim 1, 2, or 24, wherein the exonuclease has 5’ to 3’ exonuclease activity and can recognize a blunt ended dsDNA substrate, a dsDNA substrate having an

internal break in one strand, a dsDNA substrate having a 5’ overhang, and/or a dsDNA substrate having a 3’ overhang.

38. The method of claim 1, 2, or 24, wherein the exonuclease has 3’ to 5’ exonuclease activity and can recognize a blunt ended dsDNA substrate, a dsDNA substrate having an internal break in one strand, a dsDNA substrate having a 5’ overhang, and/or a dsDNA substrate having a 3’ overhang.

39. The method of claim 1, 2, or 24, wherein the exonuclease comprises a bacteriophage lambda exo protein, an Rac prophage RecE exonuclease, an Artemis protein, an Apollo protein, a DNA2 exonuclease, an Exo1 exonuclease, a herpesvirus SOX protein, UL12 exonuclease, an enterobacterial exonuclease VIII, a T7 phage exonuclease, E. coli Exonuclease III, a mammalian Trex2 exonuclease, a related protein with equivalent exonuclease activity, or a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 8, 9, 136, 137, 138, 139, 140, 141, 142, 143, 144, or 145.

40. The method of claim 1, 2, or 24, wherein the exonuclease comprises a T7 phage exonuclease, E. coli Exonuclease III, a related protein with equivalent exonuclease activity, or a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 143 or 144.

41. The method of claim 1, 2, or 24, wherein the single stranded DNA binding protein (SSB) and the SSAP are obtained from the same host organism.

42. The method of any one of claims 1-41, where the eukaryotic cell is a mammalian cell or a plant cell.

43. The method of claim 42, wherein the plant cell is haploid, diploid, or polyploid.

44. The method of claim 43, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

45. The method of claim 44, further comprising the step of isolating and/or growing a plant cell, propagule, or plant obtained from the plant cell comprising the genome

modification, wherein the genome of the plant cell, propagule, or plant comprises the genome modification.

46. The method of any one of claims 1-45, wherein one or more of the i) at least one sequence-specific endonuclease, ii) the donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) the single-stranded DNA annealing protein (SSAP), iv) the exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) the single stranded DNA binding protein (SSB) are provided in one or more vectors.

47. The method of claim 46, wherein the vector is an agrobacterium vector.

48. The method of any one of claims 1-47, wherein one or more of the i) at least one sequence-specific endonuclease, ii) the donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) the single-stranded DNA annealing protein (SSAP), iv) the exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) the single stranded DNA binding protein (SSB) are provided by in a chromosome.

49. The method of any one of claims 1-48, wherein one or more of the i) at least one sequence-specific endonuclease, ii) the donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) the single-stranded DNA annealing protein (SSAP), iv) the exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) the single stranded DNA binding protein (SSB) are provided by introducing a polypeptide, a DNA, an mRNA, and/or sexual crossing.

50. The method of any one of claims 1-49, wherein one or more of the i) at least one sequence-specific endonuclease, ii) the donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) the single-stranded DNA annealing protein (SSAP), iv) the exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) the single stranded DNA binding protein (SSB) are provided by a progenitor cell comprising one or more of i) - v),
wherein the progenitor cell does not comprise at least one of i) - v),

wherein the at least one of i) – v) that is not comprised by the progenitor cell is subsequently provided by delivering a polypeptide, a DNA, or an mRNA to the progenitor cell and/or sexual crossing of the progenitor cell.

51. The method of any one of claims 1-50, further comprising detecting the modification.

52. The method of claim 51, wherein detecting the modification comprises amplicon sequencing.

53. The method of any one of claims 1-52, wherein the target editing site is in a protein coding sequence or a promoter.

54. The method of any one of claims 1-53, wherein the modification of the target editing site is an insertion, a deletion, or a substitution.

55. The method of any one of claims 1-54, wherein the target editing site is a gene encoding an agronomically important trait or a gene involved in a mammalian disease.

56. A composition comprising nucleic acids encoding one or more of i) at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) a single-stranded DNA annealing protein (SSAP), iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) a single stranded DNA binding protein (SSB).

57. The composition of claim 56, wherein the nucleic acids are in one or more vectors.

58. A vector comprising nucleic acids encoding one or more of i) at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) a single-stranded DNA annealing protein (SSAP), iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) a single stranded DNA binding protein (SSB).

59. The vector of claim 58, wherein the vector comprises the donor template DNA, the sequence specific endonuclease and a polynucleotide encoding a guide RNA.

60. The vector of claim 58, wherein the vector comprises the single-stranded DNA annealing protein (SSAP), the exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and the single stranded DNA binding protein (SSB).

61. The vector of claim 58, wherein the vector comprises nucleic acids encoding i) at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) a single-stranded DNA annealing protein (SSAP), iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) a single stranded DNA binding protein (SSB).

62. A kit comprising nucleic acids encoding i) at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) a single-stranded DNA annealing protein (SSAP), iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) a single stranded DNA binding protein (SSB) and instructions for use for genetically engineering a eukaryotic cell.

63. The kit of claim 62, wherein the kit comprises a first vector and a second vector, wherein i) the first vector comprises nucleic acids comprising the donor template DNA and the sequence specific endonuclease, wherein the sequence-specific endonuclease comprises a polynucleotide encoding an RNA-guided nuclease and a polynucleotide encoding a guide RNA; and
ii) the second vector comprises the single-stranded DNA annealing protein (SSAP), the exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and the single stranded DNA binding protein (SSB).

64. The kit of any one of claims 62-63, further comprising an agent for detecting genetically engineered cells.

65. A cell comprising i) at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) a single-stranded DNA annealing protein (SSAP), iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) a single stranded DNA binding protein (SSB).

66. A cell comprising nucleic acids encoding i) at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) a single-stranded DNA annealing protein (SSAP), iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) a single stranded DNA binding protein (SSB).

67. The cell of claim 65 or 66, wherein the cell is a plant or mammalian cell.

68. The cell of any one of claims 65-67, wherein the cell is a host cell.

69. A genetically engineered cell produced by the method of any one of claims 1-55.

70. The vector according to claim 58, wherein the sequence-specific endonuclease is operably linked to an inducible promoter.

71. The method of claim 1, 2, or 24, wherein the sequence-specific endonuclease is a nickase.