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. The method of claim 1, wherein the sequence-specific endonuclease comprises an RNA-guided nuclease or a polynucleotide encoding an RNA-guided nuclease and a guide RNA or a polynucleotide encoding a guide RNA.~~

~~3. The method of claim 2, 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, or an engineered nuclease.~~

~~4. The method of claim 1, 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.~~

~~5. The method of claim 1, wherein the genome editing molecules comprise one or more sequence-specific endonucleases or sequence-specific endonucleases and guide RNAs that cleave a single DNA strand at two distinct DNA sequences in the target editing site.~~

6. The method of claim 5, wherein the sequence-specific endonucleases comprise at least one Cas9 nickase , Cas12a nickase, Cas12i, a zinc finger nickase, a TALE nickase, or a combination thereof.

7. The method of claim 5, 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.

8. The method of claim 1, wherein the donor DNA molecule is provided on a circular DNA vector, geminivirus replicon, or as a linear DNA fragment.

9. The method of claim 1, wherein the donor DNA molecule is flanked by copies of an endonuclease recognition sequence.

10. The method of claim 1, 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 a protospacer adjacent motif (PAM) sequence.

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

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

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

14. The method of claim 13, wherein the RecT/ Redβ- family protein comprises a Rac bacterial prophage RecT protein, a bacteriophage λ beta protein, a bacteriophage SPP1 35 protein, a related protein with equivalent SSAP activity, or a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 1, 2, or 3.

15. The method of claim 13, 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.

16. The method of claim 13, 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.

17. The method of claim 1, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.

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

19. The method of claim 1, 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.

20. The method of claim 1, 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.

21. The method of claim 1, 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, Exonuclease III, a 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 .

22. The method of claim 5, 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.

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

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

25. The method of claim 1, 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.

26. The method of claim 1, 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.

27. The method of claim 1, 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.

28. The method of claim 1, 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.

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

30. The method of claim 1, 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.

31. The method of any one of claims 1 to 30, where the eukaryotic cell is a mammalian cell or a plant cell.

32. The method of claim 31, wherein the plant cell is haploid, diploid, or polyploid.

33. The method of claim 32, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

34. The method of claim 31, wherein the cell is a plant cell and the SSAP, the exonuclease, and/or the single stranded DNA binding protein 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.

35. The method of claim 31, 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.

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

(a) a eukaryotic cell;

(b) HDR promoting agents comprising 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); and

(c) genome editing molecule(s) comprising 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;

wherein the eukaryotic cell is associated with, contacts, and/or contains and effective amount of the HDR promoting agents and the genome editing molecule(s).

37. The system of claim 36, wherein the genome editing molecules and/or 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.

~~38. The system of claim 37, 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, or an engineered nuclease.~~

~~39. The system of claim 36, 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.~~

~~40. The system of claim 36, wherein the genome editing molecules comprise one or more sequence-specific endonucleases or sequence-specific endonucleases and guide RNAs that cleave a single DNA strand at two distinct DNA sequences in the target editing site.~~

~~41. The system of claim 40, wherein the sequence-specific endonucleases comprise at least one Cas9 nickase , Cas12a nickase, Cas12i, a zinc finger nickase, a TALE nickase, or a combination thereof.~~

~~42. The system of claim 40, 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.~~

~~43. The system of claim 36, wherein the donor DNA molecule is provided on a plasmid or a geminivirus genome.~~

~~44. The system of claim 36, wherein the donor DNA molecule is flanked by an endonuclease recognition sequence.~~

~~45. The system of claim 36, 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.~~

~~46. The system of claim 36, wherein the sequence-specific endonuclease provides a 5' overhang at the target editing site following cleavage.~~

~~47. The system of claim 36, 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 by at least 2-fold in comparison to a control.~~

~~48. The system of claim 36, wherein the SSAP provides for DNA strand exchange and base pairing of complementary DNA strands of homologous DNA molecules.~~

~~49. The system of claim 36, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.~~

~~50. The system of claim 49, 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.~~

~~51. The system of claim 49, wherein the RecT/ Redβ- family protein comprises a bacteriophage λ beta protein, a bacteriophage SPP1 35 protein, a Rac bacterial prophage RecT protein, or related protein with equivalent SSAP activity.~~

~~52. The system of claim 49 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.~~

~~53. The system of claim 49, 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.~~

~~54. The system of claim 49, 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.~~

~~55. The system of claim 36, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.~~

56. The system of claim 36, wherein a linear dsDNA molecule comprising a phosphorylated 5’ terminus is a preferred substrate of the exonuclease.

57. The system of claim 36, 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.

58. The system of claim 36, 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.

59. The system of claim 36, 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 .

60. The system of claim 40, 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.

61. The system of claim 36, wherein the single stranded DNA binding protein (SSB) and the SSAP are obtained from the same host organism.

62. The system of claim 36, wherein the single stranded DNA binding protein (SSB) is a bacterial SSB or optionally an *Enterobacteriaceae* sp. SSB.

63. The system of claim 62, 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.

64. The system of claim 36, 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.

65. The system of claim 36, wherein the frequency of HDR is increased by at least 2-fold in comparison to a control system 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.

66. The system of claim 36, wherein the frequency of non-homologous end-joining (NHEJ) is maintained or decreased by at least 2-fold in comparison to a control system 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.

67. The system of claim 36, 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).

68. The system of claim 36, 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.

69. The system of any one of claims 36 to 68, where the eukaryotic cell is a mammalian cell or a plant cell.

70. The system of claim 69, wherein the plant cell is haploid, diploid, or polyploid.

71. The system of claim 69, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

72. The system of claim 69, wherein the cell is a plant cell and the SSAP, the exonuclease, and/or the single stranded DNA binding protein 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.

~~73. The system of claim 69, wherein the system provides for isolating and/or growing a plant cell, propagule, or plant obtained from the plant cell comprising the genome modification, and wherein the genome of the plant cell, propagule, or plant comprises the genome modification.~~

~~74~~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.

~~75. The method of claim 74, wherein the genome editing molecules and/or 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.~~

~~76. The method of claim 75, 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, or an engineered nuclease~~

~~77. The method of claim 74, 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.~~

~~78. The method of claim 74, wherein the genome editing molecules comprise one or more sequence-specific endonucleases or sequence-specific endonucleases and guide RNAs that cleave a single DNA strand at two distinct DNA sequences in the target editing site.~~

~~79. The method of claim 78, wherein the sequence-specific endonucleases comprise at least one Cas9 nickase , Cas12a nickase, Cas12i, a zinc finger nickase, a TALE nickase, or a combination thereof.~~

~~80. The method of claim 78, 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.~~

~~81. The method of claim 74, wherein the donor DNA molecule is provided in a plasmid or a geminivirus genome.~~

~~82. The method of claim 74, wherein the donor DNA molecule is flanked by an endonuclease recognition sequence.~~

~~83~~. The method of claim 1 or ~~2~~74, 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.

~~84~~. The method of claim 1 or ~~2~~74, wherein the sequence-specific endonuclease provides a 5’ overhang at the target editing site following cleavage.

~~85~~. The method of claim 1 or ~~2~~74, wherein the SSAP provides for DNA strand exchange and base pairing of complementary DNA strands of homologous DNA molecules.

~~86~~. The method of claim ~~7~~1 or 2, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.

~~87~~. The method of claim ~~86~~, 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.

~~88~~8. The method of claim ~~86~~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.

~~89~~9. The method of claim 6~~86~~, 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.

~~90~~10. The method of claim 6~~86~~, 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.

~~91. The method of claim 74, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.~~

~~92. The method of claim 74, wherein a linear dsDNA molecule comprising a phosphorylated 5' terminus is a preferred substrate of the exonuclease.~~

~~93. The method of claim 74, 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.~~

~~94. The method of claim 74, 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.~~

~~95. The method of claim 74, 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.~~

~~96. The method of claim 78, 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.~~

~~97. The method of claim 74, wherein the single stranded DNA binding protein (SSB) and the SSAP are obtained from the same host organism.~~

~~98~~11. The method of claim 1 or 2~~74~~, wherein the single stranded DNA binding protein (SSB) is a bacterial SSB or optionally an *Enterobacteriaceae* sp. SSB.

~~99~~12. The method of claim 11~~98~~, 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.

~~100~~13. The method of claim 1 or 2~~74~~, 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.

~~101~~14. The method of claim 1 or 2~~74~~, 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.

~~102~~15. The method of claim 1 or 2~~74~~, 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.

~~103~~16. The method of claim 1 or 2~~74~~, 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).

~~104~~17. The method~~system~~ of claim 1 or 2~~74~~, 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.

~~105~~18. The method of any one of claims 1-17~~74 to 104~~, where the eukaryotic cell is a mammalian cell or a plant cell.

~~106~~19. The method of claim 18~~105~~, wherein the plant cell is haploid, diploid, or polyploid.

~~107~~20. The method of claim ~~105~~19, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

~~108~~21. The method of claim ~~105~~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.

~~109~~22. The method of claim ~~105~~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.

~~110~~23. The method of any one of claims 1-22~~30, the system of any one of claims 36 to 68, or the method of any one of claims 74-104~~, 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.

~~111~~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.

~~112~~25. The method of claim 1, 2, or 24~~111~~, 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.

~~113~~26. The method of claim ~~112~~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.

~~114~~27. The method of claim 1, 2, or 24~~111~~, 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.

~~115~~28. The method of claim 1, 2, or 24~~111~~, 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.

~~116~~29. The method of claim ~~115~~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.

~~117~~30. The method of claim ~~115~~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.

~~118~~31. The method of claim ~~111~~1, 2, or 24, wherein the donor DNA molecule is provided in a plasmid or a geminivirus genome.

~~119~~32. The method of claim 1, 2, or 24~~111~~, wherein the donor DNA molecule is flanked by an endonuclease recognition sequence.

~~120~~33. The method of claim 1, 2, or 24~~111~~, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.

~~121~~34. The method of claim ~~120~~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.

~~122~~35. The method of claim 1, 2, or 24~~111~~, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.

~~123~~36. The method of claim 1, 2, or 24~~111~~, wherein a linear dsDNA molecule comprising a phosphorylated 5’ terminus is a preferred substrate of the exonuclease.

~~124~~37. The method of claim 1, 2, or 24~~111~~, 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.

~~125~~38. The method of claim 1, 2, or 24~~111~~, 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.

~~126~~39. The method of claim 1, 2, or 24~~111~~, 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.

~~127~~40. The method of claim 1, 2, or 24~~111~~, 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.

~~128~~41. The method of claim 1, 2, or 24~~111~~, wherein the single stranded DNA binding protein (SSB) and the SSAP are obtained from the same host organism.

~~129~~42. The method of any one of claims 1-41~~111 to 128~~, where the eukaryotic cell is a mammalian cell or a plant cell.

~~130~~43. The method of claim 42~~129~~, wherein the plant cell is haploid, diploid, or polyploid.

~~131~~44. The method of claim 43~~130~~, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

~~132~~45. The method of claim 44~~131~~, 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.

~~133~~46. The method of any one of claims ~~111-132~~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.

~~135~~47. The method of claim ~~133~~46, wherein the vector is an agrobacterium vector.

~~136~~48. The method of any one of claims 1-47~~111-132~~, 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.

~~137~~49. The method of any one of claims 1-48~~111-132~~, 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.

~~138~~50. The method of any one of claims 1-49~~111-132~~, 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.

~~139~~51. The method of any one of claims 1-50~~111-138~~, further comprising detecting the modification.

~~140~~52. The method of claim ~~139~~51, wherein detecting the modification comprises amplicon sequencing.

~~141~~53. The method of any one of claims 1-52~~111-140~~, wherein the target editing site is in a protein coding sequence or a promoter.

~~142~~54. The method of any one of claims 1-53~~111-141~~, wherein the modification of the target editing site is an insertion, a deletion, or a substitution.

~~143~~55. The method of any one of claims 1-54~~111-142~~, wherein the target editing site is a gene encoding an agronomically important trait or a gene involved in a mammalian disease.

144. A method for producing a eukaryotic cell with a genetically modified target editing site comprising:

(a) providing at least one sequence-specific endonuclease which cleaves a DNA sequence at least one endonuclease recognition sequence in said target editing site or at least one polynucleotide encoding said at least one sequence-specific endonuclease, and

(b) providing at least one donor molecule comprising at least one double-stranded DNA sequence, wherein (i) said DNA sequence has a homology of at least 90% over a length of at least 50 nucleotides to sequences flanking the target editing site and (ii) wherein said donor sequence comprises at least one modification in comparison to said target editing site; and

(c) providing at least one Homology Directed Repair (HDR) promoting agent comprising

(i) at least one single-stranded DNA annealing protein (SSAP), and

(ii) at least one exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and

(iii) at least one single stranded DNA binding protein (SSB);

and whereby the at least one sequence-specific endonucleases, the at least one donor molecule, and the at least one HDR promoting agent introduce said modification into said target editing site of said eukaryotic cell; and

(d) isolating a eukaryotic cell comprising a modification in said target editing site.

145. The method of claim 144, wherein the modification in selected from the group consisting of an insertion of one or more nucleotides, a deletion of one or more nucleotides, or a substitution of one or more nucleotides.

146. The method of claim 144, wherein a portion of the target editing site is deleted by using two sequence specific cleavages in said target editing site, and is replaced by a sequence provide by the donor molecule.

147. The method any one of claims 144-146, wherein said donor sequence is in a vector flanked by endonuclease recognition sequences.

~~148. The method of any one of claims 144-147, further comprises propagating the eukaryotic cell comprising the modification.~~

~~149. A method of producing a genetically modified organism comprising the steps of~~

~~(i) producing a genetically modified eukaryotic cell by any of claim 144-148, and~~

~~(ii) regenerating said cell into an organism.~~

~~150. The organism of claim 149, wherein the organism is selected from the group consisting of plants and non-human animals.~~

~~151~~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).

~~152~~57. The composition of claim 56~~151~~, wherein the nucleic acids are in one or more vectors.

~~153~~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).

~~154~~59. The vector of claim ~~153~~58, wherein the vector comprises the donor template DNA, the sequence specific endonuclease and a polynucleotide encoding a guide RNA.

~~155~~60. The vector of claim 58~~153~~, 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).

~~156~~61. The vector of claim 58~~153~~, 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).

~~157~~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.

~~158~~63. The kit of claim 62~~157~~, 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).

~~159~~64. The kit of any one of claims 62-63~~157-158~~, further comprising an agent for detecting genetically engineered cells.

~~160~~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).

~~161~~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).

~~162~~67. The cell of claim 65~~160~~ or 66~~161~~, wherein the cell is a plant or mammalian cell.

~~163~~68. The cell of any one of claims 65-67~~160-162~~, wherein the cell is a host cell.

~~164~~69. A genetically engineered cell produced by the method of any one of claims 1-55~~1-35 or 74-149~~.

~~165. A progenitor eukaryotic cell or organism for genetic engineering at a target editing site, comprising a subset of i) at least one sequence-specific endonuclease, ii) a donor template 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 cell does not comprises at least one of i)- v), wherein providing the cell or organism with the at least one of i)- v) that is not comprised in the progenitor cell or organism results in modification of the target editing site by the donor template molecule.~~

~~166. The progenitor eukaryotic cell or organism of claim 165, wherein the donor template is a double-stranded DNA molecule.~~

~~167. The progenitor cell of claim 165, wherein the cell is a germline cell.~~

~~168. The progenitor eukaryotic cell or organism of claim 165, wherein the progenitor eukaryotic cell is a progenitor plant cell and the at least one of i) – v) that is not comprised by the progenitor plant cell or plant is supplied by transformation.~~

~~169. The progenitor organism of claim 165, wherein the organism is a plant and wherein the at least one of i) – v) that is not comprised by the progenitor plant is supplied by sexual~~

~~crossing to a second plant comprising the at least one of i) – v) that is not comprised by the progenitor plant.~~

~~170. The progenitor eukaryotic cell of claim 165, wherein the progenitor eukaryotic cell is an animal cell, and wherein at least one of i) – v) that is not comprised by the progenitor cell is supplied by transfection.~~

~~171. The progenitor organism of claim 165, wherein the progenitor organism is a non-human animal and the at least one of i) – v) that is not comprised by the non-human animal is supplied by sexual crossing to a non-human animal comprising the at least one of i) – v) that is not comprised by the non-human animal.~~

~~172~~70. The vector according to claim 58~~153~~, wherein the sequence-specific endonuclease is operably linked to an inducible promoter.

~~173~~71. The method of claim 1, 2, or 24~~111~~, wherein the sequence-specific endonuclease is a nickase.