IMPROVED HOMOLOGY DEPENDENT REPAIR GENOME EDITING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/866,317, filed on June 25, 2019, the content of which is hereby incorporated by reference in its entirety for all purposes.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 165362000640SEQLIST.TXT, date recorded: June 24, 2020, size: 284 KB).

FIELD OF THE INVENTION

[0003] The present application is related to methods, kits, and compositions for gene editing.

BACKGROUND

[0004] Homology-Directed Repair (HDR) is a genome editing method that can be used for precise replacement of a target genomic DNA site with the sequence from a provided DNA template containing the desired replacement sequence. While the results of HDR are quite desirable, it does not work well for a number of reasons. One of the biggest problems is its low overall occurrence frequency, especially when compared to the alternative non-homologous end-joining (NHEJ) repair mechanism often triggered by the genome editing molecules that cleave targeted editing sites in the genome. While most cells may have several pathways that could mediate HDR, some of them are most active during the cell cycle, diminishing the success rate of HDR in typical cell culture conditions.

[0005] In prokaryotic hosts such as *E. coli,* homologous gene replacements can be effected with bacteriophage λ Red homologous recombination systems which comprise a bacteriophage λ exonuclease, a bacteriophage λ Beta protein, a single-stranded DNA annealing protein (SSAP) which facilitates annealing of complementary DNA strands, and a DNA template (Murphy, 2016). Bacteriophage λ Red homologous recombination systems have been combined with CRISPR-Cas9 systems in prokaryotes to effect recombination at target sequences in bacterial genomes (Jiang *et al.*, 2013; Wang *et al.*, 2016).

SUMMARY

[0006] Disclosed herein are methods, systems, eukaryotic cells (*e.g.,* plant cells or mammalian cells), and compositions (*e.g.,* cell culture compositions, nucleic acids, vectors, kits, or cells) that can provide for increased frequencies of modification of a target editing site of the eukaryotic cell genome with a donor template polynucleotide by Homology-Directed Repair (HDR) in comparison to a control. Features of such methods, systems, eukaryotic cells (*e.g.,* plant cells or mammalian cells), and compositions (*e.g.,* cell culture compositions, nucleic acids, vectors, kits, or cells) that can provide for such increased frequencies of HDR include provision of 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) in combination with genome editing molecules comprising at least one sequence-specific endonuclease which cleaves a target editing site in a eukaryotic cell genome and a donor template DNA molecule having homology to the target editing site. In certain embodiments, the donor template DNA molecule is flanked by copies of an endonuclease recognition sequence.

[0007] Methods provided herein include methods 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.

[0008] Methods provided herein also include methods for making a eukaryotic cell having a genomic modification, comprising: 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 isolating or propagating a eukaryotic cell comprising the genome modification.

[0009] Systems provided herein include systems 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).

[0010] Methods provided herein also include 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.

[0011] Methods provided herein also include 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.

[0012] Compositions provided herein include 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).

[0013] Vectors provided herein include 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).

[0014] Kits provided herein include 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.

[0015] Cells provided herein include 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).

[0016] Cells provided herein also include 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1** shows a schematic diagram of the vector pRS08t Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes a high copy number origin of replication (High Copy Ori), Cas expression cassette (tomato SlUBI10 promoter, Cas nuclease coding sequence (Cas nuclease CDS), and HSP terminator), guide RNA expression cassette (*A. thaliana* U6 promoter (AtU6), sequence encoding a guide RNA, and 35S promoter), mGFP6 sequence, pea rbcS E9 terminator, ANT1 donor template, and spectinomycin resistance marker (SpnR).

[0018] **FIG. 2** shows a schematic diagram of the vector pRS045. Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes an ampicillin resistance marker (AmpR), HDR promoting agents expression cassette (PcUbi promoter, c2 nuclear localization sequence (NLS) fused to an *E. coli* SSB coding sequence (*E. coli* SSB CDS), pea 3A terminator, tomato SlUBI10 promoter, c2 NLS fused to a SSAP coding sequence (Red Beta CDS), HSP terminator, 2x 35S promoter, c2 NLS fused to an exonuclease coding sequence (Red Exo CDS), and 35S terminator), and pUC origin of replication (pUC ori).

[0019] **FIG. 3** shows a schematic diagram of the vector pAP046. Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes a high copy number origin of replication (High Copy Ori), Cas expression cassette (tomato SlUBI10 promoter, Cas nuclease coding sequence (Cas nuclease CDS), and HSP terminator), guide RNA and ribozyme expression cassette (35S promoter, sequence encoding a

hammerhead (HH) ribozyme, sequence encoding a guide RNA, sequence encoding a hepatitis delta virus (HDV) ribozyme, and 35S terminator), HDR promoting agents expression cassette (PcUbi promoter, c2 NLS fused to an *E. coli* SSB coding sequence (*E. coli* SSB CDS), pea 3A terminator, tomato SlUBI10 promoter, c2 NLS fused to a SSAP coding sequence (Red Beta CDS), HSP terminator, 2x 35S promoter, c2 NLS fused to an exonuclease coding sequence (Red Exo CDS), and 35S terminator), ANT1 donor template, and spectinomycin resistance marker (SpnR).

[0020] **FIG. 4** shows a schematic diagram of the vector pRS148. Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes a high copy number origin of replication (High Copy Ori), Cas expression cassette (tomato SlUBI10 promoter, Cas nuclease coding sequence (Cas nuclease CDS), and HSP terminator), guide RNA and ribozyme expression cassette (35S promoter, sequence encoding a hammerhead (HH) ribozyme, sequence encoding a guide RNA, sequence encoding a hepatitis delta virus (HDV) ribozyme, and 35S terminator), and spectinomycin resistance marker (SpnR).

[0021] **FIG. 5** shows a schematic diagram of the vector pRS192. Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes a high copy number origin of replication (High Copy Ori), HDR promoting agent expression cassette (PcUbi promoter, c2 NLS fused to an *E. coli* SSB coding sequence (*E. coli* SSB CDS), pea 3A terminator, tomato SlUBI10 promoter, c2 NLS fused to a SSAP coding sequence (Red Beta CDS), HSP terminator, 2x 35S promoter, c2 NLS fused to an exonuclease coding sequence (Red Exo CDS), and 35S terminator), ANT1 donor template, and ampicillin resistance marker (AmpR).

[0022] **FIG. 6** shows a schematic diagram of the vector pTC801. Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes a high copy number origin of replication (High Copy Ori), Cas expression cassette (maize ubiquitin (ZmUbi) promoter, Cas nuclease coding sequence (Cas nuclease CDS), and HSP terminator), a guide RNA and ribozyme expression cassette (35S promoter, sequence encoding a hammerhead (HH) ribozyme, sequences encoding a guide RNA 1 and 2, sequence encoding a hepatitis delta virus (HDV) ribozyme, and 35S terminator), a HDR promoting agents expression cassette (*Oryza sativa* actin (OsActin) promoter, c2 NLS fused to an *E. coli* SSB coding sequence (*E. coli* SSB CDS), pea 3A terminator, *Panicum virgatum* ubiquitin (PvUbi1) promoter, c2 NLS fused to a SSAP coding sequence (Red Beta CDS), pea rbcS E9

terminator, *O. sativa* ubiquitin (OsUB1) promoter, c2 NLS fused to an exonuclease coding sequence (Red Exo CDS), and tobacco extensin (NtEXT) terminator), *SPX* donor template, and spectinomycin resistance marker (SpnR).

[0023] **FIG. 7** shows a schematic diagram of the vector pAB156. Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes a kanamycin resistance marker (KanR), left T-DNA border, a hygromycin resistance cassette (2x 35S promoter, hygromycin phosphotransferase (hygR) coding sequence, and 35S terminator), a Cas expression cassette (tomato SlUBI10 promoter, Cas nuclease coding sequence (Cas nuclease CDS), and HSP terminator), a guide RNA and ribozyme expression cassette (35S promoter, sequence encoding a guide RNA, sequence encoding a hammerhead (HH) ribozyme, sequence encoding a hepatitis delta virus (HDV) ribozyme, and 35S terminator), a HDR promoting agents expression cassette (PcUbi4 promoter, c2 NLS fused to an *E. coli* SSB coding sequence (*E. coli* SSB CDS), pea 3A terminator, AtUbi10 promoter, c2 NLS fused to a SSAP coding sequence (Red Beta CDS), pea rbcS E9 terminator, HaUbiCh4 promoter, c2 NLS fused to an exonuclease coding sequence (Red Exo CDS), and Ext3' terminator), GFP donor template, right T-DNA border, and STA region from pVS1.

[0024] **FIG. 8** shows a schematic diagram of the designed insertion regions of superbinary T-DNA vectors pIN1757 (lower) and pIN1576 (upper). pIN1757 includes a left T-DNA border, NOS terminator, PAT for glufosinate selection, 35S promoter, a Cas expression cassette (maize ubiquitin (ZmUbi) promoter, Cas nuclease coding sequence (Cas nuclease CDS), and HSP terminator), a guide RNA expression cassette (wheat U6 (TaU6) promoter, sequence encoding a guide RNA (Gln1-3 Pro-2), and Pol III terminator), Gln1-3 donor template, and right T-DNA border. Additionally, vector pIN1756 includes an HDR promoting agents expression cassette (*O. sativa* actin (OsActin promoter + intron) promoter, *E. coli* SSB coding sequence (SSB), pea 3A terminator; *P. virgatum* ubiquitin (PvUbi1 promoter + intron) promote, an SSAP coding sequence (beta), pea rbcS E9 terminator; *O. sativa* ubiquitin (OsUB1) promoter, an exonuclease coding sequence (Exo), and tobacco extensin (NtEXT) terminator).

[0025] **FIG. 9A-9B** show schematic diagrams of vectors and expression cassettes for transforming tomato cotyledons. **FIG. 9A** shows a schematic diagram of the vector pIN1705. Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes a kanamycin resistance marker (KanR), left T-DNA border, a 5-enolpyruvylshikimate-3-phosphate (EPSPS) synthase expression cassette (*i.e.*, the EPSPS

coding sequence (CDS) under control of the *A. thaliana* ubiquitin promoter (AtUbi10) and pea rbcS E9 terminator), a Cas expression cassette (tomato SlUBI10 promoter, Cas nuclease coding sequence (Cas nuclease CDS), and HSP terminator), a guide RNA and ribozyme expression cassette (35S promoter, sequence encoding a hammerhead (HH) ribozyme, sequence encoding a guide RNA, sequence encoding a hepatitis delta virus (HDV) ribozyme, 35S terminator), a HDR promoting agents expression cassette (PcUbi promoter, c2 NLS fused to an *E. coli* SSB coding sequence (*E. coli* SSB CDS), pea 3A terminator, tomato SlUBI10 promoter, c2 NLS fused to a SSAP coding sequence (Red Beta CDS), HSP terminator, 2x 35S promoter, c2 NLS fused to an exonuclease coding sequence (Red Exo CDS), and 35S terminator), ANT1 donor template, right T-DNA border, STA region from pVS1, pVS1 origin of replication (ori), and an origin of replication (ori). **FIG. 9B** shows schematic diagrams of the regions between the left and right borders of *Agrobacterium* T-DNA vectors for chromosomal integration into the genome of tomato cotyledons. Shown, from top to bottom, are regions of the pIN1703, pIN1704, and pIN1705 vectors. CS indicates cut sites, EPSPS indicates the EPSPS expression cassette, CasS indicates the Cas expression cassette, ANT1 donor indicates the donor template, HDR agents indicates the HDR promoting agents expression cassette encoding the SSAP, SSB, and exonuclease, and GFP indicates the green fluorescent protein coding sequence.

[0026] **FIG. 10** shows a schematic diagram of a vector for expression in humans. Length in base pairs is indicated by the labels outside of the vector. Beginning at base pair 1, the vector includes a high copy number origin of replication (High Copy Ori), a Cas expression cassette (CAG promoter, Cas nuclease coding sequence (Cas nuclease CDS), and rabbit beta-globin (rb globin) terminator), a guide RNA expression cassette (*H. sapiens* U6 (HsU6) promoter, sequence encoding a guide RNA), a HDR promoting agents expression cassette (*H. sapiens* EF1a promoter, SV40 NLS linked to an *E. coli* SSB coding sequence (*E. coli* SSB CDS), human growth hormone (hGH) terminator, *H. sapiens* ACTB (hACTB) promoter, SV40 NLS linked to a SSAP coding sequence (Red Beta CDS), bovine growth hormone (bGH) terminator, CMV promoter, SV40 NLS linked to a exonuclease coding sequence (Red Exo CDS), and SV40 polyA signal), EMX1 FRT donor template, and spectinomycin resistance marker (SpnR).

DETAILED DESCRIPTION

I. DEFINITIONS

[0027] Unless otherwise stated, nucleic acid sequences in the text of this specification are given, when read from left to right, in the 5' to 3' direction. Nucleic acid sequences may be provided as DNA or as RNA, as specified; disclosure of one necessarily defines the other, as well as necessarily defines the exact complements, as is known to one of ordinary skill in the art. Where a term is provided in the singular, the inventors also contemplate embodiments described by the plural of that term.

[0028] The phrase "allelic variant" as used herein refers to a polynucleotide or polypeptide sequence variant that occurs in a different strain, variety, or isolate of a given organism.

[0029] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0030] As used herein, the terms "Cpf1" and "Cas12a" are used interchangeably herein to refer to the same RNA directed nuclease.

[0031] As used herein, the phrase "genome-editing molecules" refers to one or more sequence-specific endonuclease(s) or polynucleotide(s) encoding the sequence-specific endonuclease(s) that cleave at least one DNA sequence at an endonuclease recognition site.

[0032] As used herein, an "exogenous" agent or molecule refers to any agent or molecule from an external source that is provided to or introduced into a system, composition, a eukaryotic or plant cell culture, reaction system, or a eukaryotic or plant cell. In certain embodiments, the exogenous agent (*e.g.*, polynucleotide, protein, or compound) from the external source can be an agent that is also found in a eukaryotic or plant cell. In certain embodiments, the exogenous agent (*e.g.*, polynucleotide, protein, or compound) from the external source can be an agent that is heterologous to the eukaryotic or plant cell.

[0033] As used herein, a "heterologous" agent or molecule refers: (i) to any agent or molecule that is not found in a wild-type, untreated, or naturally occurring composition,

eukaryotic cell, or plant cell; and/or (ii) to a polynucleotide or peptide sequence located in, *e.g.*, a genome or a vector, in a context other than that in which the sequence occurs in nature. For example, a promoter that is operably linked to a gene other than the gene that the promoter is operably linked to in nature is a heterologous promoter.

[0034] As used herein, the terms "include," "includes," and "including" are to be construed as at least having the features to which they refer while not excluding any additional unspecified features.

[0035] The term "homologous recombination" as used herein refers to the exchange of DNA fragments between two DNA molecules at the sites of homology. The frequency of homologous recombination is influenced by a number of factors. Different organisms vary with respect to the amount of homologous recombination and the relative proportion of homologous to non-homologous recombination. Generally, the length of the region of homology affects the frequency of homologous recombination events: the longer the region of homology, the greater the frequency. The length of the homology region needed to observe homologous recombination is also species-variable. In many cases, at least 5 kb of homology has been utilized, but homologous recombination has been observed with as little as 25-50 bp of homology.

[0036] As used herein Homology-directed repair (HDR) means a method of DNA repair that results in precite editing of a target editing site by incorporating a provided donor sequence.

[0037] As used herein, phrases such as "frequency of HDR," "HDR frequency," and the like refer to the number of HDR-mediated events at a target editing site in comparison to the total number target-editing sites analyzed. The total number of target editing sites is the sum of: (a) target editing sites having NHEJ-mediated events; (b) target editing sites having no changes; and (c) target editing sites having HDR-mediated events. HDR-mediated events include precise insertions of heterologous sequences into a target editing site that do not contain any unintended nucleotide insertions, deletions, or substitutions in either the inserted heterologous sequence, the homologous sequences that flank the heterologous insert, or in the sequences located at the junction of the heterologous sequence and the homologous sequences.

[0038] As used herein, the phrase "eukaryotic cell" refers to any cell containing a nucleus and thus includes mammalian (*e.g.*, human, livestock, and companion animal cells), insect

cells, reptile cells, plant cells (*e.g.,* monocot and dicot plant cells), yeast cells, and fungal cells (*e.g.,* filamentous and non-filamentous fungi).

[0039] A "modified nucleotide" or "edited nucleotide" refers to a nucleotide sequence of interest that comprises at least one alteration when compared to its non-modified nucleotide sequence. Such "alterations" include, for example: (i) replacement of at least one nucleotide, (ii) a deletion of at least one nucleotide, (iii) an insertion of at least one nucleotide, or (iv) any combination of (i)-(iii).

[0040] As used herein, the phrase "plant cell" can refer either a plant cell having a plant cell wall or to a plant cell protoplast lacking a plant cell wall.

[0041] The term "polynucleotide" where used herein is a nucleic acid molecule containing two (2) or more nucleotide residues. Polynucleotides are generally described as single- or double-stranded. Where a polynucleotide contains double-stranded regions formed by intra- or intermolecular hybridization, the length of each double-stranded region is conveniently described in terms of the number of base pairs. Embodiments of the systems, methods, and compositions provided herein can employ or include: (i) one or more polynucleotides of 2 to 25 residues in length, one or more polynucleotides of more than 26 residues in length, or a mixture of both. Polynucleotides can comprise single- or double-stranded RNA, single- or double-stranded DNA, double-stranded DNA/RNA hybrids, chemically modified analogues thereof, or a mixture thereof. In certain embodiments, a polynucleotide can include a combination of ribonucleotides and deoxyribonucleotides (*e.g.*, synthetic polynucleotides consisting mainly of ribonucleotides but with one or more terminal deoxyribonucleotides or synthetic polynucleotides consisting mainly of deoxyribonucleotides but with one or more terminal dideoxyribonucleotides), or can include non-canonical nucleotides such as inosine, thiouridine, or pseudouridine. In certain embodiments, the polynucleotide includes chemically modified nucleotides (see, *e.g.*, Verma and Eckstein (1998) *Annu. Rev. Biochem.,* 67:99-134). Chemically modified nucleotides that can be used in the polynucleotides provided herein include: (i) phosphorothioate, phosphorodithioate, or methylphosphonate internucleotide linkage modifications of the phosphodiester backbone; (ii) nucleosides comprising modified bases and/or modified sugars; and/or (iii) detectable labels including a fluorescent moiety (*e.g.*, fluorescein or rhodamine or a fluorescence resonance energy transfer or FRET pair of chromophore labels) or other label (*e.g.*, biotin or an isotope). Polynucleotides provided or used herein also include modified nucleic acids,

particularly modified RNAs, which are disclosed in US Patent 9,464,124, which is incorporated herein by reference in its entirety.

[0042] A "recombinant AAV vector (rAAV vector)" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of AAV origin) that are flanked by at least one, and in some embodiments two, AAV inverted terminal repeat sequences (ITRs). Such rAAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper virus (or that is expressing suitable helper functions) and that is expressing AAV rep and cap gene products (*i.e.* AAV Rep and Cap proteins). When a rAAV vector is incorporated into a larger polynucleotide (*e.g.*, in a chromosome or in another vector such as a plasmid used for cloning or transfection), men the rAAV vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of AAV packaging functions and suitable helper functions. A rAAV vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, particularly an AAV particle. A rAAV vector can be packaged into an AAV virus capsid to generate a "recombinant adeno-associated viral particle (rAAV particle)".

[0043] A "recombinant adenoviral vector" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of adenovirus origin) that are flanked by at least one adenovirus inverted terminal repeat sequence (ITRs). In some embodiments, the recombinant nucleic acid is flanked by two inverted terminal repeat sequences (ITRs). Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that is expressing essential adenovirus genes deleted from the recombinant viral genome (*e.g.,* El genes, E2 genes, E4 genes, *etc.*). When a recombinant viral vector is incorporated into a larger polynucleotide (*e.g.*, in a chromosome or in another vector such as a plasmid used for cloning or transfection), men the recombinant viral vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of adenovirus packaging functions. A recombinant viral vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, for example, an adenovirus particle. A recombinant viral vector can be packaged into an adenovirus virus capsid to generate a "recombinant adenoviral particle."

[0044] A "recombinant lentivirus vector" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of lentivirus origin) that are flanked by at least one lentivirus terminal repeat sequences (LTRs). In some embodiments, the recombinant nucleic acid is flanked by two lentiviral terminal repeat sequences (LTRs). Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper functions. A recombinant lentiviral vector can be packaged into a lentivirus capsid to generate a "recombinant lentiviral particle."

[0045] A "recombinant herpes simplex vector (recombinant HSV vector)" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of HSV origin) that are flanked by HSV terminal repeat sequences. Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper functions. When a recombinant viral vector is incorporated into a larger polynucleotide (*e.g.*, in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the recombinant viral vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of HSV packaging functions. A recombinant viral vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, for example, an HSV particle. A recombinant viral vector can be packaged into an HSV capsid to generate a "recombinant herpes simplex viral particle."

[0046] As used herein, the phrase "target editing site" refers to a DNA sequence that is modified by a donor nucleic acid.

[0047] As used herein, the phrase "target gene" can refer to a gene located in the genome that is to be modified by gene editing molecules provided in a system, method, composition and/or eukaryotic cell provided herein. Embodiments of target genes include (protein-) coding sequence, non-coding sequence, and combinations of coding and non-coding sequences. Modifications of a target gene include nucleotide substitutions, insertions, and/or deletions in one or more elements of a gene that include a transcriptional enhancer or promoter, a 5' or 3' untranslated region, a mature or precursor RNA coding sequence, an intron, a splice donor and/or acceptor, a protein coding sequence, a polyadenylation site, and/or a transcriptional terminator. In certain embodiments, all copies or all alleles of a given target gene in a diploid or polyploid plant cell are modified to provide homozygosity of the

modified target gene in the plant cell. In embodiments, where a desired trait is conferred by a loss-of-function mutation that is introduced into the target gene by gene editing, a plant cell, population of plant cells, plant, or seed is homozygous for a modified target gene with the loss-of-function mutation. In other embodiments, only a subset of the copies or alleles of a given target gene are modified to provide heterozygosity of the modified target gene in the plant cell. In certain embodiments where a desired trait is conferred by a dominant mutation that is introduced into the target gene by gene editing, a plant cell, population of plant cells, plant, or seed is heterozygous for a modified target gene with the dominant mutation. Traits imparted by such modifications to certain plant target genes include improved yield, resistance to insects, fungi, bacterial pathogens, and/or nematodes, herbicide tolerance, abiotic stress tolerance (*e.g.,* drought, cold, salt, and/or heat tolerance), protein quantity and/or quality, starch quantity and/or quality, lipid quantity and/or quality, secondary metabolite quantity and/or quality, and the like, all in comparison to a control plant that lacks the modification. The plant having a genome modified by gene editing molecules provided in a system, method, composition and/or plant cell provided herein differs from a plant having a genome modified by traditional breeding (*i.e.*, crossing of a male parent plant and a female parent plant), where unwanted and random exchange of genomic regions as well as random mitotically or meiotically generated genetic and epigenetic changes in the genome typically occurs during the cross and are then found in the progeny plants. Thus, in embodiments of the plant (or plant cell) with a modified genome, the modified genome is more than 99.9% identical to the original (unmodified) genome. In embodiments, the modified genome is devoid of random mitotically or meiotically generated genetic or epigenetic changes relative to the original (unmodified) genome. In embodiments, the modified genome includes a difference of epigenetic changes in less than 0.01% of the genome relative to the original (unmodified) genome. In embodiments, the modified genome includes: (a) a difference of DNA methylation in less than 0.01% of the genome, relative to the original (unmodified) genome; or (b) a difference of DNA methylation in less than 0.005% of the genome, relative to the original (unmodified) genome; or (c) a difference of DNA methylation in less than 0.001% of the genome, relative to the original (unmodified) genome. In embodiments, the gene of interest is located on a chromosome in the plant cell, and the modified genome includes: (a) a difference of DNA methylation in less than 0.01% of the portion of the genome that is contained within the chromosome containing the gene of interest, relative to the original (unmodified) genome; or (b) a difference of DNA methylation in less than

0.005% of the portion of the genome that is contained within the chromosome containing the gene of interest, relative to the original (unmodified) genome; or (c) a difference of DNA methylation in less than 0.001% of the portion of the genome that is contained within the chromosome containing the gene of interest, relative to the original (unmodified) genome. In embodiments, the modified genome has not more unintended changes in comparison to the original (unmodified) genome than 1 ×10^-8 mutations per base pair per replication. In certain embodiments, the modified genome has not more unintended changes than would occur at the natural mutation rate. Natural mutation rates can be determined empirically or are as described in the literature (Lynch, M., 2010; Clark *et al.*, 2005).

[0048] A "vector," as used herein, refers to a recombinant plasmid that comprises a nucleic acid to be delivered into a host cell, either *in vitro* or *in vivo*.

[0049] To the extent to which any of the preceding definitions is inconsistent with definitions provided in any patent or non-patent reference incorporated herein by reference, any patent or non-patent reference cited herein, or in any patent or non-patent reference found elsewhere, it is understood that the preceding definition will be used herein.

II. METHODS AND COMPOSITIONS

A. Methods for increasing Homology Directed Repair-mediated genome modification

[0050] Various reagents, systems, methods, and compositions that comprise HDR promoting agents (an SSAP, exonuclease, and SSB) and genome-editing molecules and that provide for increased frequencies of homology dependent repair (HDR) in eukaryotic cell gene editing experiments in comparison to control experiments are provided herein. In certain embodiments, the frequency of HDR is increased by at least 2-fold, 3-fold, 5-fold, or 10-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 the HDR promoting agents (SSAPs, exonucleases, and SSBs). In certain embodiments, the frequency of HDR is increased by at least 2-fold, 3-fold, or 5-fold to about 12-fold, 15-fold, 20-fold, 25-fold, or 30 -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 the HDR promoting agents (SSAPs, exonucleases, and SSBs). In some embodiments, the present methods can be employed on cells not undergoing mitosis or meiosis. In some embodiments, the present methods do not require DNA replication.

i. Nuclear localization signals (NLS)

[0051] Nuclear localization signals (NLS) that can direct SSAP, exonucleases, SSB, and/or gene editing molecules provided herein include monopartite and bipartite nuclear localization signals (Kosugi *et al.*, 2009). Examples of monopartite NLS that can be used include NLS that comprise at least 4 consecutive basic amino acids such as the SV40 large T antigen NLS (PKKKRKV; SEQ ID NO:11) and another class having only three basic amino acids with a K(K/R)*X*(K/R) consensus sequence (SEQ ID NO:12). Examples of bipartite NLS that can be used in the provided herein include (K/R)(K/R)$X_{10-12}$$(K/R)_{3/5}$ (SEQ ID NO:13) where $(K/R)_{3/5}$ represents at least three of either lysine or arginine of five consecutive amino acids. An NLS can also comprise a plant-specific class 5 NLS having a consensus sequence of LGKR(K/R)(W/F/Y) (SEQ ID NO:14). Examples of specific NLS that can be used further include the maize opaque-2 nuclear localization signal (SEQ ID NO:10, a bhendi yellow vein mosaic virus (BYVMV) c2 NLS (SEQ ID NO:15, and an extended SV40 large T antigen NLS (SEQ ID NO:16).

[0052] In some embodiments, the NLS is a mammalian (such as a human NLS) In some embodiments, the NLS is an SV40 NLS. In some embodiments, the NLS is an SV40 NLS with an amino acid linker. In some embodiments, the NLS has the amino acid sequence MAPKKKRKVGGSGS (SEQ ID NO:148).

[0053] In certain embodiments, the NLS elements or other desired elements (*e.g.,* epitope tags) can be operably linked to the SSAP, exonucleases, SSB, and/or gene editing molecules provided herein via either a direct covalent linkage of the elements and domain or by a use of a linker peptide or flexible hinge polypeptide. Flexible hinge polypeptides include glycine-rich or glycine/serine containing peptide sequence. Such sequences can include, but are not limited to, a (Gly4)n sequence, a (Gly4Ser)n sequence, a Ser(Gly_4Ser)n sequence, combinations thereof, and variants thereof, wherein n is a positive integer equal to 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In certain embodiments, such glycine-rich or glycine/serine containing hinge peptides can also contain threonyl and/or alanyl residues for flexibility as well as polar lysyl and/or glutamyl residues. Other examples of hinge peptides that can be used include immunoglobulin hinge peptides (Vidarsson *et al.*, 2014).

[0054] A variety of cell-penetrating peptides (CPP) can also be used in the SSAP, exonucleases, SSB, and/or gene editing molecules provided herein. CPPs that can be used include a minimal undecapeptide protein transduction domain (corresponding to residues 47-57 of HIV- 1 TAT comprising YGRKKRRQRRR; SEQ ID NO:17); a polyarginine sequence

comprising a number of arginines sufficient to direct entry into a cell (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, or 10-50 arginines); a VP22 domain (Zender *et al.* (2002) Cancer Gene Ther. 9(6):489-96); an Drosophila Antennapedia protein transduction domain (Noguchi *et al.* (2003) Diabetes 52(7): 1732-1737); a truncated human calcitonin peptide (Trehin *et al.* (2004) Pharm. Research 21: 1248-1256); polylysine (Wender *et al.* (2000) Proc. Natl. Acad. Sci. USA 97: 13003-13008); RRQRRTSKLMKR (SEQ ID NO:18); Transportan (*e.g.*, GWTLNSAGYLLGKINLKALAALAKKIL (SEQ ID NO:19); KALAWEAKLAKALAKALAKHLAKALAKALKCEA (SEQ ID NO:20); and RQIKIWFQNRRMKWKK (SEQ ID NO:21). Exemplary CPP amino acid sequences also include YGRKKRRQRRR (SEQ ID NO:22; RKKRRQRR (SEQ ID NO:23); YARAAARQARA (SEQ ID NO:24); THRLPRRRRRR (SEQ ID NO:25); and GGRRARRRRRR (SEQ ID NO:26).

ii. Single-stranded DNA annealing proteins (SSAPs)

[0055] In certain embodiments, the single-stranded DNA annealing protein (SSAP) used in the methods, systems, cells, and cell culture compositions provided herein include proteins which promote or catalyze DNA strand exchange and base pairing of complementary DNA strands of homologous DNA molecules. Characteristics of the SSAPs used herein include stimulation of RecA dependent and independent pathways, oligomeric rings and/or filaments formation *in vitro*, ssDNA binding activity, and ATPase-independent stimulation of complementary ssDNA strand annealing. Characteristics of SSAP proteins in the RecT/Redβ-, ERF-, or RAD52-families of proteins have been disclosed in Murphy, 2016 and Iyer *et al.*, 2002. In certain embodiments, the SSAP is a member of the RecT/Redβ-family of proteins that include a Rac bacterial prophage RecT protein, a bacteriophage λ beta protein, a bacteriophage SPP1 35 protein, or related protein with equivalent SSAP activity. Characteristics of certain RecT/Redβ-family of proteins include an α + β domain with a core of five β-strands and five α-helices, Mg^{+2} dependent single strand annealing activity and conservation of two c-terminal acidic residues in most but not all members (Iyer *et al.*, 2002). In certain embodiments, 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 and optionally a conserved α + β domain with a core of five β-strands and five α-helices, Mg^{+2} dependent single strand annealing activity, and /or conservation of two c-terminal acidic residues. In certain embodiments, the SSAP is an ERF-family protein. Characteristics of

EFR-family of proteins include a conserved region of about 150 amino acid residues comprising a GuXXoYhp + YXhXXhh (SEQ ID NO:32) motif, where G is glycine, Y-tyrosine, u is a "tiny" residue (glycine, serine, alanine), h-hydrophobic (alanine, valine, leucine, isoleucine, phenylalanine, methionine), p is a polar residue (lysine, arginine, glutamate, aspartate, asparagine, threonine, serine), o is an alcohol-containing amino acid residue (serine or threonine), + is a basic residue, and X is any residue (Iyer *et al.*, 2002). ERF family proteins include a bacteriophage P22 ERF protein or a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 4, and can optionally further comprise the GuXXoYhp + YXhXXhh (SEQ ID NO:32) motif. SSAP in the ERF-family also include proteins set forth in the NCBI database on the world wide web site ncbi.nlm.nih.gov/protein under accession (gi or gene identifier) numbers 9634188, 9635694, 16804357, 12719409, 458219, 11497308, 11497280, 1497168, 11527300, 9634634, 9635643, 13491642, 6015511, 11138335, 9627938, 9628668, and 15088753. In certain embodiments, the SSAP used herein include RAD52-family proteins from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Kluyveromyces lactis* as well as variants thereof having at least 85%, 90%, 95%, 97%, 98%, or 99% sequence identity across the entire length of SEQ ID NO:5, 6, and 7, respectively; or variants having at one or more conservative and/or semi-conservative amino acid substitutions in SEQ ID NO: 5, 6, or 7. Characteristics of RAD52-family of proteins include conserved helix-hairpin-helix (HhH) motifs with DNA binding activity (Iyer *et al.*, 2002). SSAP used herein can further include proteins identified as "recombinases" that are set forth in at least Tables 1, 2, 3, 4, 5, and 6 of US Patent Application Serial Number 16/075,281, a US National Stage of PCT/US2017/016184, published as WO 2017/184227, the continents of which are incorporated herein by reference in their entireties. In certain embodiments, the SSAP can comprise an allelic variant of any of the aforementioned SSAP. In certain embodiments, any of the aforementioned SSAP can be provided to a cell by way of a nucleic acid that encodes the SSAP (*e.g.*, an expression vector, mRNA, or viral expression vector). In certain embodiments, any of the aforementioned SSAP can be provided to a cell as proteins, fusion proteins (*e.g.*, with a cell penetrating peptide and/or a nuclear localization sequence), or as polyproteins comprising protease recognition sites or self-processing protein sequences inserted between the SSAP and other proteins (*e.g.*, in combination with an SSB and/or an exonuclease).

iii. Exonucleases

[0056] In certain embodiments, the exonucleases used in the methods, systems, cells, and cell culture compositions provided herein include exonucleases with a 5' to 3' or a 3' to 5' exonuclease activity on a double-stranded DNA (dsDNA) substrate that can result in product comprising an at least partially single stranded DNA (ssDNA) having an exposed 3' terminus or an exposed 5' terminus, respectively. In certain embodiments, the exonuclease will recognize a dsDNA substrate with a blunt end, including a blunt end with a 5' phosphate group. In certain embodiments, the exonuclease will recognize a dsDNA substrate with an overhang of ssDNA (*e.g.,* a 5' or 3' ssDNA region at a terminus of a dsDNA molecule, including ends produced by endonucleases which provide staggered cuts in dsDNA substrates). In certain embodiments, the exonuclease will recognize a dsDNA substrate having an internal break in one strand (*e.g.,* a nicked dsDNA). Exonucleases with 5' to 3' exonuclease activity that can be used herein include a bacteriophage lambda exo protein (*e.g.,* SEQ ID NO:8), an Rac prophage RecE exonuclease protein (*e.g.,* SEQ ID NO:9), an Artemis protein (*e.g.,* SEQ ID NO: 136), an Apollo protein (*e.g.,* SEQ ID NO: 137), a DNA2 exonuclease protein (*e.g.,* SEQ ID NO: 138), an Exo1 exonuclease protein (*e.g.,* SEQ ID NO: 139), a herpesvirus SOX protein (*e.g.,* SEQ ID NO: 140), UL12 exonuclease protein (*e.g.,* SEQ ID NO: 141), an enterobacterial exonuclease VIII protein (*e.g.,* SEQ ID NO: 142), a T7 phage exonuclease protein (*e.g.,* SEQ ID NO:143) or a related protein with equivalent 5' to 3' 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, or 143. In certain embodiments, the exonucleases with 5' to 3' exonuclease activity provided herein include the proteins set forth in SEQ ID NO: 8, 9, 136, 137, 138, 139, 140, 141, 142, or 143 that have at least one or more conservative and/or semi-conservative amino acid substitutions in SEQ ID NO:8, 9, 136, 137, 138, 139, 140, 141, 142, or 143. Exonucleases with 3' to 5' exonuclease activity that can be used herein include an E. coli Exonuclease III protein (*e.g.,* SEQ ID NO: 144), a mammalian Trex2 exonuclease protein (*e.g.,* SEQ ID NO: 145), a related protein with equivalent 3' to 5' exonuclease activity, or a protein having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 144 or 145. In certain embodiments, the exonucleases with a 3' to 5' exonuclease activity provided herein include the proteins set forth in set forth SEQ ID NO: 144 or 145 that have at least one or more conservative and/or semi-conservative amino acid substitutions in SEQ ID NO: 144 or 145. In certain embodiments, the aforementioned exonucleases will comprise conserved DEDD catalytic

residues characteristic of the DEDD/DnaQ superfamily of exonucleases (Bernad *et al.*, 1989). In certain embodiments, any of the aforementioned exonucleases can be provided to a cell as proteins, fusion proteins (*e.g.,* with a cell penetrating peptide and/or a nuclear localization sequence), or as polyproteins comprising protease recognition sites or self-processing protein sequences inserted between the exonuclease and other proteins (*e.g.,* in combination with an SSB and/or an SSAP). In certain embodiments, the exonuclease can comprise an allelic variant of any of the aforementioned exonucleases. In certain embodiments, any of the aforementioned exonucleases can be provided to a cell by way of a nucleic acid that encodes the exonuclease (*e.g.,* an expression vector, mRNA, or viral expression vector). In some embodiments, the sequence-specific endonuclease is a nickase.

iv. Single stranded DNA binding proteins (SSBs)

[0057] Various single stranded DNA binding proteins (SSB) can be used in the methods, systems, cells, and cell culture compositions provided herein. In certain embodiments, the SSBs include a bacterial SSB or optionally an *Enterobacteriaceae* sp. SSB. In certain embodiments, 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 provided herein include the set forth in SEQ ID NO: 31, and SEQ ID NO: 34-131, and 132, as well as variants thereof having at least 85%, 90%, 95%, 97%, 98%, or 99% sequence identity across the entire length of SEQ ID NO: 31, SEQ ID NO: 34-131, or 132; or having at one or more conservative and/or semi-conservative amino acid substitutions in SEQ ID NO: 31, or SEQ ID NO: 34-131, or 132. SSB used herein can include SSB proteins that are set forth in the disclosure and at least Tables 7 and 8 of US Patent Application Serial Number 16/075,281, a US National Stage of PCT/US2017/016184, published as WO 2017/184227, the continents of which are incorporated herein by reference in their entireties. In certain embodiments, the SSB can comprise an allelic variant of any of the aforementioned SSBs. In certain embodiments, any of the aforementioned SSB can be provided to a cell by way of a nucleic acid that encodes the SSB (*e.g.,* an expression vector, mRNA, or viral expression vector). In certain embodiments, any of the aforementioned SSB can be provided to a cell as proteins, fusion proteins (*e.g.,* with a cell penetrating peptide and/or a nuclear localization sequence), or as polyproteins comprising protease recognition sites or self-processing protein sequences inserted between the SSB and other proteins (*e.g.,* in combination with an SSAP and/or an exonuclease).

[0058] In some embodiments, the SSB and SSAP used in the present methods are are from the same organism or from a phage and a bacterial host of the phage.

[0059] In some embodiments, an SSB is not required. In some embodmients, SSAP is fused with an replication protein A (RPA)-binding partner (Fanning *et al.* Nucleic acids research, 34(15), 4126–4137). In some embodiments, the SSB is an endogenous SSB. In some embodiments, an SSAP that is modified to bind to an endogenous SSB is provided.

[0060] In some embodments, the components used in the methods provided herein are provided as a fusion proteins. In some embodiments SSAP is fused with SSB. In some embodments, SSAP is fused to a replication protein A (RPA).

v. Plants, plant tissues, and plant cells

[0061] In certain embodiments, HDR is increased in isolated plant cells or plant protoplasts (*i.e.,* are not located in undissociated or intact plant tissues, plant parts, or whole plants). In certain embodiments, the plant cells are obtained from any plant part or tissue or callus. In certain embodiments, the culture includes plant cells obtained from a plant tissue, a cultured plant tissue explant, whole plant, intact nodal bud, shoot apex or shoot apical meristem, root apex or root apical meristem, lateral meristem, intercalary meristem, seedling, whole seed, halved seed or other seed fragment, zygotic embryo, somatic embryo, immature embryo, ovule, pollen, microspore, anther, hypocotyl, cotyledon, leaf, petiole, stem, tuber, root, callus, or plant cell suspension. In certain embodiments, the plant cell is derived from the L1 or L2 layer of an immature or mature embryo of a monocot plant (*e.g.,* maize, wheat, sorghum, or rice).

[0062] In certain embodiments, HDR is increased in plant cells that are located in undissociated or intact plant tissues, plant parts, plant explants, or whole plants. In certain embodiments, the plant cell can be located in an intact nodal bud, a cultured plant tissue explant, shoot apex or shoot apical meristem, root apex or root apical meristem, lateral meristem, intercalary meristem, seedling, whole seed, halved seed or other seed fragment, zygotic embryo, somatic embryo, immature embryo, ovule, pollen, microspore, anther, hypocotyl, cotyledon, leaf, petiole, stem, tuber, root, or callus. In certain embodiments, the explants used include immature embryos. Immature embryos (*e.g.,* immature maize embryos) include 1.8-2.2 mm embryos, 1-7 mm embryos, and 3-7 mm embryos. In certain embodiments, the aforementioned embryos are obtained from mature ear-derived seed, leaf bases, leaves from mature plants, leaf tips, immature inflorescences, tassels, immature ears,

and silks. In various aspects, the plant-derived explant used for transformation includes immature embryos, 1.8-2.2 mm embryos, 1-7 mm embryos, and 3.5-7 mm embryos. In an aspect, the embryos used in the disclosed methods can be derived from mature ear-derived seed, leaf bases, leaves from mature plants, leaf tips, immature inflorescences, tassel, immature ear, or silks. In certain embodiments, the plant cell is a pluripotent plant cell (*e.g.*, a stem cell or meristem cell). In certain embodiments, the plant cell is located within the L1 or L2 layer of an immature or mature embryo of a monocot plant (*e.g.*, maize, wheat, sorghum, or rice). In certain embodiments, methods of editing genomes of whole plants, seeds, embryos, explants, or meristematic tissue published in WO2018085693, which is incorporated herein by reference in its entirety, can be adapted for use in the plant cells and related systems, methods, compositions, or cultures provided herein.

[0063] In certain embodiments, the plant cells can comprise haploid, diploid, or polyploid plant cells or plant protoplasts, for example, those obtained from a haploid, diploid, or polyploid plant, plant part or tissue, or callus. In certain embodiments, plant cells in culture (or the regenerated plant, progeny seed, and progeny plant) are haploid or can be induced to become haploid; techniques for making and using haploid plants and plant cells are known in the art, see, *e.g.*, methods for generating haploids in *Arabidopsis thaliana* by crossing of a wild-type strain to a haploid-inducing strain that expresses altered forms of the centromere-specific histone CENH3, as described by Maruthachalam and Chan in "How to make haploid *Arabidopsis thaliana*", protocol available at www[dot]openwetware[dot]org/images/d/d3/Haploid_Arabidopsis_protocol[dot]pdf; (Ravi *et al.* (2014) *Nature Communications*, 5:5334, doi: 10.1038/ncomms6334). Haploids can also be obtained in a wide variety of monocot plants (*e.g.*, maize, wheat, rice, sorghum, barley) or dicot plants (*e.g.*, soybean, *Brassica* sp. including canola, cotton, tomato) by crossing a plant comprising a mutated CENH3 gene with a wildtype diploid plant to generate haploid progeny as disclosed in US Patent No. 9,215,849, which is incorporated herein by reference in its entirety. Haploid-inducing maize lines that can be used to obtain haploid maize plants and/or cells include Stock 6, MHI (Moldovian Haploid Inducer), indeterminate gametophyte (ig) mutation, KEMS, RWK, ZEM, ZMS, KMS, and well as transgenic haploid inducer lines disclosed in US Patent No. 9,677,082, which is incorporated herein by reference in its entirety. Examples of haploid cells include but are not limited to plant cells obtained from haploid plants and plant cells obtained from reproductive tissues, *e.g.*, from flowers, developing flowers or flower buds, ovaries, ovules, megaspores, anthers, pollen,

megagametophyte, and microspores. In certain embodiments where the plant cell or plant protoplast is haploid, the genetic complement can be doubled by chromosome doubling (*e.g.*, by spontaneous chromosomal doubling by meiotic non-reduction, or by using a chromosome doubling agent such as colchicine, oryzalin, trifluralin, pronamide, nitrous oxide gas, anti-microtubule herbicides, anti-microtubule agents, and mitotic inhibitors) in the plant cell or plant protoplast to produce a doubled haploid plant cell or plant protoplast wherein the complement of genes or alleles is homozygous; yet other embodiments include regeneration of a doubled haploid plant from the doubled haploid plant cell or plant protoplast. Another embodiment is related to a hybrid plant having at least one parent plant that is a doubled haploid plant provided by this approach. Production of doubled haploid plants provides homozygosity in one generation, instead of requiring several generations of self-crossing to obtain homozygous plants. The use of doubled haploids is advantageous in any situation where there is a desire to establish genetic purity (*i.e.* homozygosity) in the least possible time. Doubled haploid production can be particularly advantageous in slow-growing plants, such as fruit and other trees, or for producing hybrid plants that are offspring of at least one doubled-haploid plant.

[0064] In certain embodiments where HDR is increased in plant cells, as well as the related methods, systems, compositions, or reaction mixtures provided herein can include plant cells obtained from or located in any monocot or dicot plant species of interest, for example, row crop plants, fruit-producing plants and trees, vegetables, trees, and ornamental plants including ornamental flowers, shrubs, trees, groundcovers, and turf grasses. In certain non-limiting embodiments, the plant cells are obtained from or located in alfalfa (*Medicago sativa*), almonds (*Prunus dulcis*), apples (*Malus* x *domestica*), apricots (*Prunus armeniaca*, *P. brigantine*, *P. mandshurica*, *P. mume*, *P. sibirica*), asparagus (*Asparagus officinalis*), bananas (*Musa* spp.), barley (*Hordeum vulgare*), beans (*Phaseolus* spp.), blueberries and cranberries (*Vaccinium* spp.), cacao (*Theobroma cacao*), canola and rapeseed or oilseed rape, (*Brassica napus*), carnation (*Dianthus caryophyllus*), carrots (*Daucus carota sativus*), cassava (*Manihot esculentum*), cherry (*Prunus avium*), chickpea (*Cider arietinum*), chicory (*Cichorium intybus*), chili peppers and other capsicum peppers (*Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. pubescens*, *C. baccatum*), chrysanthemums (*Chrysanthemum* spp.), coconut (*Cocos nucifera*), coffee (*Coffea* spp. including *Coffea arabica* and *Coffea canephora*), cotton (*Gossypium hirsutum* L.), cowpea (*Vigna unguiculata*), cucumber (*Cucumis sativus*), currants and gooseberries (*Ribes* spp.), eggplant or aubergine (*Solanum melongena*),

eucalyptus (*Eucalyptus* spp.), flax (*Linum usitatissumum* L.), geraniums (*Pelargonium* spp.), grapefruit (*Citrus* x *paradisi*), grapes (*Vitus* spp.) including wine grapes (*Vitus vinifera*), guava (*Psidium guajava*), hemp and cannabis (*e.g., Cannabis sativa* and *Cannabis* spp.), hops (*Humulus lupulus*), irises (*Iris* spp.), lemon (*Citrus limon*), lettuce (*Lactuca sativa*), limes (*Citrus* spp.), maize (*Zea mays* L.), mango (*Mangifera indica*), mangosteen (*Garcinia mangostana*), melon (*Cucumis melo*), millets (*Setaria* spp, *Echinochloa* spp, *Eleusine* spp, *Panicum* spp., *Pennisetum* spp.), oats (*Avena sativa*), oil palm (*Ellis quineensis*), olive (*Olea europaea*), onion (*Allium cepa*), orange (*Citrus sinensis*), papaya (*Carica papaya*), peaches and nectarines (*Prunus persica*), pear (*Pyrus* spp.), pea (*Pisa sativum*), peanut (*Arachis hypogaea*), peonies (*Paeonia* spp.), petunias (*Petunia* spp.), pineapple (*Ananas comosus*), plantains (*Musa* spp.), plum (*Prunus domestica*), poinsettia (*Euphorbia pulcherrima*), Polish canola (*Brassica rapa*), poplar (*Populus* spp.), potato (*Solanum tuberosum*), pumpkin (*Cucurbita pepo*), rice (*Oryza sativa* L.), roses (*Rosa* spp.), rubber (*Hevea brasiliensis*), rye (*Secale cereale*), safflower (*Carthamus tinctorius* L), sesame seed (*Sesame indium*), sorghum (*Sorghum bicolor*), soybean (*Glycine max* L.), squash (*Cucurbita pepo*), strawberries (*Fragaria* spp., *Fragaria x ananassa*), sugar beet (*Beta vulgaris*), sugarcanes (*Saccharum* spp.), sunflower (*Helianthus annus*), sweet potato (*Ipomoea batatas*), tangerine (*Citrus tangerina*), tea (*Camellia sinensis*), tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum*), tulips (*Tulipa* spp.), turnip (*Brassica rapa rapa*), walnuts (*Juglans* spp. L.), watermelon (*Citrulus lanatus*), wheat (*Tritium aestivum*), or yams (*Discorea* spp.).

vi. Eukaryotic cells

[0065] In certain embodiments, the eukaryotic cells (*e.g.,* plant cells) where HDR is increased can be cells that are (a) encapsulated or enclosed in or attached to a polymer (*e.g.*, pectin, agarose, or other polysaccharide) or other support (solid or semi-solid surfaces or matrices, or particles or nanoparticles); (b) encapsulated or enclosed in or attached to a vesicle or liposome or other fluid compartment; or (c) not encapsulated or enclosed or attached. In certain embodiments, the cells can be in liquid or suspension culture, or cultured in or on semi-solid or solid media, or in a combination of liquid and solid or semi-solid media (*e.g.*, plant cells or protoplasts cultured on solid medium with a liquid medium overlay, or plant cells or protoplasts attached to solid beads or a matrix and grown with a liquid medium). In certain embodiments, the cells encapsulated in a polymer (*e.g.*, pectin, agarose, or other polysaccharide) or other encapsulating material, enclosed in a vesicle or liposome,

suspended in a mixed-phase medium (such as an emulsion or reverse emulsion), or embedded in or attached to a matrix or other solid support (*e.g.*, beads or microbeads, membranes, or solid surfaces).

[0066] In a related aspect, the disclosure provides arrangements of eukaryotic cells (*e.g.*, plant cells) having improved HDR frequencies in the systems, methods, and compositions described herein, such as arrangements of cells convenient for screening purposes or for high-throughput and/or multiplex transformation or gene editing experiments. In an embodiment, the disclosure provides an arrangement of multiple cells comprising: (a) the HDR promoting agents; and optionally (b) genome editing molecules. In certain embodiments, the arrangements of cells can further comprise at least one chemical, enzymatic, or physical delivery agent. In another embodiment, the disclosure provides an array including a plurality of containers, each including at least one cell having increased HDR-mediated genome modification frequencies. In an embodiment, the disclosure provides arrangements of cells having the HDR promoting agents and optionally the genome editing molecules, wherein the cells are in an arrayed format, for example, in multi-well plates, encapsulated or enclosed in vesicles, liposomes, or droplets (useful, (*e.g.*, in a microfluidics device), or attached discretely to a matrix or to discrete particles or beads; a specific embodiment is such an arrangement of multiple cells having increased HDR-mediated genome modification frequencies provided in an arrayed format, further including at least one genome editing molecules (*e.g.*, an RNA-guided DNA nuclease, at least one guide RNA, or a ribonucleoprotein including both an RNA-guided DNA nuclease and at least one guide RNA), which may be different for at least some locations on the array or even for each location on the array, and optionally at least one chemical, enzymatic, or physical delivery agent.

[0067] In the systems and methods provided herein, eukaryotic cells (*e.g.*, plant cells) can be exposed to one or more HDR promoting agents and/or one or more gene editing molecules in any temporal order. In certain embodiments, the HDR promoting agents and gene editing molecules are provided simultaneously. In other embodiments, the genome editing molecules are provided after the HDR promoting agents are provided. In other embodiments, the gene editing molecules are provided before the HDR promoting agents are provided. In summary, the HDR promoting agents can be provided to a eukaryotic cell (*e.g.*, a plant cell) either previous to, concurrently with, or subsequent to exposing the cell to the gene editing molecules.

[0068] Eukaryotic cells (*e.g.,* plant cells) having increased Homology Directed Repair (HDR)-mediated genome modification frequencies conferred by HDR promoting agents (*e.g.,* SSAP, exonucleases, and SSB) and/or modified DNA donor templates are provided herein. Also provided by the disclosure are compositions derived from or grown from the plant cell or plant protoplast having increased HDR-mediated genome modification frequencies, provided by the systems and methods disclosed herein; such compositions include multiple protoplasts or cells, callus, a somatic embryo, a somatic meristem, embryogenic callus, or a regenerated plant grown from the plant cell or plant protoplast having increased HDR-mediated genome modification frequencies. Increased HDR-mediated genome modification frequencies in cells that have been subjected to HDR promoting agents and/or modified DNA donor templates can be assessed by a variety of techniques. In certain embodiments, such techniques can compare the frequency of HDR observed in cells subjected to the HDR promoting agents versus the frequency of HDR in control cells that were not subjected to HDR promoting agents (*e.g.,* SSAP, exonucleases, and SSB) and/or modified DNA donor templates.

[0069] In certain embodiments, the eukaryotic cells (*e.g.,* plant cells) used in the systems, methods, and compositions provided herein can include non-dividing cells. Such non-dividing cells can include plant cell protoplasts, eukaryotic cells subjected to one or more of a genetic and/or pharmaceutically-induced cell-cycle blockage, and the like. In certain embodiments, the non-dividing cells can be induced to divide (*e.g.,* by reversing or removing a genetic or pharmaceutical cell-cycle blockages) following treatment with the HDR-promoting agents (*e.g.,* SSAP, exonucleases, and SSB) and/or gene-editing molecules that can optionally include modified DNA donor templates provided herein.

[0070] In certain embodiments, the eukaryotic cells (*e.g.,* plant cells) in used in the systems, methods, and compositions provided herein can include dividing cells. Dividing cells can include those cells found in various plant tissues including leaves, meristems, and embryos. These tissues include, but are not limited to dividing cells from young maize leaf, meristems and scutellar tissue from about 8 or 10 to about 12 or 14 days after pollination (DAP) embryos. The isolation of maize embryos has been described in several publications (Brettschneider, Becker, and Lörz 1997; Leduc *et al.* 1996; Frame *et al.* 2011; K. Wang and Frame 2009). In certain embodiments, basal leaf tissues (*e.g.,* leaf tissues located about 0 to 3 cm from the ligule of a maize plant; Kirienko, Luo, and Sylvester 2012) are targeted for HDR-mediated gene editing. Methods for obtaining regenerable plant structures and

regenerating plants from the HDR-mediated gene editing of plant cells provided herein can be adapted from methods disclosed in US Patent Application Publication No. 20170121722, which is incorporated herein by reference in its entirety and specifically with respect to such disclosure. In certain embodiments, single plant cells subjected to the HDR-mediated gene editing will give rise to single regenerable plant structures. In certain embodiments, the single regenerable plant cell structure can form from a single cell on, or within, an explant that has been subjected to the HDR-mediated gene editing.

vii. Plant regeneration

[0071] In some embodiments, methods provided herein can include the additional step of growing or regenerating a plant from a plant cell that had been subjected to the improved HDR-mediated gene editing or from a regenerable plant structure obtained from that plant cell. In certain embodiments, the plant can further comprise an inserted transgene, a target gene edit, or genome edit as provided by the methods and compositions disclosed herein. In certain embodiments, callus is produced from the plant cell, and plantlets and plants produced from such callus. In other embodiments, whole seedlings or plants are grown directly from the plant cell without a callus stage. Thus, additional related aspects are directed to whole seedlings and plants grown or regenerated from the plant cell or plant protoplast having a target gene edit or genome edit, as well as the seeds of such plants. In certain embodiments wherein the plant cell or plant protoplast is subjected to genetic modification (for example, genome editing by means of, *e.g.*, an RNA-guided DNA nuclease), the grown or regenerated plant exhibits a phenotype associated with the genetic modification. In certain embodiments, the grown or regenerated plant includes in its genome two or more genetic or epigenetic modifications that in combination provide at least one phenotype of interest. In certain embodiments, a heterogeneous population of plant cells having a target gene edit or genome edit, at least some of which include at least one genetic or epigenetic modification, is provided by the method; related aspects include a plant having a phenotype of interest associated with the genetic or epigenetic modification, provided by either regeneration of a plant having the phenotype of interest from a plant cell or plant protoplast selected from the heterogeneous population of plant cells having a target gene or genome edit, or by selection of a plant having the phenotype of interest from a heterogeneous population of plants grown or regenerated from the population of plant cells having a target gene edit or genome edit. Examples of phenotypes of interest include herbicide resistance, improved tolerance of

abiotic stress (*e.g.*, tolerance of temperature extremes, drought, or salt) or biotic stress (*e.g.*, resistance to nematode, bacterial, or fungal pathogens), improved utilization of nutrients or water, modified lipid, carbohydrate, or protein composition, improved flavor or appearance, improved storage characteristics (*e.g.*, resistance to bruising, browning, or softening), increased yield, altered morphology (*e.g.*, floral architecture or color, plant height, branching, root structure). In an embodiment, a heterogeneous population of plant cells having a target gene edit or genome edit (or seedlings or plants grown or regenerated therefrom) is exposed to conditions permitting expression of the phenotype of interest; *e.g.*, selection for herbicide resistance can include exposing the population of plant cells having a target gene edit or genome edit (or seedlings or plants grown or regenerated therefrom) to an amount of herbicide or other substance that inhibits growth or is toxic, allowing identification and selection of those resistant plant cells (or seedlings or plants) that survive treatment. Methods for obtaining regenerable plant structures and regenerating plants from plant cells or regenerable plant structures can be adapted from published procedures (Roest and Gilissen, Acta Bot. Neerl., 1989, 38(1), 1-23; Bhaskaran and Smith, Crop Sci. 30(6):1328-1337; Ikeuchi *et al.*, Development, 2016, 143: 1442-1451). Methods for obtaining regenerable plant structures and regenerating plants from plant cells or regenerable plant structures can also be adapted from US Patent Application Publication No. 20170121722, which is incorporated herein by reference in its entirety and specifically with respect to such disclosure. Also provided are heterogeneous populations, arrays, or libraries of such plants, succeeding generations or seeds of such plants grown or regenerated from the plant cells or plant protoplasts, having a target gene edit or genome edit, parts of the plants (including plant parts used in grafting as scions or rootstocks), or products (*e.g.*, fruits or other edible plant parts, cleaned grains or seeds, edible oils, flours or starches, proteins, and other processed products) made from the plants or their seeds. Embodiments include plants grown or regenerated from the plant cells having a target gene edit or genome edit, wherein the plants contain cells or tissues that do not have a genetic or epigenetic modification, *e.g.*, grafted plants in which the scion or rootstock contains a genetic or epigenetic modification, or chimeric plants in which some but not all cells or tissues contain a genetic or epigenetic modification. Plants in which grafting is commonly useful include many fruit trees and plants such as many citrus trees, apples, stone fruit (*e.g.*, peaches, apricots, cherries, and plums), avocados, tomatoes, eggplant, cucumber, melons, watermelons, and grapes as well as various ornamental plants such as roses. Grafted plants can be grafts between the same or different (generally related)

species. Additional related aspects include a hybrid plant provided by crossing a first plant grown or regenerated from a plant cell or plant protoplast having a target gene edit or genome edit and having at least one genetic or epigenetic modification, with a second plant, wherein the hybrid plant contains the genetic or epigenetic modification; also contemplated is seed produced by the hybrid plant. Also envisioned as related aspects are progeny seed and progeny plants, including hybrid seed and hybrid plants, having the regenerated plant as a parent or ancestor. The plant cells and derivative plants and seeds disclosed herein can be used for various purposes useful to the consumer or grower. The intact plant itself may be desirable, *e.g.*, plants grown as cover crops or as ornamentals. In other embodiments, processed products are made from the plant or its seeds, such as extracted proteins, oils, sugars, and starches, fermentation products, animal feed or human food, wood and wood products, pharmaceuticals, and various industrial products.

viii. Provision of HDR promoting agents to a eukaryotic cell

[0072] An SSAP, exonuclease, and/or SSB that increase HDR frequency can be provided to a eukaryotic cell (*e.g.*, a plant cell or plant protoplast) by any suitable technique. In certain embodiments, the SSAP, exonuclease, and/or SSB is provided by directly contacting a cell with the SSAP, exonuclease, and/or SSB or the polynucleotide that encodes the SSAP, exonuclease, and/or SSB. In certain embodiments, the SSAP, exonuclease, and/or SSB is provided by transporting the SSAP, exonuclease, and/or SSB or a polynucleotide that encodes SSAP, exonuclease, and/or SSB into a cell using a chemical, enzymatic, or physical agent. In certain embodiments, the SSAP, exonuclease, and/or SSB is provided by bacterially mediated (*e.g., Agrobacterium* sp., *Rhizobium* sp., *Sinorhizobium* sp., *Mesorhizobium* sp., *Bradyrhizobium* sp., *Azobacter* sp., *Phyllobacterium* sp.) transfection of a plant cell or plant protoplast with a polynucleotide encoding the SSAP, exonuclease, and/or SSB; see, *e.g.*, Broothaerts *et al.* (2005) *Nature*, 433:629 – 633. In an embodiment, the SSAP, exonuclease, and/or SSB is provided by transcription in a plant cell or plant protoplast of a DNA that encodes the SSAP, exonuclease, and/or SSB and is stably integrated in the genome of the plant cell or is provided to the plant cell or plant protoplast in the form of a plasmid or expression vector (*e.g.,* a viral vector) that encodes the SSAP, exonuclease, and/or SSB. In certain embodiments, the SSAP, exonuclease, and/or SSB is provided to the plant cell or plant protoplast as a polynucleotide that encodes SSAP, exonuclease, and/or SSB, *e.g.*, in the form of an RNA (*e.g.,* mRNA or RNA containing an internal ribosome entry site (IRES))

encoding the SSAP, exonuclease, and/or SSB. In certain embodiments, the SSAP, exonuclease, and/or SSB is provided to the plant cell or plant protoplast as a polynucleotide that encodes a polyprotein comprising in any order the SSAP, exonuclease, and/or SSB with amino acid sequences comprising protease recognition sites or self-processing protein sequences inserted between the encoded SSAP, exonuclease, and/or SSB. Examples of such protease recognition sequences include a spacer region of a plant metallothionein-like protein (PsMTa) which can be cleaved by endogenous plant proteases (Unwin *et al.*, 1998) or a recognition sequence of a specific protease (*e.g.,* the TVMV Nia proteinase; Dasgupta, *et al.*, 1998) which is also provided in the cell. Examples of such self-processing protein sequences include a foot-and-mouth disease virus (FMDV) 2A sequence (SEQ ID NO:33; Halpin, C., et al, 1999). Genome editing molecules can also be introduced into the plant cells by similar techniques.

ix. Transient expression of HDR promoting agents

[0073] In certain embodiments of the methods, systems, cells, and compositions provided herein, transient expression of the HDR promoting agents and/or genome editing molecules is used. Transient expression of an SSAP, exonuclease, and/or SSB that increase HDR frequency or genome editing molecules can be achieved by a variety of techniques. In some embodments, expression of a HDR promoting agent is inducible. In certain embodiments, the SSAP, exonuclease, SSB, and/or genome editing molecules are provided directly to the cells, systems, methods, and compositions as isolated molecules, as isolated or semi-purified products of a cell free synthetic process (*e.g., in vitro* translation), or as isolated or semi-purified products of in a cell-based synthetic process (*e.g.,* such as in a bacterial or other cell lysate). In certain embodiments, SSAP, exonuclease, SSB, and/or genome editing molecules) are targeted to the cell or cell nucleus in a manner that insures transient expression (*e.g.,* by methods adapted from Gao *et al.* 2016; or Li *et al.* 2009). In certain embodiments, the SSAP, exonuclease, SSB, and/or genome editing molecules are delivered into the cell by delivery of the SSAP, exonuclease, SSB, and/or genome editing molecule in the absence of any polynucleotide that encodes the SSAP, exonuclease, SSB, and/or genome editing molecule. Examples of exogenous agents that can be delivered in the absence of any encoding polynucleotides include SSAP, exonuclease, SSB, sequence-specific endonucleases, and RNA guides. RNA-guided DNA binding polypeptide/RNA guides can be delivered separately and/or as RNP complexes. In certain embodiments, SSAP, exonuclease, and/or

SSB proteins can be produced in a heterologous system, purified and delivered to plant cells by particle bombardment (*e.g.*, by methods adapted from Martin-Ortigosa and Wang 2014). In embodiments where the SSAP, exonuclease, and/or SSBs are delivered in the absence of any encoding polynucleotides, the delivered agent is expected to degrade over time in the absence of ongoing expression from any introduced encoding polynucleotides to result in transient expression. In certain embodiments, the SSAP, exonuclease, and/or SSB is delivered into the cell by delivery of a polynucleotide that encodes the SSAP, exonuclease, and/or SSB. In certain embodiments, SSAP, exonuclease, and/or SSB can be encoded on a bacterial plasmid and delivered to plant tissue by particle bombardment (*e.g.*, by methods adapted from Hamada *et al.* 2018; or Kirienko, Luo, and Sylvester 2012). In certain embodiments, SSAP, exonuclease, and/or SSB can be encoded on a T-DNA and transiently transferred to plant cells using agrobacterium (*e.g.*, by methods adapted from Leonelli *et al.* 2016; or Wu *et al.* 2014). In certain embodiments, SSAP, exonuclease, and/or SSB can be encoded in a viral genome and delivered to plants (*e.g.*, by methods adapted from Honig *et al.* 2015). In certain embodiments, SSAP, exonuclease, and/or SSB can be encoded in mRNA or an RNA comprising an IRES and delivered to target cells. In certain embodiments where the SSAP, exonuclease, and/or SSB comprises an RNA-guided DNA binding polypeptide and an RNA guide, the polypeptide or guide can be delivered by a combination of: (i) an encoding polynucleotide for either polypeptide or the guide; and (ii) either polypeptide or the guide itself in the absence of an encoding polynucleotide. In certain embodiments, the SSAP, exonuclease, and/or SSB is delivered into the plant cell by delivery of a polynucleotide that encodes the HDR promoting agent. In certain embodiments, the polynucleotide that encodes the SSAP, exonuclease, and/or SSB is not integrated into a plant cell genome (*e.g.*, as a polynucleotide lacking sequences that provide for integration, by agroinfiltration on an integration deficient T-DNA vector or system, or in a viral vector), is not operably linked to polynucleotides which provide for autonomous replication, and/or only provided with factors (*e.g.*, viral replication proteins) that provide for autonomous replication. Suitable techniques for transient expression including biolistic and other delivery of polynucleotides, agroinfiltration, and use of viral vectors disclosed by Canto, 2016 and others can be adapted for transient expression of the SSAP, exonuclease, and/or SSB provided herein. Transient expression of the agent encoded by a non-integrated polynucleotide effectuated by excision of the polynucleotide and/or regulated expression of the agent. In certain embodiments, the polynucleotide that encodes the SSAP, exonuclease, and/or SSB is integrated into a

eukaryotic cell genome (*e.g.,* a plant nuclear or plastid genome) and transient expression of the agent is effectuated by excision of the polynucleotide and/or regulated expression of the SSAP, exonuclease, and/or SSB. Excision of a polynucleotide encoding the agent can be provided by use of site-specific recombination systems (*e.g.,* Cre-Lox, FLP-FRT). Regulated expression of the agent can be effectuated by methods including: (i) operable linkage of the polynucleotide encoding the agent to a developmentally-regulated, de-repressible, and/or inducible promoter; and/or (ii) introduction of a polynucleotide (*e.g.,* dsRNA or a miRNA) that can induce siRNA-mediated inhibition of the agent. Suitable site-specific recombination systems as well as developmentally-regulated, de-repressible, and/or inducible promoters include those disclosed in US Patent Application Publication No. 20170121722, which is incorporated herein by reference in its entirety and specifically with respect to such disclosure.

[0074] Polynucleotides that can be used to effectuate transient expression of an SSAP, exonuclease, SSB, and/or genome editing molecules (*e.g.*, a polynucleotide encoding an SSAP, exonuclease, SSB, sequence-specific endonuclease, RNA-guided endonuclease, and/or a guide RNA) include: (a) double-stranded RNA; (b) single-stranded RNA; (c) chemically modified RNA; (d) double-stranded DNA; (e) single-stranded DNA; (f) chemically modified DNA; or (g) a combination of (a) – (f). Certain embodiments of the polynucleotide further include additional nucleotide sequences that provide useful functionality; non-limiting examples of such additional nucleotide sequences include an aptamer or riboswitch sequence, nucleotide sequence that provides secondary structure such as stem-loops or that provides a sequence-specific site for an enzyme (*e.g.*, a sequence-specific recombinase or endonuclease site), T-DNA (*e.g.*, DNA sequence encoding an SSAP, exonuclease, and/or SSB is enclosed between left and right T-DNA borders from *Agrobacterium* spp. or from other bacteria that infect or induce tumors in plants), a DNA nuclear-targeting sequence, a regulatory sequence such as a promoter sequence, and a transcript-stabilizing or -destabilizing sequence. Certain embodiments of the polynucleotide include those wherein the polynucleotide is complexed with, or covalently or non-covalently bound to, a non-nucleic acid element, *e.g.*, a carrier molecule, an antibody, an antigen, a viral movement protein, a cell-penetrating or pore-forming peptide, a polymer, a detectable label, a quantum dot, or a particulate or nanoparticulate. In some embodiments, one or more of the components provided herein is transiently expressed by induction of an inducible promoter.

x. Delivery of HDR promoting agents

[0075] Various treatments are useful in delivery of gene editing molecules and/or an SSAP, exonuclease, and/or SSB that increase HDR frequency to a eukaryotic cell (*e.g.,* a plant cell). In certain embodiments, one or more treatments is employed to deliver the HDR promoting agent (*e.g.,* comprising a polynucleotide, polypeptide or combination thereof) into a eukaryotic or plant cell, *e.g.*, through barriers such as a cell wall, a plasma membrane, a nuclear envelope, and/or other lipid bilayer. In certain embodiments, a polynucleotide-, polypeptide-, or RNP-containing composition comprising the agent(s) are delivered directly, for example by direct contact of the composition with a eukaryotic cell. Aforementioned compositions can be provided in the form of a liquid, a solution, a suspension, an emulsion, a reverse emulsion, a colloid, a dispersion, a gel, liposomes, micelles, an injectable material, an aerosol, a solid, a powder, a particulate, a nanoparticle, or a combination thereof can be applied directly to a eukaryotic cell, eukaryotic tissue, eukaryotic organ, eukaryotic organism, plant, plant part, plant cell, or plant explant (*e.g.*, through abrasion or puncture or otherwise disruption of the cell wall or cell membrane, by spraying or dipping or soaking or otherwise directly contacting, by microinjection). For example, a plant cell or plant protoplast is soaked in a liquid SSAP, exonuclease, and/or SSB- containing composition, whereby the agent is delivered to the plant cell. In certain embodiments, the agent-containing composition is delivered using negative or positive pressure, for example, using vacuum infiltration or application of hydrodynamic or fluid pressure. In certain embodiments, the agent-containing composition is introduced into a plant cell or plant protoplast, *e.g.*, by microinjection or by disruption or deformation of the cell wall or cell membrane, for example by physical treatments such as by application of negative or positive pressure, shear forces, or treatment with a chemical or physical delivery agent such as surfactants, liposomes, or nanoparticles; see, *e.g.*, delivery of materials to cells employing microfluidic flow through a cell-deforming constriction as described in US Published Patent Application 2014/0287509, incorporated by reference in its entirety herein. Other techniques useful for delivering the agent-containing composition to a eukaryotic cell, plant cell or plant protoplast include: ultrasound or sonication; vibration, friction, shear stress, vortexing, cavitation; centrifugation or application of mechanical force; mechanical cell wall or cell membrane deformation or breakage; enzymatic cell wall or cell membrane breakage or permeabilization; abrasion or mechanical scarification (*e.g.*, abrasion with carborundum or other particulate abrasive or scarification with a file or sandpaper) or chemical scarification (*e.g.*, treatment with an acid or caustic

agent); and electroporation. In certain embodiments, the agent-containing composition is provided by bacterially mediated (*e.g.*, *Agrobacterium* sp., *Rhizobium* sp., *Sinorhizobium* sp., *Mesorhizobium* sp., *Bradyrhizobium* sp., *Azobacter* sp., *Phyllobacterium* sp.) transfection of the plant cell or plant protoplast with a polynucleotide encoding the agent (*e.g.*, SSAP, exonucleases, SSB, sequence-specific endonuclease, and/or guide RNA); see, *e.g.*, Broothaerts *et al.* (2005) *Nature*, 433:629 – 633. Any of these techniques or a combination thereof are alternatively employed on the plant explant, plant part or tissue or intact plant (or seed) from which a plant cell is optionally subsequently obtained or isolated; in certain embodiments, the agent-containing composition is delivered in a separate step after the plant cell has been isolated. In certain embodiments, the aforementioned methods can also be used to introduce a genome editing molecule into the eukaryotic cell (*e.g.*, plant cell).

[0076] In embodiments, a treatment employed in delivery of a SSAP, exonuclease, and/or SSB that increase HDR frequency to a eukaryotic cell (*e.g.*, plant cell) is carried out under a specific thermal regime, which can involve one or more appropriate temperatures, *e.g.*, chilling or cold stress (exposure to temperatures below that at which normal plant growth occurs), or heating or heat stress (exposure to temperatures above that at which normal plant growth occurs), or treating at a combination of different temperatures. In certain embodiments, a specific thermal regime is carried out on the plant cell, or on a plant, plant explant, or plant part from which a plant cell or plant protoplast is subsequently obtained or isolated, in one or more steps separate from the agent delivery. In certain embodiments, the aforementioned methods can also be used to introduce a genome editing molecule into the eukaryotic cell.

[0077] In certain embodiments of the plant parts, systems, methods, and compositions provided herein, a whole plant or plant part or seed, or an isolated plant cell, a plant explant, or the plant or plant part from which a plant cell or plant protoplast is obtained or isolated, is treated with one or more delivery agents which can include at least one chemical, enzymatic, or physical agent, or a combination thereof. In certain embodiments, an SSAP, exonuclease, and/or SSB that increase HDR frequency further includes one or more than one chemical, enzymatic, or physical agents for delivery. Treatment with the chemical, enzymatic or physical agent can be carried out simultaneously with the agent delivery or in one or more separate steps that precede or follow the agent delivery. In certain embodiments, a chemical, enzymatic, or physical agent, or a combination of these, is associated or complexed with the polynucleotide composition, with the donor template polynucleotide, with the SSAP,

exonuclease, and/or SSB; examples of such associations or complexes include those involving non-covalent interactions (*e.g.*, ionic or electrostatic interactions, hydrophobic or hydrophilic interactions, formation of liposomes, micelles, or other heterogeneous composition) and covalent interactions (*e.g.*, peptide bonds, bonds formed using cross-linking agents). In non-limiting examples, the SSAP, exonuclease, and/or SSB is provided as a liposomal complex with a cationic lipid; the SSAP, exonuclease, and/or SSB is provided as a complex with a carbon nanotube; and/or SSAP, exonuclease, and/or SSB is provided as a fusion protein between the agent and a cell-penetrating peptide. Examples of agents useful for delivering the SSAP, exonuclease, and/or SSB include the various cationic liposomes and polymer nanoparticles reviewed by Zhang *et al.* (2007) *J. Controlled Release*, 123:1 – 10, and the cross-linked multilamellar liposomes described in US Patent Application Publication 2014/0356414 A1, incorporated by reference in its entirety herein. In any of the aforementioned embodiments, it is further contemplated that the aforementioned methods can also be used to introduce a genome-editing molecule into the eukaryotic cell (*e.g.,* plant cell).

[0078] In certain embodiments, the chemical agent used to deliver an SSAP, exonuclease, and/or SSB protein or polynucleotide encoding the same that can increase HDR frequency can comprise:

(a) solvents (*e.g.*, water, dimethylsulfoxide, dimethylformamide, acetonitrile, *N*-pyrrolidine, pyridine, hexamethylphosphoramide, alcohols, alkanes, alkenes, dioxanes, polyethylene glycol, and other solvents miscible or emulsifiable with water or that will dissolve phosphonucleotides in non-aqueous systems);

(b) fluorocarbons (*e.g.*, perfluorodecalin, perfluoromethyldecalin);

(c) glycols or polyols (*e.g.*, propylene glycol, polyethylene glycol);

(d) surfactants, including cationic surfactants, anionic surfactants, non-ionic surfactants, and amphiphilic surfactants, *e.g.*, alkyl or aryl sulfates, phosphates, sulfonates, or carboxylates; primary, secondary, or tertiary amines; quaternary ammonium salts; sultaines, betaines; cationic lipids; phospholipids; tallowamine; bile acids such as cholic acid; long chain alcohols; organosilicone surfactants including nonionic organosilicone surfactants such as trisiloxane ethoxylate surfactants or a silicone polyether copolymer such as a copolymer of polyalkylene oxide modified heptamethyl trisiloxane and allyloxypolypropylene glycol methylether (commercially available as SILWET L-77™ brand surfactant having CAS Number 27306-78-1 and EPA Number CAL. REG. NO. 5905-50073-AA, Momentive Performance Materials, Inc., Albany, N.Y.); specific examples of useful surfactants include

sodium lauryl sulfate, the Tween series of surfactants, Triton-X100, Triton-X114, CHAPS and CHAPSO, Tergitol-type NP-40, Nonidet P-40;

(e) lipids, lipoproteins, lipopolysaccharides;

(f) acids, bases, caustic agents;

(g) peptides, proteins, or enzymes (*e.g.*, cellulase, pectolyase, maceroenzyme, pectinase), including cell-penetrating or pore-forming peptides (*e.g.*, (BO100)2K8, Genscript; poly-lysine, poly-arginine, or poly-homoarginine peptides; gamma zein, see US Patent Application publication 2011/0247100, incorporated herein by reference in its entirety; transcription activator of human immunodeficiency virus type 1 ("HIV-1 Tat") and other Tat proteins, see, *e.g.*, www[dot]lifetein[dot]com/Cell_Penetrating_Peptides[dot]html and Järver (2012) *Mol. Therapy–Nucleic Acids*, 1:e27,1 – 17); octa-arginine or nona-arginine; poly-homoarginine (see Unnamalai *et al.* (2004) *FEBS Letters*, 566:307 – 310); see also the database of cell-penetrating peptides CPPsite 2.0 publicly available at crdd[dot]osdd[dot]net/raghava/cppsite/

(h) RNase inhibitors;

(i) cationic branched or linear polymers such as chitosan, poly-lysine, DEAE-dextran, polyvinylpyrrolidone ("PVP"), or polyethylenimine ("PEI", *e.g.*, PEI, branched, MW 25,000, CAS# 9002-98-6; PEI, linear, MW 5000, CAS# 9002-98-6; PEI linear, MW 2500, CAS# 9002-98-6);

(j) dendrimers (see, *e.g.*, US Patent Application Publication 2011/0093982, incorporated herein by reference in its entirety);

(k) counter-ions, amines or polyamines (*e.g.*, spermine, spermidine, putrescine), osmolytes, buffers, and salts (*e.g.*, calcium phosphate, ammonium phosphate);

(l) polynucleotides (*e.g.*, non-specific double-stranded DNA, salmon sperm DNA);

(m) transfection agents (*e.g.*, Lipofectin®, Lipofectamine®, and Oligofectamine®, and Invivofectamine® (all from Thermo Fisher Scientific, Waltham, MA), PepFect (see Ezzat *et al.* (2011) *Nucleic Acids Res.*, 39:5284 – 5298), TransIt® transfection reagents (Mirus Bio, LLC, Madison, WI), and poly-lysine, poly-homoarginine, and poly-arginine molecules including octo-arginine and nono-arginine as described in Lu *et al.* (2010) *J. Agric. Food Chem.*, 58:2288 – 2294);

(n) antibiotics, including non-specific DNA double-strand-break-inducing agents (*e.g.*, phleomycin, bleomycin, talisomycin); and/or

(o) antioxidants (*e.g.*, glutathione, dithiothreitol, ascorbate).

[0079] In any of the aforementioned embodiments, it is further contemplated that the aforementioned chemical agents can also be used to introduce a genome-editing molecule into the eukaryotic cell (*e.g.,* plant cell).

[0080] In certain embodiments, the chemical agent is provided simultaneously with the SSAP, exonuclease, and/or SSB that increase HDR frequency. In certain embodiments, SSAP, exonuclease, and/or SSB is covalently or non-covalently linked or complexed with one or more chemical agents; for example, an SSAP, exonuclease, SSB and/or sequence-specific endonuclease can be covalently linked to a peptide or protein (*e.g.*, a cell-penetrating peptide or a pore-forming peptide) or non-covalently complexed with cationic lipids, polycations (*e.g.*, polyamines), or cationic polymers (*e.g.*, PEI). In certain embodiments, the SSAP, exonuclease, and/or SSB is complexed with one or more chemical agents to form, *e.g.*, a solution, liposome, micelle, emulsion, reverse emulsion, suspension, colloid, or gel. In any of the aforementioned embodiments, it is further contemplated that genome editing molecules comprising polynucleotides and/or polypeptides can be also be delivered as described above.

[0081] In certain embodiments, the physical agent for delivery of an SSAP, exonuclease, and/or SSB that increase HDR frequency is at least one selected from the group consisting of particles or nanoparticles (*e.g.*, particles or nanoparticles made of materials such as carbon, silicon, silicon carbide, gold, tungsten, polymers, or ceramics) in various size ranges and shapes, magnetic particles or nanoparticles (*e.g.*, silenceMag Magnetotransfection™ agent, OZ Biosciences, San Diego, CA), abrasive or scarifying agents, needles or microneedles, matrices, and grids. In certain embodiments, particulates and nanoparticulates are useful in delivery of the SSAP, exonuclease, and/or SSB. Useful particulates and nanoparticles include those made of metals (*e.g.*, gold, silver, tungsten, iron, cerium), ceramics (*e.g.*, aluminum oxide, silicon carbide, silicon nitride, tungsten carbide), polymers (*e.g.*, polystyrene, polydiacetylene, and poly(3,4-ethylenedioxythiophene) hydrate), semiconductors (*e.g.*, quantum dots), silicon (*e.g.*, silicon carbide), carbon (*e.g.*, graphite, graphene, graphene oxide, or carbon nanosheets, nanocomplexes, or nanotubes), and composites (*e.g.*, polyvinylcarbazole/graphene, polystyrene/graphene, platinum/graphene, palladium/graphene nanocomposites). In certain embodiments, such particulates and nanoparticulates are further covalently or non-covalently functionalized, or further include modifiers or cross-linked materials such as polymers (*e.g.*, linear or branched polyethylenimine, poly-lysine), polynucleotides (*e.g.*, DNA or RNA), polysaccharides, lipids, polyglycols (*e.g.*, polyethylene glycol, thiolated polyethylene glycol), polypeptides or proteins, and detectable labels (*e.g.*, a

fluorophore, an antigen, an antibody, or a quantum dot). In various embodiments, such particulates and nanoparticles are neutral, or carry a positive charge, or carry a negative charge. Embodiments of compositions including particulates include those formulated, *e.g.*, as liquids, colloids, dispersions, suspensions, aerosols, gels, and solids. Embodiments include nanoparticles affixed to a surface or support, *e.g.*, an array of carbon nanotubes vertically aligned on a silicon or copper wafer substrate. Embodiments include polynucleotide compositions including particulates (*e.g.*, gold or tungsten or magnetic particles) delivered by a Biolistic-type technique or with magnetic force. The size of the particles used in Biolistics is generally in the "microparticle" range, for example, gold microcarriers in the 0.6, 1.0, and 1.6 micrometer size ranges (see, *e.g.*, instruction manual for the Helios® Gene Gun System, Bio-Rad, Hercules, CA; Randolph-Anderson *et al.* (2015) "Sub-micron gold particles are superior to larger particles for efficient Biolistic® transformation of organelles and some cell types", Bio-Rad US/EG Bulletin 2015), but successful Biolistics delivery using larger (40 nanometer) nanoparticles has been reported in cultured animal cells; see O'Brian and Lummis (2011) *BMC Biotechnol.*, 11:66 – 71. Other embodiments of useful particulates are nanoparticles, which are generally in the nanometer (nm) size range or less than 1 micrometer, *e.g.*, with a diameter of less than about 1 nm, less than about 3 nm, less than about 5 nm, less than about 10 nm, less than about 20 nm, less than about 40 nm, less than about 60 nm, less than about 80 nm, and less than about 100 nm. Specific, non-limiting embodiments of nanoparticles commercially available (all from Sigma-Aldrich Corp., St. Louis, MO) include gold nanoparticles with diameters of 5, 10, or 15 nm; silver nanoparticles with particle sizes of 10, 20, 40, 60, or 100 nm; palladium "nanopowder" of less than 25 nm particle size; single-, double-, and multi-walled carbon nanotubes, *e.g.*, with diameters of 0.7 – 1.1, 1.3 – 2.3, 0.7 – 0.9, or 0.7 – 1.3 nm, or with nanotube bundle dimensions of 2 – 10 nm by 1- 5 micrometers, 6 – 9 nm by 5 micrometers, 7 – 15 nm by 0.5 – 10 micrometers, 7 – 12 nm by 0.5 – 10 micrometers, 110 – 170 nm by 5 – 9 micrometers, 6 – 13 nm by 2.5 – 20 micrometers. In certain embodiments, physical agents for delivery of an SSAP, exonuclease, and/or SSBs can include materials such as gold, silicon, cerium, or carbon, *e.g.*, gold or gold-coated nanoparticles, silicon carbide whiskers, carborundum, porous silica nanoparticles, gelatin/silica nanoparticles, nanoceria or cerium oxide nanoparticles (CNPs), carbon nanotubes (CNTs) such as single-, double-, or multi-walled carbon nanotubes and their chemically functionalized versions (*e.g.*, carbon nanotubes functionalized with amide, amino, carboxylic acid, sulfonic acid, or polyethylene glycol moieties), and graphene or graphene

oxide or graphene complexes. Such physical agents that can be adapted for delivery of SSAP, exonuclease, and/or SSBs include those disclosed in Wong *et al.* (2016) *Nano Lett.*, 16:1161 – 1172; Giraldo *et al.* (2014) *Nature Materials*, 13:400-409; Shen *et al.* (2012) *Theranostics*, 2:283 – 294; Kim *et al.* (2011) *Bioconjugate Chem.*, 22:2558 – 2567; Wang *et al.* (2010) *J. Am. Chem. Soc. Comm.*, 132:9274 – 9276; Zhao *et al.* (2016) *Nanoscale Res. Lett.*, 11:195 – 203; and Choi *et al.* (2016) *J. Controlled Release*, 235:222 – 235. See also, for example, the various types of particles and nanoparticles, their preparation, and methods for their use, *e.g.*, in delivering polynucleotides and polypeptides to cells, disclosed in US Patent Application Publications 2010/0311168, 2012/0023619, 2012/0244569, 2013/0145488, 2013/0185823, 2014/0096284, 2015/0040268, 2015/0047074, and 2015/0208663, all of which are incorporated herein by reference in their entirety. In any of the aforementioned embodiments, it is further contemplated that genome editing molecules comprising polynucleotides and/or polypeptides can be also be delivered as described above.

[0082] In some embodiments "provided" as used herein includes bringing together the components in a nucleus of a cell. In some embodiments, providing of one or more components is in the form of delivery of a polypeptide. In some embodiments, delivery of one or more components is in the form of a polypeptide complexed with a polynucleotide. In some embodiments, delivery of one or more components is in the form of a ribonucleoprotein (RNP). In some embodiments, Cas and guide RNA are delivered as ribonucleoproteins. In some embodiments the RNP is delivered to a cell using lipofection or electroporation. In some embodiments, the polypeptide or RNP is delivered to a cell through biolistics. In some embodiments, the polypeptide or RNP is delivered to a cell through PEG-mediated transfection. In some embodiments, components are delivered by sexual crossing.

[0083] In some embodiments, the components are provided as RNA or as DNA. For example in some embodiments, one or more components are provided as mRNA. In some embodiments, the mRNA encodes a protein that is one of the components. In some embodiments, the mRNA is translated in the cell to produce one or more components.

[0084] In some embodiments, one or more components are provided as a nucleic acid integrated into a chromosome.

[0085] In some embodiments, 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). In some embodiments, the progenitor cell is any one of the cells described herein, *e.g.*, a plant, animal, fungal, or other eukaryotic cell. In some embodiments, the progenitor cell does not comprise at least one of the sequence-specific endonuclease, the donor template DNA molecule, the SSAP, the exonuclease, and the SSB protein. In some embodiments, the at least one of the sequence-specific endonuclease, the donor template DNA molecule, the SSAP, the exonuclease, and the SSB protein 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. In some embodiments, components are provided as shown in **Table A**, below.

Table A: Combinations of components provided by progenitor cell or by delivery and/or sexual crossing of the progenitor cell

Combination Number	Component(s) Provided by progenitor Cell	Component(s) Provided by Delivery and/or sexual crossing of the progenitor cell
1	Donor template DNA molecule SSAP Exonuclease SSB	Sequence-specific endonuclease
2	Sequence-specific endonuclease SSAP Exonuclease SSB	Donor template DNA molecule
3	Sequence-specific endonuclease Donor template DNA molecule Exonuclease SSB	SSAP
4	Sequence-specific endonuclease Donor template DNA molecule SSAP SSB	Exonuclease
5	Sequence-specific endonuclease Donor template DNA molecule SSAP Exonuclease	SSB
6	SSAP Exonuclease 9SSB	Sequence-specific endonuclease Donor template DNA molecule
7	Donor template DNA molecule Exonuclease SSB	Sequence-specific endonuclease SSAP
8	Donor template DNA molecule SSAP SSB	Sequence-specific endonuclease Exonuclease
9	Donor template DNA molecule SSAP Exonuclease	Sequence-specific endonuclease SSB

10	SSAP Exonuclease SSB	Donor template DNA molecule Sequence-specific endonuclease
11	Sequence-specific endonuclease Exonuclease SSB	Donor template DNA molecule SSAP
12	Sequence-specific endonuclease SSAP SSB	Donor template DNA molecule Exonuclease
13	Sequence-specific endonuclease SSAP Exonuclease	Donor template DNA molecule SSB
14	Donor template DNA molecule Exonuclease SSB	SSAP Sequence-specific endonuclease
15	Sequence-specific endonuclease Exonuclease SSB	SSAP Donor template DNA molecule
16	Sequence-specific endonuclease Donor template DNA molecule SSB	SSAP Exonuclease
17	Sequence-specific endonuclease Donor template DNA molecule Exonuclease	SSAP SSB
18	Donor template DNA molecule SSAP SSB	Exonuclease Sequence-specific endonuclease
19	Sequence-specific endonuclease SSAP SSB	Exonuclease Donor template DNA molecule
20	Sequence-specific endonuclease Donor template DNA molecule SSB	Exonuclease SSAP
21	Sequence-specific endonuclease Donor template DNA molecule SSAP	Exonuclease SSB
22	Donor template DNA molecule SSAP Exonuclease	SSB Sequence-specific endonuclease
23	Sequence-specific endonuclease SSAP Exonuclease	SSB Donor template DNA molecule
24	Sequence-specific endonuclease Donor template DNA molecule Exonuclease	SSB SSAP
25	Sequence-specific endonuclease Donor template DNA molecule SSAP	SSB Exonuclease
26	Sequence-specific endonuclease Donor template DNA molecule	SSAP Exonuclease SSB
27	Sequence-specific endonuclease SSAP	Donor template DNA molecule Exonuclease SSB
28	Sequence-specific endonuclease Exonuclease	Donor template DNA molecule SSAP SSB
29	Sequence-specific endonuclease SSB	Donor template DNA molecule SSAP

		Exonuclease
30	Donor template DNA molecule Sequence-specific endonuclease	SSAP Exonuclease SSB
31	Donor template DNA molecule SSAP	Sequence-specific endonuclease Exonuclease SSB
32	Donor template DNA molecule Exonuclease	Sequence-specific endonuclease SSAP SSB
33	Donor template DNA molecule SSB	Sequence-specific endonuclease SSAP Exonuclease
34	SSAP Sequence-specific endonuclease	Donor template DNA molecule Exonuclease SSB
35	SSAP Donor template DNA molecule	Sequence-specific endonuclease Exonuclease SSB
36	SSAP Exonuclease	Sequence-specific endonuclease Donor template DNA molecule SSB
37	SSAP SSB	Sequence-specific endonuclease Donor template DNA molecule Exonuclease
38	Exonuclease Sequence-specific endonuclease	Donor template DNA molecule SSAP SSB
39	Exonuclease Donor template DNA molecule	Sequence-specific endonuclease SSAP SSB
40	Exonuclease SSAP	Sequence-specific endonuclease Donor template DNA molecule SSB
41	Exonuclease SSB	Sequence-specific endonuclease Donor template DNA molecule SSAP
42	SSB Sequence-specific endonuclease	Donor template DNA molecule SSAP Exonuclease
43	SSB Donor template DNA molecule	Sequence-specific endonuclease SSAP Exonuclease
44	SSB SSAP	Sequence-specific endonuclease Donor template DNA molecule Exonuclease
45	SSB Exonuclease	Sequence-specific endonuclease Donor template DNA molecule SSAP
46	Sequence-specific endonuclease	Donor template DNA molecule SSAP Exonuclease SSB
47	Donor template DNA molecule	Sequence-specific endonuclease SSAP Exonuclease SSB
48	SSAP	Sequence-specific endonuclease Donor template DNA molecule

		Exonuclease SSB
49	Exonuclease	Sequence-specific endonuclease Donor template DNA molecule SSAP SSB
50	SSB	Sequence-specific endonuclease Donor template DNA molecule SSAP Exonuclease

xi. Gene editing molecules

[0086] In certain embodiments wherein the gene editing molecules comprise a gRNA (or polynucleotide encoding the gRNA) is provided in a composition that further includes an RNA guided DNA binding polypeptide that is nuclease activity deficient (or a polynucleotide that encodes the same), one or more one chemical, enzymatic, or physical agent can similarly be employed. In certain embodiments, the RNA guide and the nuclease activity deficient RNA-guided DNA binding polypeptide (ndRGDBP) or polynucleotide encoding the same) are provided separately, *e.g.*, in a separate composition. Such compositions can include other chemical or physical agents (*e.g.*, solvents, surfactants, proteins or enzymes, transfection agents, particulates or nanoparticulates), such as those described above as useful in the polynucleotide compositions. For example, porous silica nanoparticles are useful for delivering a DNA recombinase into maize cells; see, *e.g.*, Martin-Ortigosa *et al.* (2015) *Plant Physiol.*, 164:537 – 547, and can be adapted to providing a ndRGDBP or polynucleotide encoding the same into a maize or other plant cell. In one embodiment, the polynucleotide composition includes a gRNA and the ndRGDBP, and further includes a surfactant and a cell-penetrating peptide (CPP) which can be operably linked to the ndRGDBP. In an embodiment, the polynucleotide composition includes a plasmid or viral vector that encodes both the gRNA and the ndRGDBP, and further includes a surfactant and carbon nanotubes. In an embodiment, the polynucleotide composition includes multiple gRNAs and an mRNA encoding the ndRGDBP, and further includes particles (*e.g.,* gold or tungsten particles), and the polynucleotide composition is delivered to a plant cell or plant protoplast by Biolistics. In any of the aforementioned embodiments, it is further contemplated that other polynucleotides of interest including genome editing molecules can also be delivered before, during, or after delivery of the gRNA and the ndRGDBP.

[0087] In certain embodiments, the plant, plant explant, or plant part from which a plant cell is obtained or isolated is treated with one or more chemical, enzymatic, or physical agent(s) in the process of obtaining, isolating, or treating the plant cell. In certain

embodiments, the plant cell, plant, plant explant, or plant part is treated with an abrasive, a caustic agent, a surfactant such as Silwet L-77 or a cationic lipid, or an enzyme such as cellulase. In any of the aforementioned embodiments, it is further contemplated that other polynucleotides of interest including genome editing molecules can also be delivered before, during, or after delivery of the HDR promoting agents.

[0088] In certain embodiments, one or more than one chemical, enzymatic, or physical agent, separately or in combination with the polynucleotide composition encoding the SSAP, exonuclease, and/or SSB that increase HDR frequency, is provided/applied at a location in the plant or plant part other than the plant location, part, or tissue from which the plant cell is treated, obtained, or isolated. In certain embodiments, the polynucleotide composition is applied to adjacent or distal cells or tissues and is transported (*e.g.*, through the vascular system or by cell-to-cell movement) to the meristem from which plant cells are subsequently isolated. In certain embodiments, the polynucleotide-containing composition is applied by soaking a seed or seed fragment or zygotic or somatic embryo in the polynucleotide-containing composition, whereby the polynucleotide is delivered to the plant cell. In certain embodiments, a flower bud or shoot tip is contacted with a polynucleotide-containing composition, whereby the polynucleotide is delivered to cells in the flower bud or shoot tip from which desired plant cells are obtained. In certain embodiments, a polynucleotide-containing composition is applied to the surface of a plant or of a part of a plant (*e.g.*, a leaf surface), whereby the polynucleotide(s) are delivered to tissues of the plant from which desired plant cells are obtained. In certain embodiments a whole plant or plant tissue is subjected to particle- or nanoparticle-mediated delivery (*e.g.*, Biolistics or carbon nanotube or nanoparticle delivery) of a polynucleotide-containing composition, whereby the polynucleotide(s) are delivered to cells or tissues from which plant cells are subsequently obtained. In any of the aforementioned embodiments, it is further contemplated that other polynucleotides of interest including genome editing molecules can also be delivered before, during, or after delivery of the HDR promoting agents.

[0089] Genome editing molecules include gene editing molecules for inducing a genetic modification in the plant cells having increased HDR-mediated genome modification frequencies provided herein. In certain embodiments, such genome editing molecules can include: (i) a polynucleotide selected from the group consisting of an RNA guide for an RNA-guided nuclease, a DNA encoding an RNA guide for an RNA-guided nuclease; (ii) a nuclease selected from the group consisting of an RNA-guided nuclease, an RNA-guided

DNA endonuclease, a type II Cas nuclease, a Cas9, a nCas9, a type V Cas nuclease, a Cas12a, a nCas12a, a CasY, a CasX, a Cas12b, a Cas12c, Cas12i, Cas14, an engineered nuclease, a codon-optimized nuclease, a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TAL-effector nuclease), Argonaute, a meganuclease or engineered meganuclease; (iii) a polynucleotide encoding one or more nucleases capable of effectuating site-specific cleavage of a target nucleotide sequence; and/or (iv) a donor template DNA molecule. In certain embodiments, at least one delivery agent is selected from the group consisting of solvents, fluorocarbons, glycols or polyols, surfactants; primary, secondary, or tertiary amines and quaternary ammonium salts; organosilicone surfactants; lipids, lipoproteins, lipopolysaccharides; acids, bases, caustic agents; peptides, proteins, or enzymes; cell-penetrating peptides; RNase inhibitors; cationic branched or linear polymers; dendrimers; counter-ions, amines or polyamines, osmolytes, buffers, and salts; polynucleotides; transfection agents; antibiotics; chelating agents such as ammonium oxalate, EDTA, EGTA, or cyclohexane diamine tetraacetate, non-specific DNA double-strand-break-inducing agents; and antioxidants; particles or nanoparticles, magnetic particles or nanoparticles, abrasive or scarifying agents, needles or microneedles, matrices, and grids. In certain embodiments, the eukaryotic cell (*e.g.,* plant cell), system, method, or composition comprising the cells provided herein further includes (a) at least one cell having at least one Cas9, nCas9, Cas12a, nCas12a, a CasY, a CasX, a Cas12b, Cas12c, or a Cas12i nuclease or nickase; (b) at least one guide RNA; and (c) optionally, at least one chemical, enzymatic, or physical delivery agent.

[0090] Gene editing molecules of use in the cells, systems, methods, compositions, and reaction mixtures provided herein include molecules capable of introducing a double-strand break ("DSB") in double-stranded DNA, such as in genomic DNA or in a target gene located within the genomic DNA as well as accompanying guide RNA or donor template polynucleotides. Examples of such gene editing molecules include: (a) a nuclease selected from the group consisting of an RNA-guided nuclease, an RNA-guided DNA endonuclease, a type II Cas nuclease, a Cas9, a nCas9 nickase, a type V Cas nuclease, a Cas12a nuclease, a nCas12a nickase, a CasY, a CasX, a Cas12b, a Cas12c, Cas12i, Cas14 an engineered nuclease, a codon-optimized nuclease, a zinc-finger nuclease (ZFN) or nickase, a transcription activator-like effector nuclease (TAL-effector nuclease) or nickase, an Argonaute, and a meganuclease or engineered meganuclease; (b) a polynucleotide encoding one or more nucleases capable of effectuating site-specific alteration (such as introduction of

a DSB) of a target editing site; (c) a guide RNA (gRNA) for an RNA-guided nuclease, or a DNA encoding a gRNA for an RNA-guided nuclease; and (d) donor template polynucleotides.

[0091] CRISPR-type genome editing can be adapted for use in the eukaryotic cells (*e.g.*, plant cells), systems, methods, and compositions provided herein in several ways. CRISPR elements, *i.e.*, gene editing molecules comprising CRISPR endonucleases and CRISPR single-guide RNAs or polynucleotides encoding the same, are useful in effectuating genome editing without remnants of the CRISPR elements or selective genetic markers occurring in progeny. In certain embodiments, the CRISPR elements are provided directly to the eukaryotic cell (*e.g.*, plant cells), systems, methods, and compositions as isolated molecules, as isolated or semi-purified products of a cell free synthetic process (*e.g.*, *in vitro* translation), or as isolated or semi-purified products of in a cell-based synthetic process (*e.g.*, such as in a bacterial or other cell lysate). In certain embodiments, genome-inserted CRISPR elements are useful in plant lines adapted for use in the systems, methods, and compositions provide herein. In certain embodiments, plants or plant cells used in the systems, methods, and compositions provided herein can comprise a transgene that expresses a CRISPR endonuclease (*e.g.*, a Cas9, a Cpf1-type or other CRISPR endonuclease). In certain embodiments, one or more CRISPR endonucleases with unique PAM recognition sites can be used. Guide RNAs (sgRNAs or crRNAs and a tracrRNA) to form an RNA-guided endonuclease/guide RNA complex which can specifically bind sequences in the gDNA target editing site that are adjacent to a protospacer adjacent motif (PAM) sequence. The type of RNA-guided endonuclease typically informs the location of suitable PAM sites and design of crRNAs or sgRNAs. G-rich PAM sites, *e.g.*, 5'-NGG are typically targeted for design of crRNAs or sgRNAs used with Cas9 proteins. T-rich PAM sites (*e.g.*, 5'-TTTV [1], where "V" is A, C, or G) are typically targeted for design of crRNAs or sgRNAs used with Cas12a proteins (*e.g.*, SEQ ID NO:27, 28, 29, and 30). Cpf1 endonuclease and corresponding guide RNAs and PAM sites are disclosed in US Patent Application Publication 2016/0208243 A1, which is incorporated herein by reference for its disclosure of DNA encoding Cpf1 endonucleases and guide RNAs and PAM sites. Introduction of one or more of a wide variety of CRISPR guide RNAs that interact with CRISPR endonucleases integrated into a plant genome or otherwise provided to a plant is useful for genetic editing for providing desired phenotypes or traits, for trait screening, or for gene editing mediated trait introgression (*e.g.*, for introducing a trait into a new genotype without backcrossing to a recurrent parent or with

limited backcrossing to a recurrent parent). Multiple endonucleases can be provided in expression cassettes with the appropriate promoters to allow multiple genome editing in a spatially or temporally separated fashion in either in chromosome DNA or episome DNA.

[0092] CRISPR technology for editing the genes of eukaryotes is disclosed in US Patent Application Publications 2016/0138008A1 and US2015/0344912A1, and in US Patents 8,697,359, 8,771,945, 8,945,839, 8,999,641, 8,993,233, 8,895,308, 8,865,406, 8,889,418, 8,871,445, 8,889,356, 8,932,814, 8,795,965, and 8,906,616. Cpf1 endonuclease and corresponding guide RNAs and PAM sites are disclosed in US Patent Application Publication 2016/0208243 A1. Other CRISPR nucleases useful for editing genomes include Cas12b and Cas12c (see Shmakov *et al.* (2015) *Mol. Cell*, 60:385 – 397) and CasX and CasY (see Burstein *et al.* (2016) *Nature*, doi:10.1038/nature21059). Plant RNA promoters for expressing CRISPR guide RNA and plant codon-optimized CRISPR Cas9 endonuclease are disclosed in International Patent Application PCT/US2015/018104 (published as WO 2015/131101 and claiming priority to US Provisional Patent Application 61/945,700). Methods of using CRISPR technology for genome editing in plants are disclosed in US Patent Application Publications US 2015/0082478A1 and US 2015/0059010A1 and in International Patent Application PCT/US2015/038767 A1 (published as WO 2016/007347 and claiming priority to US Provisional Patent Application 62/023,246). All of the patent publications referenced in this paragraph are incorporated herein by reference in their entirety. In certain embodiments, an RNA-guided endonuclease that leaves a blunt end following cleavage of the target editing site at the endonuclease recognition sequence is used. Blunt-end cutting RNA-guided endonucleases include Cas9, Cas12c, and Cas12h (Yan *et al.*, 2019). In certain embodiments, an RNA-guided endonuclease that leaves a staggered single stranded DNA overhanging end following cleavage of the endonuclease recognition sequence is used. Staggered-end cutting RNA-guided endonucleases include Cas12a, Cas12b, and Cas12e.

[0093] The methods, systems, compositions, eukaryotic cells (*e.g.,* plant cells) can also use sequence-specific endonucleases or sequence-specific endonucleases and guide RNAs that cleave a single DNA strand in a dsDNA at an endonuclease recognition sequence within the target editing site. Such cleavage of a single DNA strand in a dsDNA target editing site is also referred to herein and elsewhere as "nicking" and can be effected by various "nickases" or systems that provide for nicking. Nickases that can be used include nCas9 (Cas9 comprising a D10A amino acid substitution), nCas12a (*e.g.,* Cas12a comprising an R1226A

amino acid substitution; Yamano *et al.*, 2016), Cas12i (Yan *et al.* 2019), a zinc finger nickase *e.g.,* as disclosed in Kim *et al.*, 2012), a TALE nickase (*e.g.,* as disclosed in Wu *et al.*, 2014), or a combination thereof. In certain embodiments, systems that provide for nicking can comprise a Cas nuclease (*e.g.,* Cas9 and/or Cas12a) and guide RNA molecules that have at least one base mismatch to DNA sequences in the target editing site (Fu *et al.*, 2019). In certain embodiments, genome modifications can be introduced into the target editing site by creating single stranded breaks (*i.e.,* "nicks") in genomic locations separated by no more than about 10, 20, 30, 40, 50, 60, 80, 100, 150, or 200 base pairs of DNA. In certain illustrative and non-limiting embodiments, two nickases (*i.e.,* a CAS nuclease which introduces a single stranded DNA break including nCas9, nCas12a, Cas12i, zinc finger nickases, TALE nickases, combinations thereof, and the like) or nickase systems can directed to make cuts to nearby sites separated by no more than about 10, 20, 30, 40, 50, 60, 80 or 100 base pairs of DNA. In instances where an RNA guided nickase and an RNA guide are used, the RNA guides are adjacent to PAM sequences that are sufficiently close (*i.e.,* separated by no more than about 10, 20, 30, 40, 50, 60, 80, 100, 150, or 200 base pairs of DNA). In any of the aforementioned embodiments where a nickase or nickase system is used, an exonuclease with 5' to 3' or 3' to 5' exonuclease activity that can recognize dsDNA substrate having an internal break in one strand can be used. In certain embodiments, 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 can be used in conjunction with the nickase or nickase system, an SSAP, and an SSB.

[0094] For the purposes of gene editing, CRISPR arrays can be designed to contain one or multiple guide RNA sequences corresponding to a desired target DNA sequence; see, for example, Cong *et al.* (2013) *Science,* 339:819–823; Ran *et al.* (2013) *Nature Protocols*, 8:2281 – 2308. At least 16 or 17 nucleotides of gRNA sequence are required by Cas9 for DNA cleavage to occur; for Cpf1 at least 16 nucleotides of gRNA sequence are needed to achieve detectable DNA cleavage and at least 18 nucleotides of gRNA sequence were reported necessary for efficient DNA cleavage *in vitro*; see Zetsche *et al.* (2015) *Cell*, 163:759 – 771. In practice, guide RNA sequences are generally designed to have a length of 17 – 24 nucleotides (frequently 19, 20, or 21 nucleotides) and exact complementarity (*i.e.*, perfect base-pairing) to the targeted gene or nucleic acid sequence; guide RNAs having less than 100% complementarity to the target sequence can be used (*e.g.*, a gRNA with a length of

20 nucleotides and 1 – 4 mismatches to the target sequence) but can increase the potential for off-target effects. The design of effective guide RNAs for use in plant genome editing is disclosed in US Patent Application Publication 2015/0082478 A1, the entire specification of which is incorporated herein by reference. More recently, efficient gene editing has been achieved using a chimeric "single guide RNA" ("sgRNA"), an engineered (synthetic) single RNA molecule that mimics a naturally occurring crRNA-tracrRNA complex and contains both a tracrRNA (for binding the nuclease) and at least one crRNA (to guide the nuclease to the sequence targeted for editing); see, for example, Cong *et al.* (2013) *Science*, 339:819 – 823; Xing *et al.* (2014) *BMC Plant Biol.*, 14:327 – 340. Chemically modified sgRNAs have been demonstrated to be effective in genome editing; see, for example, Hendel *et al.* (2015) *Nature Biotechnol.*, 985 – 991. The design of effective gRNAs for use in plant genome editing is disclosed in US Patent Application Publication 2015/0082478 A1, the entire specification of which is incorporated herein by reference.

[0095] Other sequence-specific endonucleases capable of effecting site-specific modification of a target nucleotide sequence in the systems, methods, and compositions provided herein include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TAL-effector nucleases or TALENs), Argonaute proteins, and a meganuclease or engineered meganuclease. Zinc finger nucleases (ZFNs) are engineered proteins comprising a zinc finger DNA-binding domain fused to a nucleic acid cleavage domain, *e.g.*, a nuclease. The zinc finger binding domains provide specificity and can be engineered to specifically recognize any desired target DNA sequence. For a review of the construction and use of ZFNs in plants and other organisms, see, *e.g.*, Urnov *et al.* (2010) *Nature Rev. Genet.*, 11:636 – 646. The zinc finger DNA binding domains are derived from the DNA-binding domain of a large class of eukaryotic transcription factors called zinc finger proteins (ZFPs). The DNA-binding domain of ZFPs typically contains a tandem array of at least three zinc "fingers" each recognizing a specific triplet of DNA. A number of strategies can be used to design the binding specificity of the zinc finger binding domain. One approach, termed "modular assembly", relies on the functional autonomy of individual zinc fingers with DNA. In this approach, a given sequence is targeted by identifying zinc fingers for each component triplet in the sequence and linking them into a multifinger peptide. Several alternative strategies for designing zinc finger DNA binding domains have also been developed. These methods are designed to accommodate the ability of zinc fingers to contact neighboring fingers as well as nucleotide bases outside their target triplet. Typically, the engineered zinc finger DNA

binding domain has a novel binding specificity, compared to a naturally-occurring zinc finger protein. Engineering methods include, for example, rational design and various types of selection. Rational design includes, for example, the use of databases of triplet (or quadruplet) nucleotide sequences and individual zinc finger amino acid sequences, in which each triplet or quadruplet nucleotide sequence is associated with one or more amino acid sequences of zinc fingers which bind the particular triplet or quadruplet sequence. See, *e.g.*, US Patents 6,453,242 and 6,534,261, both incorporated herein by reference in their entirety. Exemplary selection methods (*e.g.*, phage display and yeast two-hybrid systems) are well known and described in the literature. In addition, enhancement of binding specificity for zinc finger binding domains has been described in US Patent 6,794,136, incorporated herein by reference in its entirety. In addition, individual zinc finger domains may be linked together using any suitable linker sequences. Examples of linker sequences are publicly known, *e.g.*, see US Patents 6,479,626; 6,903,185; and 7,153,949, incorporated herein by reference in their entirety. The nucleic acid cleavage domain is non-specific and is typically a restriction endonuclease, such as FokI. This endonuclease must dimerize to cleave DNA. Thus, cleavage by FokI as part of a ZFN requires two adjacent and independent binding events, which must occur in both the correct orientation and with appropriate spacing to permit dimer formation. The requirement for two DNA binding events enables more specific targeting of long and potentially unique recognition sites. FokI variants with enhanced activities have been described; see, *e.g.*, Guo *et al.* (2010) *J. Mol. Biol.*, 400:96 - 107.

[0096] Transcription activator like effectors (TALEs) are proteins secreted by certain *Xanthomonas* species to modulate gene expression in host plants and to facilitate the colonization by and survival of the bacterium. TALEs act as transcription factors and modulate expression of resistance genes in the plants. Recent studies of TALEs have revealed the code linking the repetitive region of TALEs with their target DNA-binding sites. TALEs comprise a highly conserved and repetitive region consisting of tandem repeats of mostly 33 or 34 amino acid segments. The repeat monomers differ from each other mainly at amino acid positions 12 and 13. A strong correlation between unique pairs of amino acids at positions 12 and 13 and the corresponding nucleotide in the TALE-binding site has been found. The simple relationship between amino acid sequence and DNA recognition of the TALE binding domain allows for the design of DNA binding domains of any desired specificity. TALEs can be linked to a non-specific DNA cleavage domain to prepare sequence-specific endonucleases referred to as TAL-effector nucleases or TALENs. As in the

case of ZFNs, a restriction endonuclease, such as FokI, can be conveniently used. For a description of the use of TALENs in plants, see Mahfouz *et al.* (2011) *Proc. Natl. Acad. Sci. USA*, 108:2623 – 2628 and Mahfouz (2011) *GM Crops*, 2:99 – 103.

[0097] Argonautes are proteins that can function as sequence-specific endonucleases by binding a polynucleotide (*e.g.*, a single-stranded DNA or single-stranded RNA) that includes sequence complementary to a target nucleotide sequence) that guides the Argonaut to the target nucleotide sequence and effects site-specific alteration of the target nucleotide sequence; see, *e.g.*, US Patent Application Publication 2015/0089681, incorporated herein by reference in its entirety.

[0098] In some embodiments, the endonuclease binds to an endonuclease recognition sequence. In some embodiments, the endonuclease cleaves the endonuclease recognition sequence. In some embodiments, the term "endonuclease recognition sequence" is used interchangeably with an endonuclease cleavage site sequence.

[0099] In some embodiments, an endonuclease is not required. In some embodiments, the method is carried out by providing a compound that non-specificially introduces a double strand break. Exemplary double strand break inducing compounds include hydroquinone (HQ), benzoquinone (BQ), benzenetriol (BT), hydrogen peroxide (H2O2), bleomycin (BLM) or sodium ascorbate (Vit C) are used to introduce a double strand break.

[0100] Donor template DNA molecules used in the methods, systems, eukaryotic cells (*e.g.*, plant cells), and compositions provided herein include DNA molecules comprising, from 5' to 3', a first homology arm, a replacement DNA, and a second homology arm, wherein the homology arms containing sequences that are partially or completely homologous to genomic DNA (gDNA) sequences flanking an endonuclease recognition sequence in the gDNA and wherein the replacement DNA can comprise an insertion, deletion, or substitution of 1 or more DNA base pairs relative to the target gDNA. In certain embodiments, a donor DNA template homology arm can be about 20, 50, 100, 200, 400, or 600 to about 800, or 1000 base pairs in length. In certain embodiments, a donor template DNA molecule can be delivered to a eukaryotic cell (*e.g.*, a plant cell) in a circular (*e.g.*, a plasmid or a viral vector including a geminivirus vector) or a linear DNA molecule. In certain embodiments, a circular or linear DNA molecule that is used can comprise a modified donor template DNA molecule comprising, from 5' to 3', a first copy of an endonuclease recognition sequence, the first homology arm, the replacement DNA, the second homology arm, and a second copy of the endonuclease recognition sequence. Without seeking to be

limited by theory, such modified DNA donor template molecules can be cleaved by the same sequence-specific endonuclease that is used to cleave an endonuclease recognition sequences within the target eiditng site genomic DNA of the eukaryotic cell to release a donor template DNA molecule that can participate in HDR-mediated genome modification of the target editing site in the eukaryotic cell genome. In certain embodiments, the donor DNA template can comprise a linear DNA molecule comprising, from 5' to 3', a cleaved endonuclease recognition sequence, the first homology arm, the replacement DNA, the second homology arm, and a cleaved endonuclease recognition sequence. In certain embodiments, the cleaved endonuclease sequence can comprise a blunt DNA end or a blunt DNA end that can optionally comprise a 5' phosphate group. In certain embodiments, the cleaved endonuclease sequence comprises a DNA end having a single-stranded 5' or 3' DNA overhang. Such cleaved endonuclease recognition sequences can be produced by either cleaving an intact target sequence or by synthesizing a copy of the cleaved target sequence-specific endonuclease recognition sequence. Donor DNA templates can be synthesized either chemically or enzymatically (*e.g.,* in a polymerase chain reaction (PCR)).

[0101] Use of donor templates other than double-stranded DNA are also contemplated. For example in some embodiments, a precursor of a double stranded DNA is provided. In some embodiments, an RNA template of a reverse transcriptase is provided. In some embodiments, a revise transcriptase is provided in addition to an RNA. In some embodiments, the method comprises use of a single stranded DNA donor template. In some a single or double stranded RNA template is used. In some embodiments, the method comprises use of a DNA/RNA hybrid. In some embodiments, a PNA is used to generate the donor template.

[0102] In some embodiments, more than one donor template is provided. In some embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more donor templates are provided. In some embodments, the donor templates target the same gene. In some embodiments, the donor templates target different genes in the same pathway. In some embodiments, the donor templates target multiple genes that perform the same function.

[0103] Other genome editing molecules used in plant cells and methods provided herein can be used on plants or cells having transgenes or vectors comprising the same. Such transgenes can confer useful traits that include herbicide tolerance, pest tolerance (*e.g.,* tolerance to insects, nematodes, or plant pathogenic fungi and bacteria), improved yield, increased and/or qualitatively improved oil, starch, and protein content, improved abiotic

stress tolerance (*e.g.*, improved or enhanced water use efficiency or drought tolerance, osmotic stress tolerance, high salinity stress tolerance, heat stress tolerance, enhanced cold tolerance, including cold germination tolerance), and the like. Such transgenes include both transgenes that confer the trait by expression of an exogenous protein as well as transgenes that confer the trait by inhibiting expression of endogenous plant genes (*e.g.*, by inducing an siRNA response which inhibits expression of the endogenous plant genes). Transgenes that can provide such traits are disclosed in US Patent Application Publication Nos. 20170121722 and 20170275636, which are each incorporated herein by reference in their entireties and specifically with respect to such disclosures.

[0104] In some embodiments, one or more polynucleotides or vectors driving expression of one or more polynucleotides encoding any of the aforementioned SSAP, exonuclease, and/or SSBs and/or genome editing molecules are introduced into a eukaryotic cell (*e.g.*, plant cell). In certain embodiments, a polynucleotide vector comprises a regulatory element such as a promoter operably linked to one or more polynucleotides encoding SSAP, exonuclease, and/or SSBs or genome editing molecules. In such embodiments, expression of these polynucleotides can be controlled by selection of the appropriate promoter, particularly promoters functional in a eukaryotic cell (*e.g.*, plant cell); useful promoters include constitutive, conditional, inducible, and temporally or spatially specific promoters (*e.g.*, a tissue specific promoter, a developmentally regulated promoter, or a cell cycle regulated promoter). Developmentally regulated promoters that can be used in plant cells include Phospholipid Transfer Protein (PLTP), fructose-1,6-bisphosphatase protein, NAD(P)-binding Rossmann-Fold protein, adipocyte plasma membrane-associated protein-like protein, Rieske [2Fe-2S] iron-sulfur domain protein, chlororespiratory reduction 6 protein, D-glycerate 3-kinase, chloroplastic-like protein, chlorophyll a-b binding protein 7, chloroplastic-like protein, ultraviolet-B-repressible protein, Soul heme-binding family protein, Photosystem I reaction center subunit psi-N protein, and short-chain dehydrogenase/reductase protein that are disclosed in US Patent Application Publication No. 20170121722, which is incorporated herein by reference in its entirety and specifically with respect to such disclosure. In certain embodiments, the promoter is operably linked to nucleotide sequences encoding multiple guide RNAs, wherein the sequences encoding guide RNAs are separated by a cleavage site such as a nucleotide sequence encoding a microRNA recognition/cleavage site or a self-cleaving ribozyme (see, *e.g.*, Ferré-D'Amaré and Scott (2014) *Cold Spring Harbor Perspectives Biol.*, 2:a003574). In certain embodiments, the promoter is an RNA polymerase

III promoter operably linked to a nucleotide sequence encoding one or more guide RNAs. In certain embodiments, the promoter operably linked to one or more polynucleotides is a constitutive promoter that drives gene expression in eukaryotic cells (*e.g.*, plant cells). In certain embodiments, the promoter drives gene expression in the nucleus or in an organelle such as a chloroplast or mitochondrion. Examples of constitutive promoters for use in plants include a CaMV 35S promoter as disclosed in US Patents 5,858,742 and 5,322,938, a rice actin promoter as disclosed in US Patent 5,641,876, a maize chloroplast aldolase promoter as disclosed in US Patent 7,151,204, and the nopaline synthase (NOS) and octopine synthase (OCS) promoters from *Agrobacterium tumefaciens*. In certain embodiments, the promoter operably linked to one or more polynucleotides encoding elements of a genome-editing system is a promoter from figwort mosaic virus (FMV), a RUBISCO promoter, or a pyruvate phosphate dikinase (PPDK) promoter, which is active in photosynthetic tissues. Other contemplated promoters include cell-specific or tissue-specific or developmentally regulated promoters, for example, a promoter that limits the expression of the nucleic acid targeting system to germline or reproductive cells (*e.g.*, promoters of genes encoding DNA ligases, recombinases, replicases, or other genes specifically expressed in germline or reproductive cells). In certain embodiments, the genome alteration is limited only to those cells from which DNA is inherited in subsequent generations, which is advantageous where it is desirable that expression of the genome-editing system be limited in order to avoid genotoxicity or other unwanted effects. All of the patent publications referenced in this paragraph are incorporated herein by reference in their entirety.

[0105] Expression vectors or polynucleotides provided herein may contain a DNA segment near the 3′ end of an expression cassette that acts as a signal to terminate transcription and directs polyadenylation of the resultant mRNA, and may also support promoter activity. Such a 3’ element is commonly referred to as a “3′-untranslated region” or “3′-UTR” or a “polyadenylation signal.” In some cases, plant gene-based 3’ elements (or terminators) consist of both the 3’-UTR and downstream non-transcribed sequence (Nuccio *et al.*, 2015). Useful 3′ elements include: *Agrobacterium tumefaciens* nos 3′, tml 3′, tmr 3′, tms 3′, ocs 3′, and tr7 3′ elements disclosed in U.S. Pat. No. 6,090,627, incorporated herein by reference, and 3′ elements from plant genes such as the heat shock protein 17, ubiquitin, and fructose-1,6-biphosphatase genes from wheat (*Triticum aestivum*), and the glutelin, lactate dehydrogenase, and beta-tubulin genes from rice (*Oryza sativa*), disclosed in US Patent Application Publication 2002/0192813 A1, incorporated herein by reference.

[0106] In certain embodiments, a vector or polynucleotide comprising an expression cassette includes additional components, *e.g.*, a polynucleotide encoding a drug resistance or herbicide gene or a polynucleotide encoding a detectable marker such as green fluorescent protein (GFP) or *beta*-glucuronidase (*gus*) to allow convenient screening or selection of cells expressing the vector or polynucleotide. Selectable markers include genes that confer resistance to herbicidal compounds, such as glyphosate, sulfonylureas, glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). Such selectable marker genes and selective agents include the maize HRA gene (Lee *et al.*, 1988, EMBO J 7:1241-1248) which confers resistance to sulfonylureas and imidazolinones, the CP4 gene that confers resistance to glyphosate (US Reissue Patent RE039247, specifically incorporated herein by reference in its entirety and with respect to such genes and related selection methods), the GAT gene which confers resistance to glyphosate (Castle *et al.*, 2004, Science 304:1151-1154), genes that confer resistance to spectinomycin such as the aadA gene (Svab *et al.*, 1990, Plant Mol Biol. 14:197-205) and the bar gene that confers resistance to glufosinate ammonium (White *et al.*, 1990, Nucl. Acids Res. 25:1062), and PAT (or moPAT for corn, see Rasco-Gaunt *et al.*, 2003, Plant Cell Rep. 21:569-76; also see Sivamani *et al.*, 2019) and the PMI gene that permits growth on mannose-containing medium (Negrotto *et al.*, 2000, Plant Cell Rep. 22:684-690).

[0107] In certain embodiments, a counter-selectable marker can be used in the eukaryotic cells (*e.g.*, plant), methods, systems, and compositions provided herein. Such counter-selectable markers can in certain embodiments be incorporated into any DNA that is not intended for insertion into a host cell genome at target editing sites. In such embodiments, non-limiting examples of DNAs with counter-selectable markers include any DNA molecules that are linked to DNAs encoding HDR-promoting agents (*e.g.*, SSB, SSAP, and/or exonucleases), gene-editing molecules, and/or donor template DNA molecules. Vectors or DNA molecules comprising donor template DNA molecules wherein the counter-selectable marker is linked to the donor template DNA and optionally separated from the donor template DNA by a target editing site sequence. Examples of counter-selectable markers that can be used in Plants include cytosine deaminase genes (*e.g.*, used in conjunction with 5-fluorocytosine; Schlaman and Hooykaas, 1997), phosphonate ester hydrolases (*e.g.*, used in conjunction with phosphonate esters of glyphosate including glycerol glyphosate; Dotson, *et al.* 1996), a nitrate reductase (*e.g.*, used in conjunction with chlorate on media containing ammonia as a sole nitrogen source; Nussaume, *et al.* 1991).

[0108] In certain embodiments, the use of a selectable marker is obviated by the increased frequency of HDR provided by the HDR promoting agents (*i.e.*, SSAP, exonuclease, and/or SSBs) and/or modified template DNA molecules. In such embodiments, a selectable marker and/or a counter-selectable marker can be omitted from any of a donor template DNA molecule, a plasmid used to deliver a donor-template or other DNA molecule, or any other vector (*e.g.*, viral vector) or polynucleotide used in the cells, system, method, or composition provided herein.

B. Methods of genetic engineering

[0109] In one aspect, the present disclosure provides a method of genetic engineering of a eukaryotic cell. In some embodiments, the method comprises providing 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). In some embodiments, the method comprises delivering a nucleic acid 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).

[0110] In another aspect, the present disclosure provides a method of genetic engineering of a eukaryotic cell. In some embodiments, the method comprises 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), and iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product.

[0111] In another aspect,the method comprises i) a double strand break inducing compound, 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), and iv) an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product.

i. Genetic modifications

[0112] The genetic engineering may be a reduction in gene function (*i.e.* activity in the encoded gene product). This may require a corresponding repair template, as discussed herein, to provide the defective sequence or it may be through induction of a DSB. In particular, the gene perturbation is a gene knockdown. In some embodiments, the cell is a plant or an animal cell. In some embodiments, the genetic engineering is introduction of a stop codon within the gene. In some embodiments the genetic engineering is a mutation in the promoter or start codon.

[0113] Alternatively, the genetic engineering may be an increase in gene function (*i.e.* activity in the encoded gene product). This may require a corresponding repair template, as discussed herein, to provide the corrected sequence. In some embodiments, the genetic engineering is a substitution of one or more nucleotides in a protein coding gene.

[0114] In some embodiments the target editing site is located in a promoter region. In one embodiment the nucleotide sequence can be a promoter wherein the editing of the promoter results in any one of the following or any one combination of the following: an increased promoter activity, an increased promoter tissue specificity, a decreased promoter activity, a decreased promoter tissue specificity, a mutation of DNA binding elements and/or a deletion or addition of DNA binding elements.

[0115] In one embodiment the nucleotide sequence can be a regulatory sequence in the genome of a cell. A regulatory sequence is a segment of a nucleic acid molecule which is capable of increasing or decreasing the expression of specific genes within an organism. Examples of regulatory sequences include, but are not limited to, transcription activators, transcriptions repressors, and translational repressors, splicing factors, miRNAs, siRNA, artificial miRNAs, a CAAT box, a CCAAT box, a Pribnow box, a TATA box, SECIS elements and polyadenylation signals. In some embodiments the editing of a regulatory element results in altered protein translation, RNA cleavage, RNA splicing, or transcriptional termination.

[0116] In one embodiment, the guide polynucleotide/Cas endonuclease system can be used to insert a component of the TET operator repressor/operator/inducer system, or a component of the sulphonylurea (Su) repressor/operator/inducer system into plant genomes to generate or control inducible expression systems.

[0117] In another embodiment, the guide polynucleotide/Cas endonuclease system can be used to allow for the deletion of a promoter or promoter element, wherein the promoter

deletion (or promoter element deletion) results in any one of the following or any one combination of the following: a permanently inactivated gene locus, an increased promoter activity (increased promoter strength), an increased promoter tissue specificity, a decreased promoter activity, a decreased promoter tissue specificity, a new promoter activity, an inducible promoter activity, an extended window of gene expression, a modification of the timing or developmental progress of gene expression, a mutation of DNA binding elements and/or an addition of DNA binding elements. Promoter elements to be deleted can be, but are not limited to, promoter core elements, promoter enhancer elements or 35 S enhancer elements. The promoter or promoter fragment to be deleted can be endogenous, artificial, pre-existing, or transgenic to the cell that is being edited.

[0118] In one embodiment the nucleotide sequence to be modified can be a terminator wherein the editing of the terminator comprises replacing the terminator (also referred to as a "terminator swap" or "terminator replacement") or terminator fragment with a different terminator (also referred to as replacement terminator) or terminator fragment (also referred to as replacement terminator fragment), wherein the terminator replacement results in any one of the following or any one combination of the following: an increased terminator activity, an increased terminator tissue specificity, a decreased terminator activity, a decreased terminator tissue specificity, a mutation of DNA binding elements and/or a deletion or addition of DNA binding elements." The terminator (or terminator fragment) to be modified can be a terminator (or terminator fragment) that is endogenous, artificial, pre-existing, or transgenic to the cell that is being edited. The replacement terminator (or replacement terminator fragment) can be a terminator (or terminator fragment) that is endogenous, artificial, pre-existing, or transgenic to the cell that is being edited.

[0119] The terminator (or terminator element) to be inserted can be a terminator (or terminator element) that is endogenous, artificial, pre-existing, or transgenic to the cell that is being edited.

[0120] In another embodiment, the guide polynucleotide/Cas endonuclease system can be used to allow for the deletion of a terminator or terminator element, wherein the terminator deletion (or terminator element deletion) results in any one of the following or any one combination of the following: an increased terminator activity (increased terminator strength), an increased terminator tissue specificity, a decreased terminator activity, a decreased terminator tissue specificity, a mutation of DNA binding elements and/or an

addition of DNA binding elements. The terminator or terminator fragment to be deleted can be endogenous, artificial, pre-existing, or transgenic to the cell that is being edited.

[0121] Modifications include 5′ cap, a 3′ polyadenylated tail, a riboswitch sequence, a stability control sequence, a sequence that forms a dsRNA duplex, a modification or sequence that targets the guide poly nucleotide to a subcellular location, a modification or sequence that provides for tracking, a modification or sequence that provides a binding site for proteins, a Locked Nucleic Acid (LNA), a 5-methyl dC nucleotide, a 2,6-Diaminopurine nucleotide, a 2′-Fluoro A nucleotide, a 2′-Fluoro U nucleotide; a 2′-O-Methyl RNA nucleotide, a phosphorothioate bond, linkage to a cholesterol molecule, linkage to a polyethylene glycol molecule, linkage to a spacer 18 molecule, a 5′ to 3′ covalent linkage, or any combination thereof. These modifications can result in at least one additional beneficial feature, wherein the additional beneficial feature is selected from the group of a modified or regulated stability, a subcellular targeting, tracking, a fluorescent label, a binding site for a protein or protein complex, modified binding affinity to complementary target editing site, modified resistance to cellular degradation, and increased cellular permeability.

[0122] In some embodiments, the genomic sequence of interest to be modified is a polyubiquitination site, wherein the modification of the polyubiquitination sites results in a modified rate of protein degradation. The ubiquitin tag condemns proteins to be degraded by proteasomes or autophagy. Proteasome inhibitors are known to cause a protein overproduction. Modifications made to a DNA sequence encoding a protein of interest can result in at least one amino acid modification of the protein of interest, wherein said modification allows for the polyubiquitination of the protein (a post translational modification) resulting in a modification of the protein degradation.

[0123] In some embodiments, the target editing site is located in a gene coding region. In some embodiments, the target sequence is located in an intragenic region. In some embodiments, the target sequence is located in the telomeres.

[0124] In some embodiments, the method provided herein results of modification of one or more nucleotides at a target editing site.

[0125] In some embodiments, the modification to the target editing site is a substitution of one or more nucleotides. In some embodiments the modification to the target editing site is a substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides.

[0126] In some embodiments, the modification to the target editing site is a deletion of one or more nucleotides. In some embodiments the modification to the target editing site is a substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides.

[0127] In some embodiments, the modification to the target editing site is an insertion of one or more nucleotides. In some embodiments the modification to the target editing site is a substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides.

[0128] In some embodiments, a target editing site is modified by a donor sequence that has one or more insertions, deletions, or substitutions compared to the target editing site. In some embodiments, the target editing site is replaced by the donor sequence.

[0129] By manipulation of a target sequence, Applicants also mean the epigenetic manipulation of a target editing site. This may be of the chromatin state of a target sequence, such as by modification of the methylation state of the target editing site (*i.e.* addition or removal of methylation or methylation patterns or CpG islands), histone modification, increasing or reducing accessibility to the target editing site, or by promoting 3D folding.

[0130] Also provided is a method of interrogating function of one or more genes in one or more animal or plant cells, comprising introducing a genetic perturbation using the methods provided herein and determining changes in expression of the one or more genes in the altered cells, thereby interrogating the function of the one or more genes. In some embodiments, the genetic perturbation is a loss of function mutation.

[0131] In some embodiments, the method comprises using multiple donor DNAs with different modifications (*i.e.*, insertions, deletions, or substitutions) to the same target. In some embodiments, the multiple donor DNAs target promoger regions or coding sequences. In some embodiments, cells with different modifications can be subesequently screened for a particular phenotype.

ii. Genetic engineering of mammals

[0132] Also provided herein are methods of genetic editing of a mammalian cell. In some embodiments, the genetic editing is of a genetic locus involved in a genetic condition or disease. In some embodiments, the disease or disorder is caused by a mutation in an enzyme. In some embodiments, the genetic condition is a metabolic disorder.

[0133] Exemplary conditions and genes are Amyloid neuropathy (TTR, PALB); Amyloidosis (APOA1, APP, AAA, CVAP, AD1, GSN, FGA, LYZ, TTR, PALB); Cirrhosis (KRT18, KRT8, CIRH1A, NAIC, TEX292, KIAA1988); Cystic fibrosis (CFTR, ABCC7,

CF, MRP7); Glycogen storage diseases (SLC2A2, GLUT2, G6PC, G6PT, G6PT1, GAA, LAMP2, LAMPB, AGL, GDE, GBE1, GYS2, PYGL, PFKM); Hepatic adenoma, 142330 (TCF1, HNF1A, MODY3), Hepatic failure, early onset, and neurologic disorder (SCOD1, SCOl), Hepatic lipase deficiency (LIPC), Hepatoblastoma, cancer and carcinomas (CTNNB1, PDGFRL, PDGRL, PRLTS, AXIN1, AXIN, CTNNB1, TP53, P53, LFS1, IGF2R, MPRI, MET, CASP8, MCH5; Medullary cystic kidney disease (UMOD, HNFJ, FJHN, MCKD2, ADMCKD2); Phenylketonuria (PAH, PKU1, QDPR, DHPR, PTS); Polycystic kidney and hepatic disease (FCYT, PKHD1, ARPKD, PKD1, PKD2, PKD4, PKDTS, PRKCSH, G19P1, PCLD, SEC63). Other preferred targets include any one or more of include one or more of: PCSK9; Hmgcr; SERPINA1; ApoB; LDL; Huntington disease (Huntington), Hemochromatosis (HEF), Duchenne muscular dystrophy (Dystrophin), Sickle cell anemia (Beta Globin), and Tay-Sachs (hexosaminidase A)

[0134] It will be appreciated that where reference is made to a method of modifying an organism or mammal including human or a non-human mammal or organism by manipulation of a target editing site in a genomic locus of interest, this may apply to the organism (or mammal) as a whole or just a single cell or population of cells from that organism (if the organism is multicellular). In the case of humans, for instance, Applicants envisage, inter alia, a single cell or a population of cells and these may preferably be modified ex vivo and then re-introduced. In this case, a biopsy or other tissue or biological fluid sample may be necessary. Stem cells are also particularly preferred in this regard. But, of course, in vivo embodiments are also envisaged.

[0135] The method may be *ex vivo* or *in vitro*, for instance in a cell culture or in an ex vivo or in vitro model (such as an organoid or 'animal or plant cell on a chip'). Alternatively, the method may be in vivo, in which case it may also include isolating the first population of cells from the subject, and transplanting the second population of cells (back) into the subject. Gene perturbation may be for one or more, or two or more, or three or more, or four or more genes.

[0136] In some embodiments of the present invention a knock out model can be produced.

[0137] In some embodiments, delivery is in the form of a vector which may be a viral vector, such as a lenti- or baculo- or preferably adeno-viral/adeno-associated viral vectors, but other means of delivery are known (such as yeast systems, microvesicles, gene guns/means of attaching vectors to gold nanoparticles) and are provided. A vector may mean

not only a viral or yeast system (for instance, where the nucleic acids of interest may be operably linked to and under the control of (in terms of expression, such as to ultimately provide a processed RNA) a promoter), but also direct delivery of nucleic acids into a host cell. While in herein methods the vector may be a viral vector and this is advantageously an AAV, other viral vectors as herein discussed can be employed, such as lentivirus. For example, baculoviruses may be used for expression in insect cells. These insect cells may, in turn be useful for producing large quantities of further vectors, such as AAV or lentivirus vectors adapted for delivery of the present invention.

iii. Genetic engineering of plants

[0138] In some embodiments provided herein is a method of genetically engineering a plant. Polynucleotides/polypeptides of interest include, but are not limited to, herbicide-tolerance coding sequences, insecticidal coding sequences, nematicidal coding sequences, antimicrobial coding sequences, antifungal coding sequences, antiviral coding sequences, abiotic and biotic stress tolerance coding sequences, or sequences modifying plant traits such as yield, grain quality, nutrient content, starch quality and quantity, nitrogen fixation and/or utilization, fatty acids, and oil content and/or composition. More specific polynucleotides of interest include, but are not limited to, genes that improve crop yield, polypeptides that improve desirability of crops, genes encoding proteins conferring resistance to abiotic stress, such as drought, nitrogen, temperature, salinity, toxic metals or trace elements, or those conferring resistance to toxins such as pesticides and herbicides, or to biotic stress, such as attacks by fungi, viruses, bacteria, insects, and nematodes, and development of diseases associated with these organisms. General categories of genes of interest include, for example, those genes involved in information, such as zinc fingers, those involved in communication, such as kinases, and those involved in housekeeping, such as heat shock proteins. More specific categories of transgenes, for example, include genes encoding important traits for agronomics, insect resistance, disease resistance, herbicide resistance, fertility or sterility, grain characteristics, and commercial products. Genes of interest include, generally, those involved in oil, starch, carbohydrate, or nutrient metabolism as well as those affecting kernel size, sucrose loading, and the like that can be stacked or used in combination with other traits.

[0139] Agronomically important traits such as oil, starch, and protein content can be genetically altered in addition to using traditional breeding methods. Modifications include increasing content of oleic acid, saturated and unsaturated oils, increasing levels of lysine and

sulfur, providing essential amino acids, and also modification of starch. Hordothionin protein modifications are described in U.S. Pat. Nos. 5,703,049, 5,885,801, 5,885,802, and 5,990,389, herein incorporated by reference. Another example is lysine and/or sulfur rich seed protein encoded by the soybean 2S albumin described in U.S. Pat. No. 5,850,016, and the chymotrypsin inhibitor from barley, described in Williamson *et al.* (1987) *Eur. J. Biochem.* 165:99-106, the disclosures of which are herein incorporated by reference.

[0140] Commercial traits can also be encoded on a polynucleotide of interest that could increase for example, starch for ethanol production, or provide expression of proteins. Another important commercial use of transformed plants is the production of polymers and bioplastics such as described in U.S. Pat. No. 5,602,321. Genes such as β-Ketothiolase, PHBase (polyhydroxybutyrate synthase), and acetoacetyl-CoA reductase (see Schubert *et al.* (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs).

[0141] Derivatives of the coding sequences can be made by site-directed mutagenesis to increase the level of preselected amino acids in the encoded polypeptide. For example, the gene encoding the barley high lysine polypeptide (BHL) is derived from barley chymotrypsin inhibitor, U.S. application Ser. No. 08/740,682, filed Nov. 1, 1996, and WO 98/20133, the disclosures of which are herein incorporated by reference. Other proteins include methionine-rich plant proteins such as from sunflower seed (Lilley *et al.* (1989) *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, ed. Applewhite (American Oil Chemists Society, Champaign, Ill.), pp. 497-502; herein incorporated by reference); corn (Pedersen *et al.* (1986) *J. Biol. Chem.* 261:6279; Kirihara *et al.* (1988) *Gene* 71:359; both of which are herein incorporated by reference); and rice (Musumura *et al.* (1989) *Plant Mol. Biol.* 12:123, herein incorporated by reference). Other agronomically important genes encode latex, Floury 2, growth factors, seed storage factors, and transcription factors.

[0142] Polynucleotides that improve crop yield include dwarfing genes, such as Rht1 and Rht2 (Peng *et al.* (1999) *Nature* 400:256-261), and those that increase plant growth, such as ammonium-inducible glutamate dehydrogenase. Polynucleotides that improve desirability of crops include, for example, those that allow plants to have reduced saturated fat content, those that boost the nutritional value of plants, and those that increase grain protein. Polynucleotides that improve salt tolerance are those that increase or allow plant growth in an environment of higher salinity than the native environment of the plant into which the salt-tolerant gene(s) has been introduced.

[0143] Polynucleotides/polypeptides that influence amino acid biosynthesis include, for example, anthranilate synthase (AS; EC 4.1.3.27) which catalyzes the first reaction branching from the aromatic amino acid pathway to the biosynthesis of tryptophan in plants, fungi, and bacteria. In plants, the chemical processes for the biosynthesis of tryptophan are compartmentalized in the chloroplast. See, for example, US Pub. 20080050506, herein incorporated by reference. Additional sequences of interest include Chorismate Pyruvate Lyase (CPL) which refers to a gene encoding an enzyme which catalyzes the conversion of chorismate to pyruvate and pHBA. The most well characterized CPL gene has been isolated from *E. coli* and bears the GenBank accession number M96268. See, U.S. Pat. No. 7,361,811, herein incorporated by reference.

[0144] These polynucleotide sequences of interest may encode proteins involved in providing disease or pest resistance. By "disease resistance" or "pest resistance" is intended that the plants avoid the harmful symptoms that are the outcome of the plant-pathogen interactions. Pest resistance genes may encode resistance to pests that have great yield drag such as rootworm, cutworm, European Corn Borer, and the like. Disease resistance and insect resistance genes such as lysozymes or cecropins for antibacterial protection, or proteins such as defensins, glucanases or chitinases for antifungal protection, or *Bacillus thuringiensis* endotoxins, protease inhibitors, collagenases, lectins, or glycosidases for controlling nematodes or insects are all examples of useful gene products. Genes encoding disease resistance traits include detoxification genes, such as against fumonisin (U.S. Pat. No. 5,792,931); avirulence (avr) and disease resistance (R) genes (Jones *et al.* (1994) *Science* 266:789; Martin *et al.* (1993) *Science* 262:1432; and Mindrinos *et al.* (1994) *Cell* 78:1089); and the like. Insect resistance genes may encode resistance to pests that have great yield drag such as rootworm, cutworm, European Corn Borer, and the like. Such genes include, for example, *Bacillus thuringiensis* toxic protein genes (U.S. Pat. Nos. 5,366,892; 5,747,450; 5,736,514; 5,723,756; 5,593,881; and Geiser *et al.* (1986) Gene 48:109); and the like.

[0145] An "herbicide resistance protein" or a protein resulting from expression of an "herbicide resistance-encoding nucleic acid molecule" includes proteins that confer upon a cell the ability to tolerate a higher concentration of an herbicide than cells that do not express the protein, or to tolerate a certain concentration of an herbicide for a longer period of time than cells that do not express the protein. Herbicide resistance traits may be introduced into plants by genes coding for resistance to herbicides that act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides, genes coding for resistance to

herbicides that act to inhibit the action of glutamine synthase, such as phosphinothricin or basta (*e.g.*, the bar gene), glyphosate (*e.g.*, the EPSP synthase gene and the GAT gene), HPPD inhibitors (*e.g.*, the HPPD gene) or other such genes known in the art. See, for example, U.S. Pat. Nos. 7,626,077, 5,310,667, 5,866,775, 6,225,114, 6,248,876, 7,169,970, 6,867,293, and U.S. Provisional Application No. 61/401,456, each of which is herein incorporated by reference. The bar gene encodes resistance to the herbicide basta, the nptII gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS-gene mutants encode resistance to the herbicide chlorsulfuron.

[0146] Additional selectable markers include genes that confer resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). See for example, Yarranton, (1992) *Curr Opin Biotech* 3:506-11; Christopherson *et al.*, (1992) *Proc. Natl. Acad. Sci.* USA 89:6314-8; Yao *et al.*, (1992) *Cell* 71:63-72; Reznikoff, (1992) *Mol Microbiol* 6:2419-22; Hu *et al.*, (1987) *Cell* 48:555-66; Brown *et al.*, (1987) Cell 49:603-12; Figge *et al.*, (1988) Cell 52:713-22; Deuschle *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:5400-4; Fuerst *et al.*, (1989) Proc. Natl. Acad. Sci. *USA* 86:2549-53; Deuschle *et al.*, (1990) *Science* 248:480-3; Gossen, (1993) *Ph.D. Thesis, University of Heidelberg*; Reines *et al.*, (1993) *Proc. Natl. Acad. Sci. USA* 90:1917-21; Labow *et al.*, (1990) *Mol Cell Biol* 10:3343-56; Zambretti *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89:3952-6; Baim *et al.*, (1991) *Proc. Natl. Acad. Sci. USA* 88:5072-6; Wyborski *et al.*, (1991) *Nucleic Acids Res* 19:4647-53; Hillen and Wissman, (1989) *Topics Mol Struc Biol* 10:143-62; Degenkolb *et al.*, (1991) *Antimicrob Agents Chemother* 35:1591-5; Kleinschnidt *et al.*, (1988) Biochemistry 27:1094-104; Bonin, (1993) *Ph.D. Thesis, University of Heidelberg*; Gossen *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-51; Oliva *et al.*, (1992) *Antimicrob Agents Chemother* 36:913-9; Hlavka *et al.*, (1985) *Handbook of Experimental Pharmacology*, Vol. 78 (Springer-Verlag, Berlin); Gill *et al.*, (1988) *Nature* 334:721-4. Commercial traits can also be encoded on a gene or genes that could increase for example, starch for ethanol production, or provide expression of proteins. Another important commercial use of transformed plants is the production of polymers and bioplastics such as described in U.S. Pat. No. 5,602,321. Genes such as β-Ketothiolase, PHBase (polyhydroxyburyrate synthase), and acetoacetyl-CoA reductase (see Schubert *et al.* (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhyroxyalkanoates (PHAs).

[0147] Exogenous products include plant enzymes and products as well as those from other sources including prokaryotes and other eukaryotes. Such products include enzymes,

cofactors, hormones, and the like. The level of proteins, particularly modified proteins having improved amino acid distribution to improve the nutrient value of the plant, can be increased. This is achieved by the expression of such proteins having enhanced amino acid content.

[0148] In some embodiments, the eukaryotic cell is engineered to produce one or more exogenous proteins in a biosynthetic pathway. In some embodiments, the biosynthetic pathway is for biofuel production. In some embodiments, the biosynthetic pathway is for an alcohol. In some embodiments, the biosynthetic pathway is for ethanol. In some embodiments, the biosynthetic pathway is for production of a small molecule. In some embodiments, the biosynthetic pathway is for production of a drug. In some embodiments, the biosynthetic pathway is for production of a sterol. In some embodiments, the biosynthetic pathway is for a hormone. In some embodiments, the biosynthetic pathway is for production of a peptide. In some embodiments, the biosynthetic pathway is for a terpene.

[0149] In some embodiments, the eukaryotic cell is engineered such that is its progeny can no longer replicate. In some embodiments, the eukaryotic cell is a pathogenic cell.

[0150] The transgenes, recombinant DNA molecules, DNA sequences of interest, and polynucleotides of interest can be comprise one or more DNA sequences for gene silencing. Methods for gene silencing involving the expression of DNA sequences in plant are known in the art include, but are not limited to, cosuppression, antisense suppression, double-stranded RNA (dsRNA) interference, hairpin RNA (hpRNA) interference, intron-containing hairpin RNA (ihpRNA) interference, transcriptional gene silencing, and micro RNA (miRNA) interference.

iv. Detection

[0151] One of ordinary skill in the art will appreciate that the genetic modification of the target editing site can be detected by various means. In some embodiments, the method further comprises sequencing a cell. In some embodiments, the method comprises detecting a reporter gene. In some embodiments, the method comprises selecting a cell using a selectable marker.

[0152] Examples of selectable markers include, but are not limited to, DNA segments that comprise restriction enzyme sites; DNA segments that encode products which provide resistance against otherwise toxic compounds including antibiotics, such as, spectinomycin, ampicillin, kanamycin, tetracycline, Basta, neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT)); DNA segments that encode products which are

otherwise lacking in the recipient cell (*e.g.*, tRNA genes, auxotrophic markers); DNA segments that encode products which can be readily identified (*e.g.*, phenotypic markers such as β-galactosidase, GUS; fluorescent proteins such as green fluorescent protein (GFP), cyan (CFP), yellow (YFP), red (RFP), and cell surface proteins); the generation of new primer sites for PCR (*e.g.*, the juxtaposition of two DNA sequence not previously juxtaposed), the inclusion of DNA sequences not acted upon or acted upon by a restriction endonuclease or other DNA modifying enzyme, chemical, *etc.*; and, the inclusion of a DNA sequences required for a specific modification (*e.g.*, methylation) that allows its identification.

[0153] Additional selectable markers include genes that confer resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). See for example, Yarranton, (1992) *Curr Opin Biotech* 3:506-11; Christopherson *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89:6314-8; Yao *et al.*, (1992) Cell 71:63-72; Reznikoff, (1992) *Mol Microbiol* 6:2419-22; Hu *et al.*, (1987) *Cell* 48:555-66; Brown *et al.*, (1987) *Cell* 49:603-12; Figge *et al.*, (1988) Cell 52:713-22; Deuschle *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:5400-4; Fuerst *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:2549-53; Deuschle *et al.*, (1990) *Science* 248:480-3; Gossen, (1993) Ph.D. Thesis, University of Heidelberg; Reines *et al.*, (1993) *Proc. Natl. Acad. Sci. USA* 90:1917-21; Labow *et al.*, (1990) *Mol Cell Biol* 10:3343-56; Zambretti *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89:3952-6; Baim *et al.*, (1991) *Proc. Natl. Acad. Sci. USA* 88:5072-6; Wyborski *et al.*, (1991) *Nucleic Acids Res* 19:4647-53; Hillen and Wissman, (1989) *Topics Mol Struc Biol* 10:143-62; Degenkolb *et al.*, (1991) *Antimicrob Agents Chemother* 35:1591-5; Kleinschnidt *et al.*, (1988) *Biochemistry* 27:1094-104; Bonin, (1993) Ph.*D. Thesis, University of Heidelberg*; Gossen *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-51; Oliva *et al.*, (1992) *Antimicrob Agents Chemother* 36:913-9; Hlavka *et al.*, (1985) *Handbook of Experimental Pharmacology*, Vol. 78 (Springer-Verlag, Berlin); Gill *et al.*, (1988) *Nature* 334:721-4.

C. Nucleic acids

[0154] In one aspect, the present disclosure provides a nucleic acid that encodes an HDR promoting agent. In some embodiments, provided herein is 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). In some embodiments, the nucleic acids are in one or more vectors. In some embodiments, the nucleic acids are in one vector.

[0155] In some embodiments, the nucleic acid encodes at least one sequence-specific endonuclease. In some embodiments, the nucleic acid comprises a donor template DNA molecule having homology to the target editing site. In some embodiments, the nucleic acid encodes an HDR promoting agent. In some embodiments, the nucleic acid encodes a single-stranded DNA annealing protein (SSAP). In some embodiments, the nucleic acid encodes an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product. In some embodiments, the nucleic acid encodes a single stranded DNA binding protein (SSB). In some embodiments, the nucleic acid is an expression construct or a vector. In some embodiments, an expression construct or a vector comprises the nucleic acid.

[0156] In some embodiments, the nucleic acid encodes a gene-editing molecule. In some embodiments, the nucleic acid encodes a sequence-specific endonuclease. In some embodiments, the nucleic acid encodes a 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. In some embodiments, nucleic acid encodes 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. In some embodiments, the nucleic acid encodes a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TAL-effector nuclease), Argonaute, a meganuclease, or engineered meganuclease. In some embodiments, the nucleic acid encodes 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. In some embodiments, the nucleic acid encodes a sequence-specific endonuclease that comprises at least one Cas9 nickase, Cas12a nickase, Cas12i, a zinc finger nickase, a TALE nickase, or a combination thereof. In some embodiments, the nucleic acid encodes a sequence-specific endonuclease that comprises Cas9 and/or Cas12a and the guide RNA molecules have at least one base mismatch to DNA sequences in the target editing site.

[0157] In some embodiments, the nucleic acid comprises a donor DNA molecule. In some embodiments, the nucleic acid comprises a donor template DNA. In some

embodiments, the donor DNA molecule is provided on a circular DNA vector, geminivirus replicon, or as a linear DNA fragment. In some embodiments, the donor DNA molecule is flanked by an endonuclease recognition sequence.

[0158] In some embodiments, the donor DNA molecule comprises a modified sequence of a genomic DNA target editing site. In some embodiments, the donor DNA molecule comprises a substitution of one or more nucleotides compared to the target editing site. In some embodiments the donor DNA molecule comprises a substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides.

[0159] In some embodiments, the donor DNA molecule comprises a deletion of one or more nucleotides compared to the genomic target editing site. In some embodiments the donor DNA molecule comprises a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides.

[0160] In some embodiments, the donor DNA molecule comprises an insertion of one or more nucleotides compared to the genomic target editing site. In some embodiments the insertion is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides.

[0161] In some embodiments, the nucleic acid encodes a 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. In some embodiments, the nucleic acid encodes a sequence-specific endonuclease that provides a 5' overhang at the target-editing site following cleavage. In some embodiments, the nucleic acid encodes a SSAP that provides for DNA strand exchange and base pairing of complementary DNA strands of homologous DNA molecules. In some embodiments, the nucleic acid encodes a SSAP that comprises a RecT/Redβ-, ERF-, or RAD52-family protein. In some embodiments, the nucleic acid encodes a RecT/ Redβ- family protein comprising 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. In some embodiments, the nucleic acid encodes a ERF-family protein that 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. In some embodiments, the nucleic acid encodes a RAD52-family protein that 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.

[0162] In some embodiments, the nucleic acid encodes an exonuclease. In some embodiments, the nucleic acid encodes an exonuclease wherein a linear dsDNA molecule is a preferred substrate of the exonuclease. In some embodiments, a linear dsDNA molecule comprising a phosphorylated 5' terminus is a preferred substrate of the exonuclease. In some embodiments, 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. In some embodiments, 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. In some embodiments, 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. In some embodiments, 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.

[0163] In some embodiments, the nucleic acid encodes a single stranded DNA binding protein (SSB). In some embodiments, the nucleic acid encodes an SSB and a SSAP. In some embodiments, the nucleic acid encodes a single stranded DNA binding protein (SSB) and a SSAP obtained from the same host organism. In some embodiments, the single stranded DNA binding protein (SSB) is a bacterial SSB or optionally an *Enterobacteriaceae* sp. SSB. In some embodiments, 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. In some embodiments, 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.

[0164] In some embodiments, the nucleic acid encodes a SSAP, exonuclease, and/or SSB protein further comprising an operably linked nuclear localization signal (NLS) and/or a cell-

penetrating peptide (CPP). In some embodiments, the nucleic acid encodes proteins for expression in a plant cell. In some embodiments, 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.

[0165] In some embodiments, the nucleic acids provided herein 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) are each operably linked to a promoter. In some embodiments, the promoter is a constitutively active promoter. In some embodiments, the promoter is an inducible promoter. In some embodiments, the promoter is a plants-specific promoter. In some embodiments, the promoter is a mammalian promoter. In some embodiments, the promoter is a viral promoter. In some embodiments, the promoter is a 35S promoter. In some embodiments, the promoter is ubiquitin promoter. In some embodiments the promoter is an actin promoter. In some embodiments, the promoter is a mammalian promoter. In some embodiments, the promoter is a CAG promoter. In some embodiments, the promoter is the U6 promoter. In some embodiments, the promoter is the EF1a promoter. In some embodiments the promoter is the human ACTB promoter some embodiments, the promoter is a CMV promoter. In some embodiments, the promoter is a U6 promoter. In some embodiments, the promoter is a T7 promoter. In some embodiments, the site specific nuclease, and/or its guide RNA for CRISPR/Cas-based nucleases, are expressed under the control of an inducible promoter. In this configuration, the onset of the genomic editing process can be induced at a time when the concentration of the other components of the system is not rate limiting.

[0166] In some embodiments, the nucleic acids provided herein are provided in one or more vectors. In some embodiments, the nucleic acids provided herein are provided in one vector. In some embodiments, the nucleic acids provided herein are provided in two vectors. In some embodiments, the nucleic acids provided herein are provided in three vectors. In some embodiments, the nucleic acids provided herein are provided in four vectors. In some embodiments, the nucleic acids provided herein are provided in five vectors.

[0167] In some embodiments, provided herein is a vector 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). In some embodiments, provided herein is a vector encoding HDR promoting elements. In some embodiments, provided herein is a vector encoding 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). In some embodiments, provided herein is a vector encoding at least one sequence-specific endonuclease and a donor template.

[0168] Also provided herein is a first vector 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 a second vector comprising a donor template DNA and a guide RNA.

[0169] In some embodiments, the nucleic acid is optimized for expression in a particular cell type. In some embodiments, the nucleic acid is optimized for expression in a particular species. In some embodiments, the nucleic acid is optimized for expression in a plant cell. In some embodiments, the nucleic acid is optimized for expression in a mammalian cell. In some embodiments, the nucleic acid comprises a protein coding sequence, such as an exonuclease, a SSB protein, and/or a SSAP. In some embodiments, the protein coding sequence is codon-optimized for translation in a plant cell. In some embodiments, the protein coding sequence is codon-optimized for translation in a mammalian cell.

[0170] In certain embodiments, a donor DNA template homology arm can be about 20, 50, 100, 200, 400, or 600 to about 800, or 1000 base pairs in length. For example, a donor DNA template homology arm can be between about 20 to about 1000, about 50 to about 1000, about 100 to about 1000, about 200 to about 1000, or about 600 to 1000 base pairs in length. In some embodiments the donor DNA template homology arm is between about 400 to about 800 base pairs in length. In some embodiments, the donor DNA template homology arms are less than 250 base pairs in length. In some embodiments, the donor DNA template homology arms are less than 100 base pair in length.

[0171] In certain embodiments, the GC content of the donor DNA template homology arm is modified. In some embodiments, the GC content is maximized.

[0172] In some embodiments, the nucleic acids provided herein are modified for expression in a certain cell type. In some embodiments, the nucleic acids provided herein are

modified for expression in eukaryotic cells. In some embodiments, the nucleic acids are modified for expression in plant or animal cells. In some embodiments, the nucleic acids are modified for mammalian cells. In some embodiments, the nucleic acids are modified for murine or primate cells. In some embodiments, the nucleic acids are modified for human cells. In some embodiments the nucleic acids are modified for mouse cells.

[0173] Methods of modification of nucleic acid compositions for expression particular cell types are well known in the art. In some embodiments, the GC (guanine-cytosine) content of a nucleotide provided herein is modified. In some embodiments, nucleic acids provided herein are codon optimized for a particular cell type, for example for eukaryotic cells.

i. Viral vectors

[0174] In one aspect, the present disclosure provides vectors that comprises any of the nucleic acids disclosed herein for expression in a mammalian cell. In some embodiments, the vector comprises an expression construct. In some embodiments, the vector comprises a nucleic acid that encodes an HDR-promoting agent (*e.g.*, an SSAP, an exonuclease, and/or an SSB protein), a sequence-specific endonuclease, and/or a donor template DNA molecule.

[0175] In some embodiments provided herein is a vector 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/or v) a single stranded DNA binding protein (SSB).

[0176] In some embodiments, a first vector encodes 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). In some embodiments, a second vector encodes 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). In some embodiments, the first vector does not encode at least one of the sequence-specific endonuclease, the donor template DNA molecule, the SSAP, the exonuclease, and the SSB protein. In some embodiments, the at least one of the sequence-specific endonuclease, the donor template DNA molecule, the SSAP, the exonuclease, and the SSB protein that is not encoded by the first vector is encoded by the second vector. In some embodiments, the components are encoded by a first and second vector as shown in **Table B**, below.

Table B: Combinations of components encoded by a first and second vector

Combination Number	Component(s) Encoded by First Vector	Component(s) Encoded by Second Vector
1	Donor template DNA molecule SSAP Exonuclease SSB	Sequence-specific endonuclease
2	Sequence-specific endonuclease SSAP Exonuclease SSB	Donor template DNA molecule
3	Sequence-specific endonuclease Donor template DNA molecule Exonuclease SSB	SSAP
4	Sequence-specific endonuclease Donor template DNA molecule SSAP SSB	Exonuclease
5	Sequence-specific endonuclease Donor template DNA molecule SSAP Exonuclease	SSB
6	SSAP Exonuclease SSB	Sequence-specific endonuclease Donor template DNA molecule
7	Donor template DNA molecule Exonuclease SSB	Sequence-specific endonuclease SSAP
8	Donor template DNA molecule SSAP SSB	Sequence-specific endonuclease Exonuclease
9	Donor template DNA molecule SSAP Exonuclease	Sequence-specific endonuclease SSB
10	SSAP Exonuclease SSB	Donor template DNA molecule Sequence-specific endonuclease
11	Sequence-specific endonuclease Exonuclease SSB	Donor template DNA molecule SSAP
12	Sequence-specific endonuclease SSAP SSB	Donor template DNA molecule Exonuclease
13	Sequence-specific endonuclease	Donor template DNA molecule

	SSAP Exonuclease	SSB
14	Donor template DNA molecule Exonuclease SSB	SSAP Sequence-specific endonuclease
15	Sequence-specific endonuclease Exonuclease SSB	SSAP Donor template DNA molecule
16	Sequence-specific endonuclease Donor template DNA molecule SSB	SSAP Exonuclease
17	Sequence-specific endonuclease Donor template DNA molecule Exonuclease	SSAP SSB
18	Donor template DNA molecule SSAP SSB	Exonuclease Sequence-specific endonuclease
19	Sequence-specific endonuclease SSAP SSB	Exonuclease Donor template DNA molecule
20	Sequence-specific endonuclease Donor template DNA molecule SSB	Exonuclease SSAP
21	Sequence-specific endonuclease Donor template DNA molecule SSAP	Exonuclease SSB
22	Donor template DNA molecule SSAP Exonuclease	SSB Sequence-specific endonuclease
23	Sequence-specific endonuclease SSAP Exonuclease	SSB Donor template DNA molecule
24	Sequence-specific endonuclease Donor template DNA molecule Exonuclease	SSB SSAP
25	Sequence-specific endonuclease Donor template DNA molecule SSAP	SSB Exonuclease
26	Sequence-specific endonuclease Donor template DNA molecule	SSAP Exonuclease SSB
27	Sequence-specific endonuclease SSAP	Donor template DNA molecule Exonuclease SSB
28	Sequence-specific endonuclease Exonuclease	Donor template DNA molecule SSAP SSB
29	Sequence-specific endonuclease SSB	Donor template DNA molecule SSAP Exonuclease
30	Donor template DNA molecule Sequence-specific endonuclease	SSAP Exonuclease SSB
31	Donor template DNA molecule SSAP	Sequence-specific endonuclease Exonuclease SSB
32	Donor template DNA molecule Exonuclease	Sequence-specific endonuclease SSAP SSB

33	Donor template DNA molecule SSB	Sequence-specific endonuclease SSAP Exonuclease
34	SSAP Sequence-specific endonuclease	Donor template DNA molecule Exonuclease SSB
35	SSAP Donor template DNA molecule	Sequence-specific endonuclease Exonuclease SSB
36	SSAP Exonuclease	Sequence-specific endonuclease Donor template DNA molecule SSB
37	SSAP SSB	Sequence-specific endonuclease Donor template DNA molecule Exonuclease
38	Exonuclease Sequence-specific endonuclease	Donor template DNA molecule SSAP SSB
39	Exonuclease Donor template DNA molecule	Sequence-specific endonuclease SSAP SSB
40	Exonuclease SSAP	Sequence-specific endonuclease Donor template DNA molecule SSB
41	Exonuclease SSB	Sequence-specific endonuclease Donor template DNA molecule SSAP
42	SSB Sequence-specific endonuclease	Donor template DNA molecule SSAP Exonuclease
43	SSB Donor template DNA molecule	Sequence-specific endonuclease SSAP Exonuclease
44	SSB SSAP	Sequence-specific endonuclease Donor template DNA molecule Exonuclease
45	SSB Exonuclease	Sequence-specific endonuclease Donor template DNA molecule SSAP
46	Sequence-specific endonuclease	Donor template DNA molecule SSAP Exonuclease SSB
47	Donor template DNA molecule	Sequence-specific endonuclease SSAP Exonuclease SSB
48	SSAP	Sequence-specific endonuclease Donor template DNA molecule Exonuclease SSB
49	Exonuclease	Sequence-specific endonuclease Donor template DNA molecule SSAP SSB
50	SSB	Sequence-specific endonuclease Donor template DNA molecule SSAP Exonuclease

51	Sequence-specific endonuclease Donor template DNA molecule SSAP Exonuclease SSB	

[0177] In some embodiments, the sequence-specific endonuclease, the donor template DNA molecule, SSAP, exonuclease, and SSB are provided in three vectors in various combinations. For example, a first vector comprising the sequence-specific endonuclease, a second vector comprising the donor template DNA, and a third vector comprising the SSAP, exonuclease, and SSB or a first vector comprising the sequence-specific endonuclease, the donor template DNA, and the SSAP, a second vector comprising the exonuclease, and a third vector comprising the SSB.

[0178] In some embodiments, the sequence-specific endonuclease, the donor template DNA molecule, SSAP, exonuclease, and SSB are provided in four vectors in various combinations. For example a first vector comprising the sequence-specific endonuclease, a second vector comprising the donor template DNA, a third vector comprising the SSAP, and a fourth vector comprising the exonuclease and SSB or a first vector comprising the sequence-specific endonuclease and the donor template DNA, a second vector comprising the SSAP, a third vector comprising the exonuclease, and a fourth vector comprising the SSB.

[0179] In some embodiments, the sequence-specific endonuclease, the donor template DNA molecule, SSAP, exonuclease, and SSB are provided in five vectors

[0180] In some embodiments, the vector is a viral vector. In some embodiments, the vector is a parvoviral vector. In some embodiments, the vector is an adeno-associated virus (AAV) vector. In some embodiments, the vector is a recombinant AAV (rAAV) vector. In some embodiments, the vector is an adenoviral vector. In some embodiments, the vector is a retroviral vector. In some embodiments, the vector is a lentiviral vector. In some embodiments, the vector is a herpesviral vector. In some embodiments, the vector is baculoviral vector.

[0181] In some embodiments, the recombinant adenoviral vector is derived from adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHuSO, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the recombinant adenoviral vector is derived from adenovirus serotype 2 or a variant of adenoviral serotype 5. In some embodiments, the vector is a recombinant lentiviral vector. In

some embodiments, the recombinant lentiviral vector is derived from a lentivirus pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD 114 or variants therein. In some embodiments, the vector is an rHSV vector. In some embodiments, the rHSV vector is derived from rHSV-1 or rHSV-2.

[0182] In some embodiments of the above methods, the vector is a rAAV vector. In some embodiments, an expression construct encoding an HDR-promoting agent (*e.g.*, an SSAP, an exonuclease, and/or an SSB protein), a sequence-specific endonuclease, and/or a donor template DNA molecule is flanked by one or more AAV inverted terminal repeat (ITR) sequences. In some embodiments, the expression construct is flanked by two AAV ITRs. In some embodiments, the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs. In some embodiments, the AAV ITRs are AAV2 ITRs. In some embodiments, the vector further comprises a stuffier nucleic acid. In some embodiments, the stuffier nucleic acid is located between the promoter and the nucleic acid encoding the expression construct. In some embodiments, the vector is a self-complementary rAAV vector. In some embodiments, the vector comprises first nucleic acid sequence encoding an HDR-promoting agent (*e.g.*, an SSAP, an exonuclease, and/or an SSB protein), a sequence-specific endonuclease, and/or a donor template DNA molecule, and a second nucleic acid sequence encoding an HDR-promoting agent (*e.g.*, an SSAP, an exonuclease, and/or an SSB protein), a sequence-specific endonuclease, and/or a donor template DNA molecule. In some embodiments, the first nucleic acid sequence and the second nucleic acid sequence are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of the D region and comprises a mutation of the terminal resolution sequence. In some embodiments, the invention provides a cell comprising any of vectors (*e.g.*, rAAV vectors) described herein.

[0183] In some embodiments of the above methods, the vector encoding an HDR-promoting agent (*e.g.*, an SSAP, an exonuclease, and/or an SSB), a sequence-specific endonuclease, and/or a donor template DNA molecule is in a viral particle, wherein the viral particle is an AAV particle encapsidating the rAAV vector, an adenovirus particle encapsidating the recombinant adenoviral vector, a lentiviral particle encapsidating the recombinant lentiviral vector or an HSV particle encapsidating the recombinant HSV vector. In some embodiments, the viral particle is an adenovirus particle encapsidating the

recombinant adenoviral vector. In some embodiments, the adenovirus particle comprises a capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11 , 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHuSO, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the adenovirus particle comprises an adenovirus serotype 2 capsid or a variant of an adenoviral serotype S capsid. In some embodiments, the viral particle is a lentiviral particle encapsidating the recombinant lentiviral vector. In some embodiments, the lentiviral particle comprises a capsid pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In some embodiments, the viral particle is a HSV particle. In some embodiments, the HSV particle is a rHSV-1 particle or a rHSV-2 particle.

[0184] In some embodiments of the above methods, the invention provides a recombinant AAV particle comprising any of the rAAV vectors described herein. In some embodiments, the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV 10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid rAAV2/HBoVl serotype capsid. In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype. In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes. In some embodiments, the ITR is derived from AAV2 and the capsid of the rAAV particle is derived from AAV1. The invention provides a vector comprising the expression construct of any one of the embodiments described herein. In some embodiments, the expression construct encodes an HDR-promoting agent (*e.g.*, an SSAP, an exonuclease, and/or an SSB), a sequence-specific endonuclease, and/or a donor template DNA molecule. In some embodiments, the vector is a recombinant adeno-associated virus (rAAV) vector, a recombinant adenoviral vector, a recombinant lentiviral vector or a recombinant herpes simplex virus (HSV) vector. In some embodiments, the vector is a recombinant adenoviral vector. In some embodiments, the recombinant adenoviral vector is derived from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48,

AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the recombinant adenoviral vector is derived from adenovirus serotype 2 or a variant of adenoviral serotype S. In some embodiments, the vector is a recombinant lentiviral vector. In some embodiments, the recombinant lentiviral vector is derived from a lentivirus pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In some embodiments, the vector is an rHSV vector. In some embodiments, the rHSV vector is derived from rHSV-1 or rHSV-2.

[0185] In some embodiments, the vector comprises a selectable marker.

[0186] In some embodiments of the above methods, the viral particle is in a composition (*e.g.*, a pharmaceutical composition). In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

ii. Other vectors

[0187] In some embodiments, the vector is a non-viral vector. In some embodiments, the vector is a plasmid. In some embodiments, the vector is a plant transformation vector. In some embodiments, the vector is a vector for *Agrobacterium*-mediated transient expression or stable transformation in tissue cultures or plant tissues.

[0188] Exemplary systems of using recombinant plasmid vectors that are compatible with the present invention include, but are not limited to the "cointegrate" and "binary" systems. In the "cointegrate" system, the shuttle vector containing the gene of interest is inserted by genetic recombination into a non-oncogenic plasmid that contains both the cis-acting and trans-acting elements required for plant cell transformation as, for example, in the pMLJ1 shuttle vector and the non-oncogenic plasmid pGV3850. The second system is called the "binary" system in which two plasmids are used; the gene of interest is inserted into a shuttle vector containing the cis-acting elements required for plant transformation. The other necessary functions are provided in trans by the non-oncogenic plasmid as exemplified by the pBIN19 shuttle vector and the non-oncogenic plasmid PAL4404. These and other vectors useful for these systems are commercially available.

D. Cells

[0189] In one aspect, the present disclosure provides a eukaryotic cell comprising an HDR promoting agent. In some embodiments, the eukaryotic cell comprises genome-editing

molecules and an HDR promoting agent. In some embodiments the cell is a host cell. In some embodiments, the cell is a cell to be modified according to the present methods. In some embodiments, 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. In some embodiments, 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).

[0190] In another aspect, the present disclosure provides a eukaryotic cell produced by the methods provided herein. In some embodiments, modification of a target editing site of a eukaryotic cell genome comprises 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 SSAP, an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and a SSB protein. In some embodiments, the cell has a genomic signature produced by modification according to the present methods. In some embodiments, a nuclease cleavage site is removed. In some embodiments, a nucleic acid sequence tag is interested.

[0191] In some embodiments, provided herein is a host cell comprising one or more vectors comprising i) nucleic acid encoding at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) nucleic acid encoding a single-stranded DNA annealing protein (SSAP), iv) nucleic acid encoding an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) nucleic acid encoding a single stranded DNA binding protein (SSB). In some embodiments, the host cell comprises one vector encoding i) nucleic acid encoding at least one sequence-specific endonuclease, ii) a donor template DNA molecule having homology to a target editing site in the eukaryotic cell, iii) nucleic acid encoding a single-stranded DNA annealing protein (SSAP), iv) nucleic acid encoding an exonuclease which can at least partially convert a double stranded DNA substrate to a single stranded DNA product, and v) nucleic acid encoding a single stranded

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

[0192] Further, the methods of the present disclosure may be used to increase HDR-mediated genome modification in a eukaryotic cell, make a eukaryotic cell having a genomic modification, and/or genetically engineer a eukaryotic cell as described herein.

[0193] In some embodiments, the cell is an isolated cell. In some embodiments the cell is in cell culture. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is obtained from a living organism, and maintained in a cell culture. In some embodiments, the cell is a single-celled organism. In some embodiments, the cell is inside of an organism. In some embodiments, the cell is an organism. In some embodiments, the cell is a cell of a single-celled eukaryotic organism, a protozoa cell, a cell from a plant, an algal cell, (*e.g., Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Nannochloropsis gaditana*, *Chlorella pyrenoidosa*, *Sargassum patens*, *C. agardh*, and the like), seaweeds (*e.g.* kelp) a fungal cell (*e.g.,* a yeast cell, a cell from a mushroom), an animal cell, a cell from an invertebrate animal (*e.g.*, fruit fly, cnidarian, echinoderm, nematode, *etc.*), a cell from a vertebrate animal (*e.g.*, fish, amphibian, reptile, bird, mammal), a cell from a mammal (*e.g.*, an ungulate (*e.g.*, a pig, a cow, a goat, a sheep); a rodent (*e.g.*, a rat, a mouse); a non-human primate; a human; a feline (*e.g.*, a cat); a canine (*e.g.,* a dog); *etc.*), and the like. In some embodiments, the cell is a cell that does not originate from a natural organism (*e.g.*, the cell can be a synthetically made cell; also referred to as an artificial cell). In some embodiments, the cell is in a cell culture (*e.g.*, in vitro cell culture). In some embodiments, the cell is one of a collection of cells. In some embodiments, the cell is a eukaryotic cell or derived from a eukaryotic cell. In some embodiments, the cell is a plant cell or derived from a plant cell. In some embodiments, the cell is an animal cell or derived from an animal cell. In some embodiments, the cell is an invertebrate cell or derived from an invertebrate cell. In some embodiments, the cell is a vertebrate cell or derived from a vertebrate cell. In some embodiments, the cell is a mammalian cell or derived from a mammalian cell. In some embodiments, the cell is rodent cell or derived from a rodent cell. In some embodiments, the cell is a human cell or derived

from a human cell. In some embodiments, the cell is a non-human animal cell or derived from a non-human animal cell. In some embodiments, the cell is a non-human mammalian cell or derived from a non-human mammalian cell. In some embodiments, the cell is a fungal cell or derived from a fungal cell. In some embodiments, the cell is an insect cell. In some embodiments, the cell is an arthropod cell. In some embodiments, the cell is a protozoan cell. In some embodiments, the cell is a helminth cell. In some embodiments, the cell is a non-mammal animal cell. In some embodiments, the cell is a fish cell. In some embodiments, the cell is an insect cell. In some embodiments, the cell is a fruit fly cell. In some embodiments, the cell is a *Drosophila melanogaster* cell. In some embodiments, the cell is a nematode cell. In some embodiments, the cell is a *Caenorhabditis elegans* cell. In some embodiments, the cell is a roundworm cell.

[0194] In some embodiments, the cell is a progenitor cell that comprises 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), wherein the progenitor cell does not comprise at least one of i) - v), and 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. For example, in some embodiments, the progenitor cell is lacking one or more components of i)-v) and is transformed with the components which are lacking.

i. Plant cells

[0195] In some embodiments, the eukaryotic cell is a plant cell. In some embodiments, the eukaryotic cell comprising an HDR promoting agent is a plant cell. Further, the methods of the present disclosure may be used to increase HDR-mediated genome modification in a plant cell, make a plant cell having a genomic modification, and/or genetically engineer a plant cell. In some embodiments, the methods disclosed herein comprise editing a plant cell. In some embodiments, the methods disclosed herein comprise performing a genome modification in a plant cell. In some embodiments, the methods disclosed herein comprise modifying a target locus in a plant cell genome. In some embodiments, the methods disclosed herein comprise increasing HDR-mediated genome modification in a plant cell.

[0196] In certain embodiments, the cell is an isolated plant cells or plant protoplasts (*i.e.*, are not located in undissociated or intact plant tissues, plant parts, or whole plants). In certain embodiments, the plant cells are obtained from any plant part or tissue or callus. In certain embodiments, the culture includes plant cells obtained from a plant tissue, a cultured plant tissue explant, whole plant, intact nodal bud, shoot apex or shoot apical meristem, root apex or root apical meristem, lateral meristem, intercalary meristem, seedling, whole seed, halved seed or other seed fragment, zygotic embryo, somatic embryo, immature embryo, ovule, pollen, microspore, anther, hypocotyl, cotyledon, leaf, petiole, stem, tuber, root, callus, or plant cell suspension. In certain embodiments, the plant cell is derived from the L1 or L2 layer of an immature or mature embryo of a monocot plant (*e.g.*, maize, wheat, sorghum, or rice).

[0197] In certain embodiments, the plant cell is located in undissociated or intact plant tissues, plant parts, plant explants, or whole plants. In certain embodiments, the plant cell can be located in an intact nodal bud, a cultured plant tissue explant, shoot apex or shoot apical meristem, root apex or root apical meristem, lateral meristem, intercalary meristem, seedling, whole seed, halved seed or other seed fragment, zygotic embryo, somatic embryo, immature embryo, ovule, pollen, microspore, anther, hypocotyl, cotyledon, leaf, petiole, stem, tuber, root, or callus. In certain embodiments, the explants used include immature embryos. Immature embryos (*e.g.*, immature maize embryos) include 1.8-2.2 mm embryos, 1-7 mm embryos, and 3-7 mm embryos. In certain embodiments, the aforementioned embryos are obtained from mature ear-derived seed, leaf bases, leaves from mature plants, leaf tips, immature inflorescences, tassels, immature ears, and silks. In various aspects, the plant-derived explant used for transformation includes immature embryos, 1.8-2.2 mm embryos, 1-7 mm embryos, and 3.5-7 mm embryos. In an aspect, the embryos can be derived from mature ear-derived seed, leaf bases, leaves from mature plants, leaf tips, immature inflorescences, tassel, immature ear, or silks. In certain embodiments, the plant cell is a pluripotent plant cell (*e.g.*, a stem cell or meristem cell). In certain embodiments, the plant cell is located within the L1 or L2 layer of an immature or mature embryo of a monocot plant (*e.g.*, maize, wheat, sorghum, or rice).

[0198] In certain embodiments, the plant cell is a haploid, diploid, or polyploid plant cell or plant protoplasts, for example, those obtained from a haploid, diploid, or polyploid plant, plant part or tissue, or callus. In certain embodiments, plant cells in culture (or the regenerated plant, progeny seed, and progeny plant) are haploid or can be induced to become

haploid; techniques for making and using haploid plants and plant cells are known in the art, see, *e.g.*, methods for generating haploids in *Arabidopsis thaliana* by crossing of a wild-type strain to a haploid-inducing strain that expresses altered forms of the centromere-specific histone CENH3, as described by Maruthachalam and Chan in "How to make haploid *Arabidopsis thaliana*", protocol available at www[dot]openwetware[dot]org/images/d/d3/Haploid_Arabidopsis_protocol[dot]pdf; (Ravi *et al.* (2014) *Nature Communications*, 5:5334, doi: 10.1038/ncomms6334). Haploids can also be obtained in a wide variety of monocot plants (*e.g.,* maize, wheat, rice, sorghum, barley) or dicot plants (*e.g.,* soybean, *Brassica* sp. including canola, cotton, tomato) by crossing a plant comprising a mutated CENH3 gene with a wildtype diploid plant to generate haploid progeny as disclosed in US Patent No. 9,215,849, which is incorporated herein by reference in its entirety. Haploid-inducing maize lines that can be used to obtain haploid maize plants and/or cells include Stock 6, MHI (Moldovian Haploid Inducer), indeterminate gametophyte (ig) mutation, KEMS, RWK, ZEM, ZMS, KMS, and well as transgenic haploid inducer lines disclosed in US Patent No. 9,677,082, which is incorporated herein by reference in its entirety. Examples of haploid cells include but are not limited to plant cells obtained from haploid plants and plant cells obtained from reproductive tissues, *e.g.*, from flowers, developing flowers or flower buds, ovaries, ovules, megaspores, anthers, pollen, megagametophyte, and microspores. In certain embodiments where the plant cell or plant protoplast is haploid, the genetic complement can be doubled by chromosome doubling (*e.g.*, by spontaneous chromosomal doubling by meiotic non-reduction, or by using a chromosome doubling agent such as colchicine, oryzalin, trifluralin, pronamide, nitrous oxide gas, anti-microtubule herbicides, anti-microtubule agents, and mitotic inhibitors) in the plant cell or plant protoplast to produce a doubled haploid plant cell or plant protoplast wherein the complement of genes or alleles is homozygous; yet other embodiments include regeneration of a doubled haploid plant from the doubled haploid plant cell or plant protoplast. Another embodiment is related to a hybrid plant having at least one parent plant that is a doubled haploid plant provided by this approach. Production of doubled haploid plants provides homozygosity in one generation, instead of requiring several generations of self-crossing to obtain homozygous plants. The use of doubled haploids is advantageous in any situation where there is a desire to establish genetic purity (*i.e.* homozygosity) in the least possible time. Doubled haploid production can be particularly advantageous in slow-growing plants,

such as fruit and other trees, or for producing hybrid plants that are offspring of at least one doubled-haploid plant.

[0199] In certain embodiments, the plant cell is obtained from or located in any monocot or dicot plant species of interest, for example, row crop plants, fruit-producing plants and trees, vegetables, trees, and ornamental plants including ornamental flowers, shrubs, trees, groundcovers, and turf grasses. In certain non-limiting embodiments, the plant cells are obtained from or located in alfalfa (*Medicago sativa*), almonds (*Prunus dulcis*), apples (*Malus* x *domestica*), apricots (*Prunus armeniaca*, *P. brigantine*, *P. mandshurica*, *P. mume*, *P. sibirica*), asparagus (*Asparagus officinalis*), bananas (*Musa* spp.), barley (*Hordeum vulgare*), beans (*Phaseolus* spp.), blueberries and cranberries (*Vaccinium* spp.), cacao (*Theobroma cacao*), canola and rapeseed or oilseed rape, (*Brassica napus*), carnation (*Dianthus caryophyllus*), carrots (*Daucus carota sativus*), cassava (*Manihot esculentum*), cherry (*Prunus avium*), chickpea (*Cider arietinum*), chicory (*Cichorium intybus*), chili peppers and other capsicum peppers (*Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. pubescens*, *C. baccatum*), chrysanthemums (*Chrysanthemum* spp.), coconut (*Cocos nucifera*), coffee (*Coffea* spp. including *Coffea arabica* and *Coffea canephora*), cotton (*Gossypium hirsutum* L.), cowpea (*Vigna unguiculata*), cucumber (*Cucumis sativus*), currants and gooseberries (*Ribes* spp.), eggplant or aubergine (*Solanum melongena*), eucalyptus (*Eucalyptus* spp.), flax (*Linum usitatissumum* L.), geraniums (*Pelargonium* spp.), grapefruit (*Citrus* x *paradisi*), grapes (*Vitus* spp.) including wine grapes (*Vitus vinifera*), guava (*Psidium guajava*), hemp and cannabis (*e.g., Cannabis sativa* and *Cannabis* spp.), hops (*Humulus lupulus*), irises (*Iris* spp.), lemon (*Citrus limon*), lettuce (*Lactuca sativa*), limes (*Citrus* spp.), maize (*Zea mays* L.), mango (*Mangifera indica*), mangosteen (*Garcinia mangostana*), melon (*Cucumis melo*), millets (*Setaria* spp, *Echinochloa* spp, *Eleusine* spp, *Panicum* spp., *Pennisetum* spp.), oats (*Avena sativa*), oil palm (*Ellis quineensis*), olive (*Olea europaea*), onion (*Allium cepa*), orange (*Citrus sinensis*), papaya (*Carica papaya*), peaches and nectarines (*Prunus persica*), pear (*Pyrus* spp.), pea (*Pisa sativum*), peanut (*Arachis hypogaea*), peonies (*Paeonia* spp.), petunias (*Petunia* spp.), pineapple (*Ananas comosus*), plantains (*Musa* spp.), plum (*Prunus domestica*), poinsettia (*Euphorbia pulcherrima*), Polish canola (*Brassica rapa*), poplar (*Populus* spp.), potato (*Solanum tuberosum*), pumpkin (*Cucurbita pepo*), rice (*Oryza sativa* L.), roses (*Rosa* spp.), rubber (*Hevea brasiliensis*), rye (*Secale cereale*), safflower (*Carthamus tinctorius* L), sesame seed (*Sesame indium*), sorghum (*Sorghum bicolor*), soybean (*Glycine max* L.), squash (*Cucurbita pepo*), strawberries

(*Fragaria* spp., *Fragaria x ananassa*), sugar beet (*Beta vulgaris*), sugarcanes (*Saccharum* spp.), sunflower (*Helianthus annus*), sweet potato (*Ipomoea batatas*), tangerine (*Citrus tangerina*), tea (*Camellia sinensis*), tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum*), tulips (*Tulipa* spp.), turnip (*Brassica rapa rapa*), walnuts (*Juglans* spp. L.), watermelon (*Citrulus lanatus*), wheat (*Tritium aestivum*), or yams (*Discorea* spp.).

ii. Mammalian cells

[0200] In some embodiments, the eukaryotic cell comprising an HDR promoting agent is an animal cell. In some embodiments, the animal cell is a mammalian cell. Further, the methods of the present disclosure may be used to increase HDR-mediated genome modification in an animal cell, make an animal cell having a genomic modification, and/or genetically engineer an animal cell. In some embodiments, the methods may be used to increase HDR-mediated genome modification, make a cell having a genomic modification, and/or genetically engineer a mammalian cell. In some embodiments, the methods disclosed herein comprise editing an animal cell, *e.g.*, a mammalian cell. In some embodiments, the methods disclosed herein comprise performing a genome modification in an animal cell, *e.g.*, a mammalian cell. In some embodiments, the methods disclosed herein comprise modifying a target locus in an animal cell, *e.g.*, a mammalian cell. In some embodiments, the methods disclosed herein comprise increasing HDR-mediated genome modification in an animal cell, *e.g.*, a mammalian cell.

[0201] In some embodiments, the cell is an animal cell from any multicellular vertebrate or invertebrate animal. In some embodiments, the animal is a model organism used for biological, physiological, or genetic research. Accordingly, in some embodiments, the animal is selected from: mouse (*Mus musculus*), zebrafish (*Danio rerio*), fruit fly (*Drosophila melanogaster*), cat (*Felis sylvestris catus*), chicken (*Gallus gallus*), dog (*Canis lupus familiaris*), guinea pig (*Cavia porcellus*), rat (*Rattus norvegicus*) and nematode (*Caenorhabditis elegans*). In some embodiments, the animal is a domesticated or farmed animal. Accordingly, in some embodiments the animal is selected from: goat (*Capra aegagrus hircus*), pig (*Sus scrofa domesticus*), sheep (*Ovis aries*), cattle (*Bos taurus*), cat (*Felis catus*), donkey (*Equus africanus asinus*), duck (*Anas platyrhynchos domesticus*), water buffalo, including *Bubalus bubalis bubalis* and *Bubalus bubalis carabenesis*, the Western honey bee (*Apis mellifera*), including the subspecies Italian bee (*A. mellifera ligustica*), European dark bee (*A. mellifera mellifera*), Carniolan honey bee (*A. mellifera carnica*),

Caucasian honey bee (*A. mellifera caucasia*), and Greek bee (*A. mellifera cecropia*), dromedary camel (*Camelus dromedarius*), horse (*Equus ferns caballus*), silkmoth (*Bombyx mori*), pigeon (*Columba livia*), goose (*Anser domesticus* and *Anser cygnoides domesticus*), yak (*Bos grunniens*), bactrian camel (*Camelus bactrianus*), llama (*Lama glama*), alpaca (*Vicugna pacos*), guineafowl (*Numida meleagris*), ferret (*Mustela putorius furo*), turkey (*Meleagris gallopavo*) grass carp, silver carp, common carp, nile tilapia, bighead carp, catla (indian carp), crucian carp, atlantic salmon, roho labeo, milkfish, rainbow trout, wuchang bream, black carp, northern snakehead and amur catfish.

[0202] In some embodiments, the cell is derived from a cell line, *e.g.*, a mammalian cell line or a human cell line. A wide variety of cell lines for tissue culture are known in the art. Examples of cell lines include, but are not limited to, A549, HEK-293, 293T, MF7, K562, Caco-2, HeLa cells, and transgenic varieties thereof. In some embodiments, the cell is a HEK-293 cell. In some embodiments, the cell is a Chinese hamster ovary (CHO) cell. Cell lines are available from a variety of sources known to those with skill the art (see, *e.g.*, the American Type Culture Collection (ATCC) (Manassus, Va.)). In some embodiments, a cell transfected with one or more nucleic acids (such as a vector encoding HDR promoting agents) as described herein is used to establish a new cell line comprising one or more vector-derived sequences to establish a new cell line comprising modification to a target nucleic acid.

[0203] In some embodiments, the cell is a primary cell, *e.g.*, a mammalian primary cell or a human primary cell. For example, cultures of primary cells can be passaged 0 times, 1 time, 2 times, 4 times, 5 times, 10 times, 15 times or more. In some embodiments, the primary cells are harvest from an individual by any known method. For example, leukocytes may be harvested by apheresis, leukocytapheresis, density gradient separation, *etc.* Cells from tissues such as skin, muscle, bone marrow, spleen, liver, pancreas, lung, intestine, stomach, *etc.* can be harvested by biopsy. An appropriate solution may be used for dispersion or suspension of the harvested cells. Such solution can generally be a balanced salt solution, (*e.g.* normal saline, phosphate-buffered saline (PBS), Hank's balanced salt solution, *etc.*), conveniently supplemented with fetal calf serum or other naturally occurring factors, in conjunction with an acceptable buffer at low concentration. Buffers can include HEPES, phosphate buffers, lactate buffers, *etc.* Cells may be used immediately, or they may be stored (*e.g.*, by freezing). Frozen cells can be thawed and can be capable of being reused. Cells can be frozen in a

DMSO, serum, medium buffer (*e.g.*, 10% DMSO, 50% serum, 40% buffered medium), and/or some other such common solution used to preserve cells at freezing temperatures.

[0204] In some embodiments, the cell is a human cell. In some embodiments, the cell is a germline cell. In some embodiments, the cell is a somatic cell. In some embodiments, the cell is a post-mitotic cell. In some embodiments, the cell is an immune cell, such as a T cell, Natural killer (NK) cell, or a macrophage. In some embodiments, the cell is a human T cell obtained from a patient or a donor. The methods provided herein can be used to modify a target nucleic acid in a primary T cell for use in immunotherapy. In some embodiments, the methods provided herein are used to generate a CAR-T cell, *e.g.*, by editing the genome of the T cell to introduce an expression construct that expresses a chimeric antigen receptor (CAR). In some embodiments, the methods provided herein are used to *ex vivo* modify an immune cell. In some embodiments, the methods provided herein are used to *ex vivo* generate a CAR-T cell. In some embodiments, the methods disclosed herein comprise editing a human cell. In some embodiments, the methods disclosed herein comprise performing a genome modification in a human cell. In some embodiments, the methods disclosed herein comprise modifying a target locus in a human cell. In some embodiments, the methods disclosed herein comprise increasing HDR-mediated genome modification in a human cell.

[0205] In some embodiments, the cell is a stem cell or progenitor cell. In some embodiments, the cell is an un-differentiated cell. In some embodiments, the cell is a human stem cell or progenitor cell. In some embodiments, the cell is a mammalian stem cell or progenitor cell. In some embodiments, the cell is an adult stem cell, an embryonic stem cell, an induced pluripotent (iPS) cell, or a progenitor cell (*e.g.*, a cardiac progenitor cell, neural progenitor cell, *etc.*). In some embodiments, the cell is a hematopoietic stem cell (HSC). In some embodiments, the cell is a mesenchymal stem cell (MSC). In some embodiments, the cell is a neural stem cell. In some embodiments, the cell is an epithelial stem cell. Cells can include mammalian stem cells and progenitor cells, including rodent stem cells, rodent progenitor cells, human stem cells, human progenitor cells, *etc.*

[0206] In some embodiments, the cell is a diseased cell, *e.g.*, a diseased mammalian cell or a diseased human cell. A diseased cell can have altered metabolic, gene expression, and/or morphologic features. In some embodiments, the cell has a genome with a genetic variant associated with disease. In some embodiments, the cell has a SNP associated with a disease. In some embodiments, the genome of the cell has a genetic marker associated with a disease. In some embodiments, the cell has a deleterious mutation. In some embodiments, the cell has

a mutation that causes a disease. In some embodiments, the cell has a mutant allele associated with a disease. In some embodiments, the cell has a loss-of-function mutation. In some embodiments, the cell has a disease genotype. In some embodiments, the cell has a disease phenotype. In some embodiments, the cell has a genetic defect. In some embodiments, the cell has an oncogenic mutation. In some embodiments, the cell has an integrated and/or stably maintained virus. In some embodiments, a retrovirus is integrated into the genome of the cell. In some embodiments, a lentivirus is integrated into the genome of the cell. In some embodiments, the cell has a persistent viral infection. In some embodiments, the cell has HIV. In some embodiments, the cell has an integrated copy of the HIV genome. In some embodiments, the cell is infected with a virus. In some embodiments, the cell has a latent viral infection. In some embodiments, the cell is infected by a herpesvirus. In some embodiments, the cell is infected by a Human Herpesviruses 6 or 7. In some embodiments, the cell is infected by Herpes Simplex Virus Types 1 or 2. In some embodiments, the cell is infected by Varicella-Zoster Virus. In some embodiments, the cell is infected by a Human Papovavirus. In some embodiments, the cell is infected by an Epstein-Barr Virus. A diseased cell can be a cancer cell, a diabetic cell, or an apoptotic cell. A diseased cell can be a cell from a diseased subject. Exemplary diseases can include genetic disorders, infectious diseases, blood disorders, cancers, metabolic disorders, eye disorders, organ disorders, musculoskeletal disorders, cardiac disease, and the like. In some embodiments, the cell is derived from a patient. In some embodiments, the cell is modified *ex vivo*. In some embodiments, the cell is a cancer cell. In some embodiments, the cell is an embryonic cell. In some embodiments, the cell is an embryonic stem cell.

[0207] In some embodiments, the methods provided herein are used to genetically modify a diseased cell, *e.g.*, a diseased mammalian cell or a diseased human cell. In some embodiments, the methods provided herein are used to genetically modify a diseased cell. In some embodiments, the methods provided herein are used to insert a wild-type allele of a gene into a diseased cell. In some embodiments, the methods provided herein are used to correct a deleterious mutation in a diseased cell. In some embodiments, the methods provided herein are used to genetically modify an oncogene. In some embodiments, the methods provided herein are used to genetically modify an allele of a gene associated with disease. In some embodiments, the methods provided herein are used to insert a healthy allele of a gene. In some embodiments, the methods provided herein are used to insert an allele of a gene that is not associated with disease. In some embodiments, the methods provided herein are used to

remove an integrated or stably maintained virus, such as a lentivirus, a retrovirus, or a herpesvirus, from the genome of the cell.

iii. Fungal cells

[0208] In some embodiments, the eukaryotic cell is a fungal cell. In some embodiments, the eukaryotic cell comprising an HDR promoting agent is a fungal cell. Further, the methods of the present disclosure may be used to increase HDR-mediated genome modification in a fungal cell, make a fungal cell having a genomic modification, and/or genetically engineer a fungal cell. In some embodiments, the methods disclosed herein comprise editing a fungal cell. In some embodiments, the methods disclosed herein comprise performing a genome modification in a fungal cell. In some embodiments, the methods disclosed herein comprise modifying a target locus in a fungal cell. In some embodiments, the methods disclosed herein comprise increasing HDR-mediated genome modification in a fungal cell.

[0209] In some embodiments, the fungal cell is a cell derived from a multicellular fungus. In some embodiments, the cell is an ascomycete cell. In some embodiments, the cell is a single-celled fungus. In some embodiments, the cell is a yeast cell. In some embodiments, the cell is a fungal cell of the genus *Aspergillus*, *Candida*, *Cochliobolus*, *Cryphonectria*, *Cryptococcus*, *Epidermophyton*, *Fusarium*, *Kluyveromyces*, *Lachancea*, *Mucor*, *Neurospora*, *Ophiostoma*, *Penicillium*, *Pichia*, *Pneumocystis*, *Pullularia*, *Saccharomyces*, *Schizosaccharomyces*, *Tolypocladium*, *Trichoderma*, *Rhodotorula*, or *Yarrowia*. In some embodiments, the cell is a *Candida* sp. cell, such as a *C. albicans, C. auris*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, or a *C. tropicalis* cell. In some embodiments, the cell is a chytrid fungal cell, *i.e.*, a *Chytridiomycota* cell. In some embodiments, the cell is a *Batrachochytrium* sp. cell, such as a *Batrachochytrium dendrobatidis* cell. In some embodiments, the cell is a *Microsporidia* cell, such as a *Glugea* sp. or *Nosema* sp. cell. In some embodiments, the fungal cell is a parasite. In some embodiments, the cell is a *Trichophyton* sp. or *Microsporum* sp. cell, *i.e.*, a member of the genera of fungi that includes the parasitic varieties that cause tinea. In some embodiments, the cell is a filamentous fungal cell, *i.e.*, a cell from a filamentous fungus. In some embodiments, the cell is a *Cryptococcus* sp. cell, such as a *Cryptococcus neoformans* cell. In some embodiments, the cell is a *Botrytis* sp. cell, such as a *Botrytis cinerea, Botrytis allii, Botrytis anthophila, Botrytis elliptica, Botrytis fabae, Botrytis squamosal,* or a *Botrytis tracheiphila* cell.

iv. Other eukaryotic cells

[0210] In some embodiments, the eukaryotic cell comprising an HDR promoting agent is a microbial eukaryotic cell. Further, the methods of the present disclosure may be used to increase HDR-mediated genome modification in a microbial eukaryotic cell, make a microbial eukaryotic cell having a genomic modification, and/or genetically engineer a microbial eukaryotic cell. In some embodiments, the methods disclosed herein comprise editing a microbial eukaryote. In some embodiments, the methods disclosed herein comprise performing a genome modification in a microbial eukaryote. In some embodiments, the methods disclosed herein comprise modifying a target locus in a microbial eukaryote. In some embodiments, the methods disclosed herein comprise increasing HDR-mediated genome modification in a microbial eukaryote. In some embodiments, the cell is a microbial eukaryote. In some embodiments, the cell is a cell of a single-celled eukaryotic organism. In some embodiments, the cell is a protozoa cell. In some embodiments, the cell is a protist. In some embodiments, the cell is an infectious microbial eukaryote. In some embodiments, the cell is a parasitic microbial eukaryote. In some embodiments, the cell is a *Giardia* sp. cell, such as a *G. lamblia*, *G. muris*, *G. ardeae*, *G. psittaci*, *G. agilis* or *G. microti* cell. In some embodiments, the cell is a *Plasmodium* sp. cell, such as a *P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale*, or *P. knowlesi* cell. In some embodiments, the cell is a kinetoplastid cell. In some embodiments, the cell is a *Trypanosoma* sp. cell, such as a *Trypanosoma cruzi* or *Trypanosoma brucei* cell.

[0211] In some embodiments, the cell is an algal cell. In some embodiments, the algal cell is of a species of *Achnanthes, Amphiprora, Amphora, Ankistrodesmus, Asteromonas, Boekelovia, Bolidomonas, Borodinella, Botrydium, Botryococcus, Bracteococcus, Chaetoceros, Carteria, Chlamydomonas, Chlorococcum, Chlorogonium, Chlorella, Chroomonas, Chrysosphaera, Cricosphaera, Crypthecodinium, Cryptomonas, Cyclotella, Dunaliella, Ellipsoidon, Emiliania, Eremosphaera, Ernodesmius, Euglena, Eustigmatos, Franceia, Fragilaria, Fragilaropsis, Gloeothamnion, Haematococcus, Halocafeteria, Heterosigma, Hymenomonas, Isochrysis, Lepocinclis, Micractinium, Monoraphidium, Nannochloris, Nannochloropsis, Navicula, Neochloris, Nephrochloris, Nephroselmis, Nitzschia, Ochromonas, Oedogonium, Oocystis, Ostreococcus, Pavlova, Parachlorella, Pascheria, Pelagomonas, Phaeodactylum, Phagus, Picochlorum, Platymonas, Pleurochrysis, Pleurococcus, Prototheca, Pseudochlorella, Pseudoneochloris, Pseudostaurastrum, Pyramimonas, Pyrobotrys, Scenedesmus, Skeletonema, Spyrogyra, Stichococcus,*

Tetraselmis, Thalassiosira, Tribonema, Vaucheria, Viridiella, Vischeria, or *Volvox.* In some embodiments, the cell is diatom. Diatoms include members of the genera *Achnanthes, Amphora, Chaetoceros, Coscinodiscus, Cylindrotheca, Cyclotella, Cymbella, Fragilaria, Fragilaropsis, Hantzschia, Navicula, Nitzschia, Pseudo-Nitzschia, Phaeodactylum, Psammodictyon, Skeletonema, Thalassionema*, and *Thalassiosira.* In some embodiments, the cell is a eustigmatophyte such as a *Nannochloropsis* species or a species of *Monodus*, *Pseudostaurastrum*, *Vischeria*, and *Eustigmatos*. In some embodiments, the cell is an algal cell of the genus *Nannochloropsis* such as, but are not limited to, *N. gaditana*, *N. granulata*, *N. limnetica*, *N. oceanica*, *N. oculata*, and *N. salina.*

[0212] In some embodiments, the cell is a heterokont. For example, heterokonts include not only eustigmatophytes and diatoms such as those listed above but also chytrid species, including labrinthulids and thraustochytrids. In some embodiments, the cell is of a heterokont species including, but are not limited to, *Bacillariophytes*, *Eustigmatophytes*, *Labrinthulids*, and *Thraustochytrids*. In some embodiments, the cell is of a species of *Labryinthula*, *Labryinthuloides*, *Thraustochytrium*, *Schizochytrium*, *Aplanochytrium*, *Aurantiochytrium*, *Japonochytrium*, *Diplophrys*, or *Ulkenia*. For example, the strain may be a species of *Thraustochytrium*, *Schizochytrium*, *Oblongichytrium*, or *Aurantiochytrium*. In some embodiments, the cell is an opisthokont. In some embodiments, the cell is a choanoflagellate. In some embodiments, the cell is amesomycetozoea (*e.g.*, *Sphaeroforma*). In some embodiments, the cell is a unikont. In some embodiments, the cell is an amoebozoa. In some embodiments, the cell is of the genus *Acanthamoeba*, *Amoeba, Chaos*, *Dictyostelium Entamoeba*, or *Pelomyxa*.

v. Compositions of cells

[0213] Provided herein are compositions of cells. In one aspect, the methods provided herein may be used to produce a composition of eukaryotic cells. In some embodiments, the composition of eukaryotic cells may be comprised of any of the cells described herein, *e.g.*, plant, animal, fungal, or other eukaryotic cells. In some embodiments, the methods disclosed herein comprise editing a population of cells. In some embodiments, the methods disclosed herein comprise producing an edited population of cells. In some embodiments, the methods disclosed herein comprising producing an edited population of cells, wherein the proportion of edited cells in the population is about any one of 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 20-, 25-, 30-fold higher than that of a population of cells edited in

the absence of HDR promoting agents, including any value or range between these values. In some embodiments, the methods disclosed herein comprising producing an edited population of cells, wherein the proportion of edited cells in the population is 10-fold higher than that of a population of cells edited in the absence of HDR promoting agents.

[0214] In some embodiments, provided herein are compositions clonal subpopulations of cells used in the methods provided herein. In some embodiments, the clonal subpopulation is a subpopulation of a cell line. In some embodiments, the clonal subpopulation is a subpopulation of cells derived from an individual. In some embodiments, the clonal cell subpopulation is a population of cells derived from a single cell. In some embodiments, the clonal cell subpopulation has the same genetic and epigenetic profile.

[0215] In some embodiments, the methods disclosed herein comprise performing a genome modification in a population of cells. In some embodiments, the methods disclosed herein comprise producing a composition of cells with a genome modification. In some embodiments, the methods disclosed herein comprising producing a composition of cells with a genome modification, wherein the proportion of cells in the population with the genome modification is 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 20-, 25-, 30-fold higher than that of a population of cells modified in the absence of HDR promoting agents, including any value or range between these values. In some embodiments, the methods disclosed herein comprise modifying a target locus in a population of cells. In some embodiments, the methods disclosed herein comprise producing a population of cells with a modified target locus. In some embodiments, the methods disclosed herein comprise producing a population of cells with a modified target locus, wherein the proportion of cells in the population with the modified target locus is 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 20-, 25-, 30-fold higher than that of a population of cells modified in the absence of HDR promoting agents, including any value or range between these values.

E. Kits

[0216] The methods of this invention can be provided in the form of a kit. In some embodiments, the kit comprises a nucleic acid encoding an HDR promoting agent. In some embodiments, the kit 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) and instructions for use. In some embodiments, the kit provides a vector comprising the nucleic acids. In some embodiments, the kit is used to modify a target editing site of the cell using the donor template DNA molecule. In some embodiments, the kit comprises any of the vectors described herein. In some embodiments, the kit comprises vectors for increasing HDR-mediated genome modification of a target editing site of a eukaryotic cell genome, such as a plant or mammalian cell genome. In some embodiments, the kit comprises vectors for increasing HDR-mediated genome modification of a target editing site in a plant cell. In some embodiments, the kit comprises vectors for increasing HDR-mediated genome modification of a target editing site in a mammalian cell.

[0217] In some embodiments, the kit comprises instructions. In some embodiments, the instructions include instructions on transforming a cell with the nucleic acids. In some embodiments, the instructions include instructions on detecting the presence of the nucleic acids in the cell. In some embodiments, the instructions include instructions on assessing the effects of the nucleic acids in the cell.

[0218] In some embodiments, the kit comprises an agent for detecting genetically engineered cells. In some embodiments, the kit comprises instructions for using the agent to detect genetically engineered cells. In some embodiments, the agent for detecting genetically engineered cells is an assay to assess the genome of the cells, such as a PCR assay, an RT-qPCR assay, a Southern blot, or a sequencing assay. In some embodiments, the agent for detecting genetically engineered cells is a set of oligonucleotide primers, wherein certain pairs of primers specifically amplify the genetic modification, or the wild-type target locus. In some embodiments, detection of the genetically engineered cells is performed using a reporter, such as a fluorescent reporter, a transcriptional reporter, a colorimetric reporter, or a chemiluminescent reporter. Accordingly, in some embodiments, the agent for detecting genetically engineered cells is a means for detecting the reporter.

[0219] In some embodiments, provided herein is a kit for increasing Homology Directed Repair (HDR)-mediated genome modification of a target editing site of a eukaryotic cell genome, such as a plant or mammalian cell genome. In some embodiments, the kit comprises nucleic acids encoding genome-editing molecules and HDR promoting agents. In some embodiments, 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. In some embodiments, 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). In some embodiments, 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. In some embodiments, the kit comprises an agent for measuring the level of HDR-mediated genome modification of the target editing site.

[0220] In some embodiments, provided herein is a kit for making a eukaryotic cell having a genomic modification. In some embodiments, the kit comprises nucleic acids encoding genome editing molecules and Homology Directed Repair (HDR) promoting agents, 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. In some embodiments, the kit provides a means of isolating or propagating a eukaryotic cell comprising the genome modification, thereby making the eukaryotic cell having a genomic modification. In some embodiments, the kit comprises an agent for detecting the presence of the genome modification of the target editing site.

[0221] In some embodiments, provided herein is a kit for a method of genetic engineering of a eukaryotic cell. In some embodiments, the kit 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). In some embodiments, the kit comprises an agent for detecting genetic engineering of the target editing site.

Embodiments

[0222] Various embodiments of the eukaryotic cells (*e.g.*, plant cells and mammalian cells), systems, and methods provided herein are included in the following non-limiting list of embodiments.

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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 any one of embodiments 1 to 8, wherein the donor DNA molecule is flanked by copies of an endonuclease recognition sequence.

10. The method of any one of embodiments 1 to 9, 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 any one of embodiments 1 to 10, wherein the sequence-specific endonuclease provides a 5' overhang at the target editing site following cleavage.

12. The method of any one of embodiments 1 to 11, wherein the SSAP provides for DNA strand exchange and base pairing of complementary DNA strands of homologous DNA molecules.

13. The method of any one of embodiments 1 to 12, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.

14. The method of embodiment 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 embodiment 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 embodiment 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 any one of embodiments 1 to 16, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.

18. The method of embodiment 17, wherein a linear dsDNA molecule comprising a phosphorylated 5' terminus is a preferred substrate of the exonuclease.

19. The method of any one of embodiments 1 to 16, 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 any one of embodiments 1 to 16, 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 any one of embodiments 1 to 16, 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 any one of embodiments 1, 5, or 6, 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 any one of embodiments 1 to 22, wherein the single stranded DNA binding protein (SSB) and the SSAP are obtained from the same host organism.

24. The method of any one of embodiments 1 to 23, wherein the single stranded DNA binding protein (SSB) is a bacterial SSB or optionally an *Enterobacteriaceae* sp. SSB.

25. The method of embodiment 24, 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 any one of embodiments 1 to 23, 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 any one of embodiments 1 to 26, 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 any one of embodiments 1 to 26, 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 any one of embodiments 1 to 28, 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 any one of embodiments 1 to 29, 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 embodiments 1 to 30, where the eukaryotic cell is a mammalian cell or a plant cell.

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

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

34. The method of any one of embodiments 31-33, 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 any one of embodiments 31 to 34, 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 36, wherein the donor DNA molecule is provided on a plasmid or a geminivirus genome.

44. The system of any one of embodiments 36 to 43, wherein the donor DNA molecule is flanked by an endonuclease recognition sequence.

45. The system of any one of embodiments 36 to 44, 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 any one of embodiments 36 to 45, wherein the sequence-specific endonuclease provides a 5' overhang at the target editing site following cleavage.

47. The system of any one of embodiments 36 to 46, 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 any one of embodiments 36 to 47, wherein the SSAP provides for DNA strand exchange and base pairing of complementary DNA strands of homologous DNA molecules.

49. The system of embodiment 36 or 48, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.

50. The system of embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 any one of embodiments 36 to 54, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.

56. The system of any one of embodiments 36 to 54, wherein a linear dsDNA molecule comprising a phosphorylated 5' terminus is a preferred substrate of the exonuclease.

57. The system of any one of embodiments 36 to 54, 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 any one of embodiments 36 to 54, 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 any one of embodiments 36 to 58, 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 any one of embodiments 36, 40, or 41, 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 any one of embodiments 36 to 60, wherein the single stranded DNA binding protein (SSB) and the SSAP are obtained from the same host organism.

62. The system of any one of embodiments 36 to 61, wherein the single stranded DNA binding protein (SSB) is a bacterial SSB or optionally an *Enterobacteriaceae* sp. SSB.

63. The system of embodiment 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 any one of embodiments 36 to 63, 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 any one of embodiments 36 to 64, 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 any one of embodiments 36 to 64, 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 any one of embodiments 36 to 66, 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 any one of embodiments 36 to 64, 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 embodiments 36 to 68, where the eukaryotic cell is a mammalian cell or a plant cell.

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

71. The system of embodiment 69 or 70, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

72. The system of embodiment 69, 70, or 71, 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 any one of embodiments 69 to 72, 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. 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 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 embodiment 74, wherein the donor DNA molecule is provided in a plasmid or a geminivirus genome.

82. The method of any one of embodiments 74 to 81, wherein the donor DNA molecule is flanked by an endonuclease recognition sequence.

83. The method of any one of embodiments 74 to 82, 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 any one of embodiments 74 to 83, wherein the sequence-specific endonuclease provides a 5' overhang at the target editing site following cleavage.

85. The method of any one of embodiments 74 to 84, wherein the SSAP provides for DNA strand exchange and base pairing of complementary DNA strands of homologous DNA molecules.

86. The method of any one of embodiments 74 to 85, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.

87. The method of embodiment 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. The method of embodiment 86, 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. The method of embodiment 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. The method of embodiment 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 any one of embodiments 74 to 90, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.

92. The method of any one of embodiments 74 to 91, wherein a linear dsDNA molecule comprising a phosphorylated 5' terminus is a preferred substrate of the exonuclease.

93. The method of any one of embodiments 74 to 92, 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 any one of embodiments 74 to 92, 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 any one of embodiments 74 to 90, 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 embodiment 74, 78, or 79, 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 any one of embodiments 74 to 96, wherein the single stranded DNA binding protein (SSB) and the SSAP are obtained from the same host organism.

98. The method of any one of embodiments 74 to 97, wherein the single stranded DNA binding protein (SSB) is a bacterial SSB or optionally an *Enterobacteriaceae* sp. SSB.

99. The method of embodiment 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. The method of any one of embodiments 74 to 99, 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. The method of any one of embodiments 74 to 100, 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. The method of any one of embodiments 74 to 100, 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. The method of any one of embodiments 74 to 102, 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. The system of any one of embodiments 74 to 103, 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. The method of any one of embodiments 74 to 104, where the eukaryotic cell is a mammalian cell or a plant cell.

106. The method of embodiment 105, wherein the plant cell is haploid, diploid, or polyploid.

107. The method of embodiment 105 or 106, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

108. The method of embodiment 105, 106, or 107, 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. The method of any one of embodiments 105 to 108, 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. The method of any one of embodiments 1-30, the system of any one of embodiments 36 to 68, or the method of any one of embodiments 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. 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. The method of embodiment 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. The method of embodiment 112, 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. The method of embodiment 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. The method of embodiment 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. The method of embodiment 115, 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. The method of embodiment 115, 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. The method of embodiment 111, wherein the donor DNA molecule is provided in a plasmid or a geminivirus genome.

119. The method of embodiment 111, wherein the donor DNA molecule is flanked by an endonuclease recognition sequence.

120. The method of embodiment 111, wherein the SSAP comprises an RecT/Redβ-, ERF-, or RAD52-family protein.

121. The method of embodiment 120, 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. The method of embodiment 111, wherein a linear dsDNA molecule is a preferred substrate of the exonuclease.

123. The method of embodiment 111, wherein a linear dsDNA molecule comprising a phosphorylated 5' terminus is a preferred substrate of the exonuclease.

124. The method of embodiment 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. The method of embodiment 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. The method of embodiment 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. The method of embodiment 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. The method of embodiment 111, wherein the single stranded DNA binding protein (SSB) and the SSAP are obtained from the same host organism.

129. The method of any one of embodiments 111 to 128, where the eukaryotic cell is a mammalian cell or a plant cell.

130. The method of embodiment 129, wherein the plant cell is haploid, diploid, or polyploid.

131. The method of embodiment 130, wherein the plant cell is in a culture medium, in a plant, or in a plant tissue.

132. The method of embodiment 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. The method of any one of embodiments 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 in one or more vectors.

135. The method of embodiment 133, wherein the vector is an agrobacterium vector.

136. The method of any one of embodiments 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. The method of any one of embodiments 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. The method of any one of embodiments 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. The method of any one of embodiments 111-138, further comprising detecting the modification.

140. The method of embodiment 139, wherein detecting the modification comprises amplicon sequencing.

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

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

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

148. The method of any one of embodiments 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 embodiment 144-148, and

(ii) regenerating said cell into an organism.

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

151. 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. The composition of embodiment 151, wherein the nucleic acids are in one or more vectors.

153. 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. The vector of embodiment 153, wherein the vector comprises the donor template DNA, the sequence specific endonuclease and a polynucleotide encoding a guide RNA.

155. The vector of embodiment 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. The vector of embodiment 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. 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. The kit of embodiment 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. The kit of any one of embodiments 157-158, further comprising an agent for detecting genetically engineered cells.

160. 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. 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. The cell of embodiment 160 or 161, wherein the cell is a plant or mammalian cell.

163. The cell of any one of embodiments 160-162, wherein the cell is a host cell.

164. A genetically engineered cell produced by the method of any one of embodiments 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 embodiment 165, wherein the donor template is a double-stranded DNA molecule.

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

168. The progenitor eukaryotic cell or organism of embodiment 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 embodiment 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 embodiment 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 embodiment 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. The vector according to embodiment 153, wherein the sequence-specific nuclease is operably linked to an inducible promoter.

173. The method of embodiment 111, wherein the sequence-specific endonuclease is a nickase.

EXAMPLES

[0223] The examples below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation.

Example 1. Exonuclease, SSAP, and SSB expression vectors and donor DNA template sequences

[0224] This example describes the construction of plant expression vectors used to express a bacteriophage lambda exonuclease (SEQ ID NO:8), a bacteriophage lambda beta SSAP protein (SEQ ID NO:1), and an *E.coli* SSB (SEQ ID NO:31).

[0225] Plant expression constructs for expressing a Bacteriophage lambda exonuclease (SEQ ID NO:8), a bacteriophage lambda beta SSAP protein (SEQ ID NO: 1), and an *E.coli* SSB (SEQ ID NO:31) were constructed. A DNA sequence encoding a tobacco c2 nuclear localization signal (NLS) of SEQ ID NO:15 was operably linked to the DNA sequences encoding the exonuclease, the bacteriophage lambda beta SSAP protein, and the *E.coli* SSB to provide a DNA sequence encoding the c2 NLS-Exo (also known as Red-Exo), c2 NLS lambda beta SSAP (also known as Red-Beta), and c2 NLS-SSB fusion proteins that are set forth in SEQ ID NO: 135, SEQ ID NO: 134, and SEQ ID NO: 133, respectively. DNA sequences encoding the c2 NLS-Exo, c2 NLS lambda beta SSAP, and c2NLSf-SSB fusion proteins were operably linked to a 2x35S, SlUBI10, PcUBI4 promoter and a 35S, AtHSP, pea3A polyadenylation site respectively, to provide the exonuclease, SSAP, and SSB plant cell gene expression cassettes (see **FIG. 2**).

[0226] DNA donor template plasmids that targeted the promoter region of the tomato Ant1 gene for insertion of a 42 base pair heterologous sequence by HDR were constructed (**FIG. 1**). The circular DNA donor plasmid included a replacement template with desired insertion region (42 base pairs long) flanked on both sides by homology arms about 600-800 bp in length. The homology arms matched (*i.e.,* were homologous to) gDNA (genomic DNA) regions flanking the target gDNA insertion site. The replacement template region comprising

the donor DNA was flanked at each end by DNA sequences identical to the target gDNA sequence recognized by an RNA-guided nuclease. Plant expression cassettes that provided for expression of the RNA-guided sequence-specific endonuclease and a guide RNA complementary to sequences adjacent to the insertion site were also constructed (**FIG. 1**).

Example 2. Genome Editing Experiments with Tomato Protoplasts

[0227] This example describes gene editing in tomato protoplasts with both blunt- and staggered end cutting CAS nucleases in the presence and absence of an exonuclease, SSB, and SSAP.

[0228] Tomato protoplasts were isolated, cultivated, and subject to PEG-mediated transfection essentially according to published procedures (Čermák *et al.* 2017). The transfected materials included plasmids having the donor DNA template region described in Example 1, as well as expressing the gRNAs and Cas polynucleotides as indicated (**FIG. 1**). Cas polynucleotides were fused to a nuclear localization signal. The gRNA both targets a double strand break into the intended genomic DNA target and releases the replacement template from the donor plasmid (see **FIG. 1**). Some experiments were carried out with a Cas nuclease which is representative of a CAS nuclease that leaves a blunt end following cleavage of the endonuclease recognition sequence and referred to herein as a CasB nuclease. Other experiments were carried out with Cas nuclease which is representative of a CAS nuclease that leaves a staggered single stranded DNA overhanging end following cleavage of the endonuclease recognition sequence and referred to herein as a CasS nuclease.

[0229] After 48 hour of incubation of the protoplasts following transfection, gDNA was extracted from transfected samples and the target locus was amplified with primers complementary to genomic sequences flanking the introduced replacement sequence and the homology arm of the replacement template, and analyzed by amplicon sequencing.

[0230] Amplicons were sequenced using paired-end Illumina sequencing. Due to the size of the amplicon, only one read end (Read 1) of the paired-end reads covered the site of interest containing the targeted sequence insertion. Reads of interest (Read 1) were trimmed for quality and aligned to the reference amplicon. The reads had a unique molecular identifier (UMI) tag to distinguish them from some kinds of PCR duplicates, and these reads were de-duplicated from the alignment. The read that mapped to the un-edited genomic sequence (Read 2) was then checked for correct mapping to the genome. Alignments generated from Read 1s were analyzed with CrispRVariants, which described and tallied all of the sequence

alleles which differed within a 100bp window centered on the cut site (Lindsay, H. *et al.* *Nature Biotechnology* 2016 34: 701-702). CrispRVariants reported the frequency of reads of each allele in number of reads of the total alignment. Different sequence alleles were categorized as 1) wildtype sequence, SNPs, or sequencing artifacts, 2) indel mutations, or 3) precise insertion events. CrispRVariants automatically detected SNPs based on the type of mutation and its distance from the defined cut site, an additional filtering steps were used to remove any other sequence aberration that did not involve bases within 5bp on either side of the predicted cut site. These alleles were placed in category 1. All sequencing alleles which had an insertion or deletion mutation that involved any base within 5bp on either side of the cut site were determined to be indels and were placed in category 2. Successful precise gene targeting yielded a single CrispRVariants sequence allele which was identifiable by an insertion of the expected size and sequence. In **Tables 1-2**, below, the frequencies reported for % indel are the sum of all frequencies of all sequencing alleles determined to be indels. The frequencies reported for % precise are the frequency of the single precise insertion sequencing allele. The denominator for both frequencies is the sum of all reads which aligned to the reference amplicon.

[0231] Results of average measurements are summarized in **Table 1** below. CasS (1) and CasS (2), were similar treatments, except that 2-fold increase of guide RNA was used in (2) when compared to (1). "Lambda RED" refers to all three HDR promoting agents (the exonuclease, lambda beta SSAP protein, and the SSB). SD = standard deviation.

Table 1

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
CasB, gRNA, GFP, donor DNA template plasmid + Lambda RED plasmid **(all – CasB)**	8.25	3.68	1.19	0.39
CasS (1), 1X gRNA, GFP, donor DNA template plasmid + Lambda RED plasmid **(all CasS 1x)**	0.53	1.94	0.28	0.22
CasS (2), 2X gRNA, GFP, donor DNA template plasmid + Lambda RED plasmid **(all CasS 2x)**	0.43	1.91	0.38	0.33
CasB, gRNA, GFP, donor DNA template plasmid **(no Lambda Red – CasB)** (Baseline control)	29.2	0.3	1.1	0.07

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
CasS (1), 1X gRNA, GFP, donor DNA template plasmid **(no Lambda Red – CasS 1x)** (Baseline control)	6.43	0.1	0.27	0.05
CasS (2), 2X gRNA, GFP, donor DNA template plasmid **(no Lambda Red – CasS 2x)** (Baseline control)	5.42	0.13	0.98	0.06
Lambda RED plasmid + donor DNA template, GFP plasmid **(no nuclease)**	0.17	0.27	0.15	0.19
Donor DNA template, GFP plasmid **(donor only)**	0.54	0.22	0.62	0.18
Lambda RED plasmid + GFP plasmid **(Lambda Red only)**	0.51	0	0.34	0
Green fluorescent protein plasmid **(GFP only)**	0.02	0	0.04	0

[0232] Transfection of all three HDR promoting agents (*i.e.,* the SSB, the exonuclease, and the SSAP) greatly enhanced (about 10-fold) the occurrence of HDR for both the CasB blunt end nuclease experiments and the CasS staggered end cutting nuclease. The baseline was measured in the absence of all three HDR promoting agents, when the donor template (HDR) was incorporated in only 0.1-0.22% of the genome editing edits. As indicated in **Table 1**, the samples that did not contain the HDR promoting agents served as the baseline controls.

[0233] Eliminating any one or two of the three HDR promoting agents significantly diminished HDR occurrence, although in all cases it was still measurable above the baseline (**Table 2**).

Table 2

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
CasB, gRNA, GFP, donor DNA template plasmid + Lambda RED plasmid **(all – CasB)**	9.16	2.89	0.50	0.19
Lambda RED plasmid + donor DNA template, GFP plasmid **(no nuclease)**	0.04	2.11	0.03	0.78

Red-Beta, Red-Exo, Hyg plasmid + CasB, gRNA, GFP, donor DNA template plasmid **(no SSB)**	5.99	0.52	1.72	0.51
Red-Beta, SSB, Hyg plasmid + CasB, gRNA, GFP, donor DNA template plasmid **(no Exo)**	11.63	0.26	0.99	0.02
Red-Exo, SSB, GFP plasmid + CasB, gRNA, GFP, donor DNA template plasmid **(no Beta)**	10.49	0.97	1.20	0.33
SSB, GFP, Hyg plasmid + CasB, gRNA, GFP, donor DNA template plasmid **(SSB only)**	6.71	0.27	0.29	0.13
Red-Exo, GFP plasmid + CasB, gRNA, GFP, donor DNA template plasmid **(Exo only)**	12.83	0.56	1.73	0.17
Red-Beta, mCherry, Hyg plasmid + CasB, gRNA, GFP, donor DNA template plasmid **(Beta only)**	14.23	0.28	1.20	0.04
mCherry, GFP, Hyg plasmid + CasB, gRNA, GFP, donor DNA template plasmid **(CasB + no Lambda Red)** (Baseline control)	14.15	0.24	1.07	0.02
CasB, gRNA, GFP, donor DNA template plasmid **(CasB + no Lambda Red)** (Baseline control)	21.17	0.41	0.39	0.12
No transformation	0.00	0.00	0.00	0.00

[0234] CasS nuclease-mediated editing with staggered ends at target editing sites produced a higher proportion of precise editing events (HDR) than CasB nuclease-mediated editing with blunt ends at target editing sites. Accordingly, about 80% of CasS nuclease-mediated and 30% of CasB nuclease-mediated editing events were precise HDR events versus NHEJ events. The rate of generating NHEJ events was significantly decreased by the presence of the HDR promoting agents.

Example 3. Genome Editing Experiments with Maize Protoplasts

[0235] This example describes gene editing in maize protoplasts in the presence and absence of an exonuclease, SSB, and SSAP, with blunt end cutting CAS nucleases inducing two double strand breaks in close proximity, to induce sequence replacement rather than insertion.

[0236] DNA donor template plasmids are constructed that target the coding region of the maize PYL-E gene for HDR-mediated replacement of a 110 base pair sequence to introduce 7 base edits resulting in synonymous mutations and disruption of the two PAM sites targeted by the two gRNAs and 1 base edit resulting in an amino acid change. The circular DNA donor plasmid includes a replacement template with the desired modification (110 base pairs long region with 8 base modifications) flanked on both sides by homology arms about 500 bp in length. The homology arms match (*i.e.,* are homologous to) gDNA (genomic DNA) regions flanking the two gRNA target sites. The replacement template region comprising the donor DNA is flanked at each end by DNA sequence identical to one of the two target gDNA sequences recognized by an RNA-guided nuclease.

[0237] Maize protoplasts are isolated, cultivated, and subjected to PEG-mediated transfection. The transfected materials includes plasmids expressing the c2 NLS-Exo, c2 NLS lambda beta SSAP, and c2 NLS-SSB fusion proteins that are set forth in SEQ ID NO: 135, SEQ ID NO: 134, and SEQ ID NO: 133, and are operably linked to a 2x35S, ZmUBI1, OsACT1 promoter and a 35S, AtHSP, pea3A polyadenylation site respectively. The plasmids also has the donor DNA template region described above, and expressing the two gRNAs and Cas polynucleotides as indicated. Cas polynucleotides are fused to a nuclear localization signal. Each of the two gRNAs both target a double strand break into the intended genomic DNA target and a sequence flanking the replacement template on one end in order to release the replacement template from the donor plasmid. Experiments are carried out with a Cas nuclease which leaves a blunt end following cleavage of the endonuclease recognition sequence and referred to herein as a CasB nuclease.

[0238] After 48 hour of incubation of the protoplasts following transfection, gDNA is extracted from transfected samples and the target locus was amplified with primers complementary to genomic sequences flanking the introduced base modifications and the homology arm of the replacement template, and analyzed by amplicon sequencing. HDR is observed at increased levels in protoplasts transfected with the plasmids expressing the c2 NLS-Exo, c2 NLS lambda beta SSAP, and c2 NLS-SSB fusion proteins, gRNAs, and

polynucleotides encoding the Cas nuclease in comparison to the controls transfected with only the gRNAs and polynucleotides encoding the Cas nuclease.

Example 4. Biological Sequences

[0239] This example provides non-limiting embodiments of protein and nucleic acid sequences referred to herein. Biological sequences and their SEQ ID NOs are set forth in **Table 3**.

Table 3: Biological Sequences

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
1	Bacteriophage Lambda beta protein	MSTALATLAGKLAERVGMDSVDPQELITTLRQTAFKGDASDAQFI ALLIVANQYGLNPWTKEIYAFPDKQNGIVPVVGVDGWSRIINENQ QFDGMDFEQDNESCTCRIYRKDRNHPICVTEWMDECRREPFKTRE GREITGPWQSHPKRMLRHKAMIQCARLAFGFAGIYDKDEAERIVE NTAYTAERQPERDITPVNDETMQEINTLLIALDKTWDDDLLPLCS QIFRRDIRASSELTQAEAVKALGFLKQKAAEQKVAA	NCBI Reference Sequence: WP_000100844.1
2	Rac bacterial prophage RecT protein	MTKQPPIAKADLQKTQGNRAPAAVKNSDVISFINQPSMKEQLAAA LPRHMTAERMIRIATTEIRKVPALGNCDTMSFVSAIVQCSQLGLE PGSALGHAYLLPFGNKNEKSGKKNVQLIIGYRGMIDLARRSGQIA SLSARVVREGDEFSFEFGLDEKLIHRPGENEDAPVTHVYAVARLK DGGTQFEVMTRKQIELVRSLSKAGNNGPWVTHWEEMAKKTAIRRL FKYLPVSIEIQRAVSMDEKEPLTIDPADSSVLTGEYSVIDNSEE	NCBI Reference Sequence: NP_415865.1
3	Bacteriophage SPP1 35 protein	MATKKQEELKNALAQQNGAVPQTPVKPQDKVKGYLERMMPAIKDV LPKHLDADRLSRIAMNVIRTNPKLLECDTASLMGAVLESAKLGVE PGLLGQAYILPYTNYKKKTVEAQFILGYKGLLDLVRRSGHVSTIS AQTVYKNDTFEYEYGLDDKLVHRPAPFGTDRGEPVGYYAVAKMKD GGYNFLVMSKQDVEKHRDAFSKSKNREGVVYGPWADHFDAMAKKT VLRQLINYLPISVEQLSGVAADERTGSELHNQFADDDNIINVDIN TGEIIDHQEKLGGETNE	UniProtKB: locus Q38143_BPSPP, accession Q38143;

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
4	Bacteriophage P22 ERF protein	MSKEFYARLAEIQEHLNAPKNQYNSFGKYKYRSCEDILEGVKPLL KGLFLSISDEIVLIGDRYYVKATATITDGENSHSASAIAREEENK KGMDAAQVTGATSSYARKYCLNGLFGIDDAKDADTEEHKQQQNAA PAKQTKSSPSSPAPEQVLKAFSEYAATETDKKKLIERYQHDWQLL TGHDDEQTKCVQVMNIRINELKQVA	NCBI Reference Sequence: NP_059596.1; mutations in ERF are complemented by Bacteriophage Lambda Red beta protein (Poteete AR, Fenton AC. Lambda red-dependent growth and recombination of phage P22. Virology. 1984 Apr 15;134(1):161-7.) ERF-family motif underlined in bold
5	Saccharomyces cerevisiae RAD52 protein	MNEIMDMDEKKPVFGNHSEDIQTKLDKKLGPEYISKRVGFGTSRI AYIEGWRVINLANQIFGYNGWSTEVKSVVIDFLDERQGKFSIGCT AIVRVTLTSGTYREDIGYGTVENERRKPAAFERAKKSAVTDALKR SLRGFGNALGNCLYDKDFLAKIDKVKFDPPDFDENNLFRPTDEIS ESSRTNTLHENQEQQQYPNKRRQLTKVTNTNPDSTKNLVKIENTV SRGTPMMAAPAEANSKNSSNKDTDLKSLDASKQDQDDLLDDSLMF SDDFQDDDLINMGNTNSNVLTTEKDPVVAKQSPTASSNPEAEQIT FVTAKAATSVQNERYIGEESIFDPKYQAQSIRHTVDQTTSKHIPA SVLKDKTMTTARDSVYEKFAPKGKQLSMKNNDKELGPHMLEGAGN QVPRETTPIKTNATAFPPAAAPRFAPPSKVVHPNGNGAVPAVPQQ RSTRREVGRPKINPLHARKPT	NCBI Reference Sequence: NP_013680.2

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
6	Schizosaccharomyces pombe Rad22	MSFEQKQHVASEDQGHFNTAYSHEEFNFLQSSLTRKLGPEYVSRR SGPGGFSVSYIESWKAIELANEIFGFNGWSSSIRSINVDFMDENK ENGRISLGLSVIVRVTIKDGAYHEDIGYGSIDNCRGKASAFEKCK KEGTTDALKRALRNFGNSLGNCMYDKYYLREVGKMKPPTYHFDSG DLFRKTDPAARESFIKKQKTLNSTRTVNNQPLVNKGEQLAPRRAA ELNDEQTREIEMYADEELDNIFVEDDIIAHLAVAEDTAHPAANNH HSEKAGTQINNKDKGSHNSAKPVQRSHTYPVAVPQNTSDSVGNAV TDTSPKTLFDPLKPNTGTPSPKFISARAAAAAAEGVVSAPFTNNFN PRLDSPSIRKTSIIDHSKSLPVQRASVLPIIKQSSQTSPVSNNSM IRDSESIINERKENIGLIGVKRSLHDSTTSHNKSDLMRTNSDPQS AMRSRENYDATVDKKAKKG	UniProtKB/Swiss-Prot: P36592.2
7	Kluyveromyces lactis Rad52	MEDTGSGKNGKDDIQTKLDKKLGPEYISKRVGFGSSRVAYIEGWK AINLANQIFGYDGWSTEVKNVTIDFLDERQGRFSIGCTAIVRVSL ADGTFREDIGYGTVENERRKASAFERAKKSAVTDALKRSLRGFGN ALGNCLYDKDFLAKIDKVKFDPPDFDEGNLFRPADELSEMSRSNM VGDAHTEGPSLKKRSLTNEDRNAVPSAPAQQTYRSNNHTTQKRAP KAQAVTASASPNEETSNQQQDPDDLLDDSFMFSDEIQDDDLLNMN TTTNNKNSTNSSTTTTTISDEATGIISPVTFVTAKAATSLQHKDP IPSGSMFDPKFQAQSIRHTVDQSVSTPVRATILKEKGLDSDRSSI YSKFAPKGKELSGTTTNSEPYVAAPQTSATESNRSTPTRSNAQLA GPQPAPQLQGPQRTQLGRPRMLQQPNRRNVS	UniProtKB/Swiss-Prot: P41768.2
8	Bacteriophage Lambda exonuclease	MTPDIILQRTGIDVRAVEQGDDAWHKLRLGVITASEVHNVIAKPR SGKKWPDMKMSYFHTLLAEVCTGVAPEVNAKALAWGKQYENDART LFEFTSGVNVTESPIIYRDESMRTACSPDGLCSDGNGLELKCPFT SRDFMKFRLGGFEAIKSAYMAQVQYSMWVTRKNAWYFANYDPRMK REGLHYVVIERDEKYMASFDEIVPEFIEKMDEALAEIGFVFGEQW R	NCBI Reference Sequence: WP_000186853.1
9	Rac bacterial prophage RecE exonuclease	MSTKPLFLLRKAKKSSGEPDVVLWASNDFESTCATLDYLIVKSGK KLSSYFKAVATNFPVVNDLPAEGEIDFTWSERYQLSKDSMTWELK PGAAPDNAHYQGNTNVNGEDMTEIEENMLLPISGQELPIRWLAQH GSEKPVTHVSRDGLQALHIARAEELPAVTALAVSHKTSLLDPLEI RELHKLVRDTDKVFPNPGNSNLGLITAFFEAYLNADYTDRGLLTK EWMKGNRVSHITRTASGANAGGGNLTDRGEGFVHDLTSLARDVAT GVLARSMDLDIYNLHPAHAKRIEEIIAENKPPFSVFRDKFITMPG GLDYSRAIVVASVKEAPIGIEVIPAHVTEYLNKVLTETDHANPDP EIVDIACGRSSAPMPQRVTEEGKQDDEEKPQPSGTTAVEQGEAET MEPDATEHHQDTQPLDAQSQVNSVDAKYQELRAELHEARKNIPSK NPVDDDKLLAASRGEFVDGISDPNDPKWVKGIQTRDCVYQNQPET EKTSPDMNQPEPVVQQEPEIACNACGQTGGDNCPDCGAVMGDATY QETFDEESQVEAKENDPEEMEGAEHPHNENAGSDPHRDCSDETGE VADPVIVEDIEPGIYYGISNENYHAGPGISKSQLDDIADTPALYL WRKNAPVDTTKTKTLDLGTAFHCRVLEPEEFSNRFIVAPEFNRRT NAGKEEEKAFLMECASTGKTVITAEEGRKIELMYQSVMALPLGQW LVESAGHAESSIYWEDPETGILCRCRPDKIIPEFHWIMDVKTTAD IQRFKTAYYDYRYHVQDAFYSDGYEAQFGVQPTFVFLVASTTIEC GRYPVEIFMMGEEAKLAGQQEYHRNLRTLSDCLNTDEWPAIKTLS LPRWAKEYAND	NCBI Reference Sequence: AIN31810.1
10	maize opaque-2 nuclear localization signal	RKRKESNRESARRSRRSRYRKKV	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
11	SV40 large T antigen NLS	PKKKRKV	
12	Class II monopartite NLS consensus	K(K/R)X(K/R)	
13	Bipartite NLS consensus	(K/R)(K/R)X_{10-12}(K/R)$_{3/5}$	where K/R)$_{3/5}$ represents at least three of either lysine or arginine of five consecutive amino acids
14	Class 5 Plant NLS	LGKR(K/R)(W/F/Y)	
15	tobacco c2 NLS	QPSLKRMKIQPSSQP	
16	Extended SV40 Nuclear Localization Domain	ASPKKKRKVEASGS	
17	cell-penetrating peptide (CPP)	YGRKKRRQRRR	
18	cell-penetrating peptide (CPP)	RRQRRTSKLMKR	
19	cell-penetrating peptide (CPP)	GWTLNSAGYLLGKINLKALAALAKKIL	
20	cell-penetrating peptide (CPP)	KALAWEAKLAKALAKALAKHLAKALAKALKCEA	
21	cell-penetrating peptide (CPP)	RQIKIWFQNRRMKWKK	
22	cell-penetrating peptide (CPP)	YGRKKRRQRRR	
23	cell-penetrating peptide (CPP)	RKKRRQRR	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
24	cell-penetrating peptide (CPP)	YARAAARQARA	
25	cell-penetrating peptide (CPP)	THRLPRRRRRR	
26	cell-penetrating peptide (CPP)	GGRRARRRRRR	
27	As Cpf1 (wild type)	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARND HYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEE TRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKA ELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVF SAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENV KKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAG TEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNT LSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSID LTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSA KEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAAL DQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPE FSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTL ASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEK TSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSN NFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCK WIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYH ISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYW TGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKK LKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVS HEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHP ETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLD NREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVV VLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEK VGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFV DPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSF QRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFT GRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTM VALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPM DADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQEL RN	Acidaminococcus sp. (As) Cpf1

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
28	LbCpf1 (wild type)	MSKLEKFTNCYSLSKTLRFKAIPVGKTQENIDNKRLLVEDEKRAE DYKGVKKLLDRYYLSFINDVLHSIKLKNLNNYISLFRKKTRTEKE NKELENLEINLRKEIAKAFKGNEGYKSLFKKDIIETILPEFLDDK DEIALVNSFNGFTTAFTGFFDNRENMFSEEAKSTSIAFRCINENL TRYISNMDIFEKVDAIFDKHEVQEIKEKILNSDYDVEDFFEGEFF NFVLTQEGIDVYNAIIGGFVTESGEKIKGLNEYINLYNQKTKQKL PKFKPLYKQVLSDRESLSFYGEGYTSDEEVLEVFRNTLNKNSEIF SSIKKLEKLFKNFDEYSSAGIFVKNGPAISTISKDIFGEWNVIRD KWNAEYDDIHLKKKAVVTEKYEDDRRKSFKKIGSFSLEQLQEYAD ADLSVVEKLKEIIIQKVDEIYKVYGSSEKLFDADFVLEKSLKKND AVVAIMKDLLDSVKSFENYIKAFFGEGKETNRDESFYGDFVLAYD ILLKVDHIYDAIRNYVTQKPYSKDKFKLYFQNPQFMGGWDKDKET DYRATILRYGSKYYLAIMDKKYAKCLQKIDKDDVNGNYEKINYKL LPGPNKMLPKVFFSKKWMAYYNPSEDIQKIYKNGTFKKGDMFNLN DCHKLIDFFKDSISRYPKWSNAYDFNFSETEKYKDIAGFYREVEE QGYKVSFESASKKEVDKLVEEGKLYMFQIYNKDFSDKSHGTPNLH TMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVVHPANSPI ANKNPDNPKKTTTLSYDVYKDKRFSEDQYELHIPIAINKCPKNIF KINTEVRVLLKHDDNPYVIGIDRGERNLLYIVVVDGKGNIVEQYS LNEIINNFNGIRIKTDYHSLLDKKEKERFEARQNWTSIENIKELK AGYISQVVHKICELVEKYDAVIALEDLNSGFKNSRVKVEKQVYQK FEKMLIDKLNYMVDKKSNPCATGGALKGYQITNKFESFKSMSTQN GFIFYIPAWLTSKIDPSTGFVNLLKTKYTSIADSKKFISSFDRIM YVPEEDLFEFALDYKNFSRTDADYIKKWKLYSYGNRIRIFRNPKK NNVFDWEEVCLTSAYKELFNKYGINYQQGDIRALLCEQSDKAFYS SFMALMSLMLQMRNSITGRTDVDFLISPVKNSDGIFYDSRNYEAQ ENAILPKNADANGAYNIARKVLWAIGQFKKAEDEKLDKVKIAISN KEWLEYAQTSVKH	Lachnospiraceae bacterium (Lb) Cpf1

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
29	Fn Cpf1 (wild type)	MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAK DYKKAKQIIDKYHQFFIEEILSSVCISEDLLQNYSDVYFKLKKSD DDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDAKKGQE SDLIL WLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHEN RKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAIN YEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQ SGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYK MSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTV EEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDY SVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLA QISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHI SQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYITQKP YSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNK KNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIK FYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYK QSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENIS ESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDER NLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKE SVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLK EKANDVHILSIDRGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMK TNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEIAKL VIEYNAIVVFEDLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKI CPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDY KNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELE KLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNS KTGTELDYLISPVADVNGNFFDSRQAPKNMPQDADANGAYHIGLK GLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN	Francisella novicida (Fn) Cpf1

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
30	CasJ (wild type)	MQQYQVSKTVRFGLTLKNSEKKHATHLLLKDLVNVSEERIKNEIT KDDKNQSELSFFNEVIETLDLMDKYIKDWENCFYRTDQIQLTKEY YKVIAKKACFDWFWTNDRGMKFPTSSIISFNSLKSSDKSKTSDNL DRKKKILDYWKGNIFKTQKAIKDVLDITEDIQKAIEEKKSHREIN RVNHRKMGIHLIHLINDTLVPLCNGSIFFGNISKLDFCESENEKL IDFASTEKQDERKFLLSKINEIKQYFEDNGGNVPFARATLNRHTA NQKPDRYNEEIKKLVNELGVNSLVRSLKSKTIEEIKTHFEFENKN KINELKNSFVLSIVEKIQLFKYKTIPASVRFLLADYFEEQKLSTK EEALTIFEEIGKPQNIGFDYIQLKEKDNFTLKKYPLKQAFDYAWE NLARLDQNPKANQFSVDECKRFFKEVFSMEMDNINFKTYALLLAL KEKTTAFDKKGEGAAKNKSEIIEQIKGVFEELDQPFKIIANTLRE EVIKKEDELNVLKRQYRETDRKIKTLQNEIKKIKNQIKNLENSKK YSFPEIIKWIDLTEQEQLLDKNKQAKSNYQKAKGDLGLIRGSQKT SINDYFYLTDKVYRKLAQDFGKKMADLREKLLDKNDVNKIKYLSY IVKDNQGYQYTLLKPLEDKNAEIIELKSEPNGDLKLFEIKSLTSK TLNKFIKNKGAYKEFHSAEFEHKKIKEDWKNYKYNSDFIVKLKKC LSHSDMANTQNWKAFGWDLDKCKSYETIEKEIDQKSYQLVEIKLS KTTIEKWVKENNYLLLPIVNQDITAEKLKVNTNQFTKDWQHIFEK NPNHRLHPEFNIAYRQPTKDYAKEGEKRYSRFQLTGQFMYEYIPQ DANYISRKEQITLFNDKEEQKIQVETFNNQIAKILNAEDFYVIGI DRGITQLATLCVLNKNGVIQGGFEIFTREFDYTNKQWKHTKLKEN RNILDISNLKVETTVNGEKVLVDLSEVKTYLRDENGEPMKNEKGV ILTKDNLQKIKLKQLAYDRKLQYKMQHEPELVLSFLDRLENKEQI PNLLASTKLISAYKEGTAYADIDIEQFWNILQTFQTIVDKFGGIE NAKKTMEFRQYTELDASFDLKNGVVANMVGVVKFIMEKYNYKTFI ALEDLTFAFGQSIDGINGERLRSTKEDKEVDFKEQENSTLAGLGT YHFFEMQLLKKLSKTQIGNEIKHFVPAFRSTENYEKIVRKDKNVK AKIVSYPFGIVSFVNPRNTSISCPNCKNANKSNRIKKENDRILCK HNIEKTKGNCGFDTANFDENKLRAENKGKNFKYISSGDANAAYNI AVKLLEDKIFEINKK	CasJ
31	E. coli single stranded DNA binding polypeptide (SSB)	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	NCBI Reference Sequence: WP_000168305.1
32	ERF protein motif	G(G/S/A)XX(S/T)Y(A/V/L/I/M/F)(K/R/E,/D/N/T/S) (K/R)YX(A/V/L/I/M/F)XX(A/V/L/I/M/F) A/V/L/I/M/F)	
33	FMDV 2A self-processing peptide sequence	QLLNFDLLKLAGDVESNPGP	
34	single strand DNA-binding protein [Escherichia coli APEC O1]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
35	single strand DNA-binding protein [Escherichia coli UTI89]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
36	ssDNA-binding protein [Proteobacteria]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
37	ssDNA-binding protein [Escherichia]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNVGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
38	ssDNA-binding protein [Shigella flexneri]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNKFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
39	ssDNA-binding protein [Escherichia coli]	MASKGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
40	single-stranded DNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYLEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
41	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSAQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
42	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAAGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
43	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGHDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
44	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQSG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
45	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGS WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
46	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGSAQSRPQQSAPAAPSNEPPMDFDDDIPF	
47	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGSNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
48	ssDNA - binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGSNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
49	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNSGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
50	ssDNA-binding protein [Escherichia]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQSGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
51	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSTPAAPSNEPPMDFDDDIPF	
52	ssDNA - binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGQPQGGW GQSQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
53	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSCGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
54	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMXMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
55	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVVSEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
56	ssDNA - binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGVQSRPQQSAPAAPSNEPPMDFDDDIPF	
57	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGDAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
58	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQDGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
59	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYITEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
60	Single-strand DNA binding protein [Shigella dysenteriae 1617]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQLQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
61	single-stranded DNA-binding protein [Escherichia albertii]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGLDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
62	Single-stranded DNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLCKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
63	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGLDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
64	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQPAPAAPSNEPPMDFDDDIPF	
65	ssDNA-binding protein Enterobacteriaceae]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQLQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
66	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKDQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSTPAAPSNEPPMDFDDDIPF	
67	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRLQQSAPAAPSNEPPMDFDDDIPF	
68	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQLQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
69	ssDNA-binding protein [Escherichia]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQSGGAPTGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
70	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQGYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
71	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEGASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
72	single-stranded DNA-binding protein [Escherichia albertii]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSLVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQSGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
73	ssDNA-binding protein [Escherichia albertii]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSEFWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQSGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
74	ssDNA-binding protein [Escherichia coli]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGWGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPM DFDDDIPF	
75	ssDNA-binding protein [Citrobacter]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGNAGGGQQGGW GQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
76	ssDNA-binding protein [Citrobacter koseri]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGNMGGGQQQGG WGQPQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
77	single-stranded DNA-binding protein [Escherichia coli ECC-1470]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQGG WGQPQQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMD	
78	ssDNA-binding protein [Citrobacter koseri]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGVPAGGNMGGGQQQGG WGQPQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
79	single-stranded DNA-binding protein [Citrobacter koseri]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDKYITEVVVNVGGTMQMLGGRQGGGAPAGGNMGGGQQQGG WGQPQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
80	ssDNA - binding protein [Shigella]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDRYTTEVVVNVGGTMQMLGGRQGGGAPAGGNIGGGQPQQP QGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
81	ssDNA-binding protein Enterobacteriaceae]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
82	ssDNA-binding protein [Citrobacter freundii complex]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGQQQGGWGQP QQPQGGNQFSGGGQSRPQQSAPAAPSNEPPMDFDDDIPF	
83	ssDNA-binding protein [Citrobacter]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGQQQGGWGQP QQPQGGNQFSGGEQSRPQQSAPAAPSNEPPMDFDDDIPF	
84	ssDNA-binding protein [Citrobacter youngae]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQSAPAAPSNEPPMDFDDDIPF	
85	single-stranded DNA-binding protein [Citrobacter werkmanii]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQSAPAAPSNEPSMDFDDDIPF	
86	ssDNA-binding protein [Citrobacter sp. MGH109]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGQQQGGWGQP QQPQGGNQFSGGAQSRLQQSAPAAPSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
87	ssDNA-binding protein Enterobacteriaceae]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQSAPAAPSNEPPMDFDDDIPF	
88	ssDNA-binding protein [Citrobacter]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGQQQQGGWGQP QQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
89	ssDNA-binding protein [Proteobacteria]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
90	single - stranded DNA-binding protein [Escherichia coli PA5]	MPNGGAVANITLATSESWRDKATGEMKEQTEWHRVVLFGKLAEVA SEYLRKGSQVYIEGQLRTRKWTDQSGQDRYTTEVVVNVGGTMQML GGRQGGGAPAGGNIGGGQPQGGWGQPQQPQGGNQFSGGAQSRPQQ SAPAAPSNEPPMDFDDDIPF	
91	ssDNA-binding protein [Enterobacter aerogenes]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEIVVNVGGTMQMLGGRQGGGAPASGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
92	ssDNA-binding protein [Enterobacter cloacae]	MASKGVNKVILVGNLGQDPEVRYLPSGGAVCSVTLATSESWRDKA TGELKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGGGAPTGGSQNQQQGGWG RHQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDLDDDIPF	
93	ssDNA-binding protein [Enterobacter cloacae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGAEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGSQQQGGWGQP QQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
94	single-stranded DNA-binding protein [Klebsiella sp. G5]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGNMGGGQQQGG WGQPQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
95	ssDNA-binding protein [Klebsiella oxytoca]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQQGASAPAGGGQQQGGWGQ PQQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
96	ssDNA-binding protein [Enterobacteriaceae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGAEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGNMGGGQGQQG GWGQPQQPQGGNQFSGGAQSRPQQSAPAPSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
97	ssDNA-binding protein [Enterobacter lignolyticus]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGGGASAGGNMGGGQQQGG WGQPQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
98	ssDNA-binding protein [Serratia marcescens]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEQKEKTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGSLQTRKWQ DQSGQDRYTTEIVVNVGGTMQMLGGRQGGGAPAGQSAGGQSGWGQ PQQPQGGNQFSGGQQQSRPAQNSAPATSNEPPMDFDDDIPF	
99	ssDNA-binding protein [Enterobacter cloacae complex]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGSGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
100	ssDNA-binding protein [Enterobacter cloacae complex]	MASKGVNKVILVGNLGQDPEVRYLPSGGAVCSVTLATSESWRDKA TGELKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGSQNQQQGGWG QPQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
101	ssDNA-binding protein[Enterobacteriaceae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQQGAGAPAGGGQQQGGWGQ PQQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
102	ssDNA-binding protein [Enterobacteriaceae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEIVVNVGGTMQMLGGRQQGAGAPAGGGQQQGGWGQ PQQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
103	single-stranded DNA-binding protein [Enterobacter cloacae]	MASKGVNKVILVGNLGQDPEVRYLPSGSAVCSVTLATSESWRDKA TGELKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGSQNQQQGGWG QPQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
104	ssDNA-binding protein [Klebsiella oxytoca]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQQGAGAPAGGGQQQGGWGQ PQQPQGGNQYSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
105	ssDNA-binding protein [Klebsiella oxytoca]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQQGAGAPAGGGQQQGGWGQ PQQPQGGNQFSGGAQSRPQQQTPAAPSNEPPMDFDDDIPF	
106	ssDNA-binding protein [Pantoea]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGENKEITEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWQ DQGGQDRYTTEVVVNVGGTMQMLGGRQQGGASAGGAPMGGGQQSG GNNNGWGQPQQPQGGNQFSGGAQSRPQPQSAPASNNNEPPMDFDD DIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
107	single-stranded DNA-binding protein [Klebsiella oxytoca]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQQGAGAPAGGGQQQGGWGQ PQQPQGGNQFSGGAQSRPQQQAPAAPSNETPMDFDDDIPF	
108	ssDNA-binding protein [Enterobacteriaceae]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEQKEKTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGSLQTRKWQ DQSGQDRYTTEIVVNVGGTMQMLGGRQGGGAPAGQSAGGQGGWGQ PQQPQSGNQFSGGQQQSRPAQNSAPATSNEPPMDFDDDIPF	
109	ssDNA-binding protein [Klebsiella pneumoniae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKH TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
110	single-stranded DNA-binding protein [Klebsiella pneumoniae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
111	ssDNA-binding protein [Enterobacteriaceae]	ASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQT GEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWTD QSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQPQ QPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
112	ssDNA - binding protein [Klebsiella pneumoniae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
113	ssDNA-binding protein [Gammaproteobacteria]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGQQQQGGWGQP QQPQGGNQFSGGAQSRPQQQSAPAAPSNEPPMDFDDDIPF	
114	ssDNA-binding protein [Enterobacter aerogenes]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEIVVNVGGTMQMLGGRQGGGAPAGGQQQGGWGQPQ QPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
115	ssDNA-binding protein [Enterobacter aerogenes]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEIVVNVGGTMQMLGGRQGGGAPAGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
116	ssDNA - binding protein [Serratia]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEQKEKTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGSLQTRKWQ DQSGQDRYTTEIVVNVGGTMQMLGGRQGGGAPAGQSAGGQGGWGQ PQQPQGGNQFSGGQQQSRPAQNSAPAASSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
117	ssDNA-binding protein [Yokenella regensburgei]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEIVVNVGGTMQMLGGRQQGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
118	ssDNA-binding protein [Raoultella terrigena]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGAEKYTTEIVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQQQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
119	ssDNA-binding protein [Klebsiella pneumoniae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQAPSAPSNEPPMDFDDDIPF	
120	ssDNA - binding protein [Yersinia]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEQKEKTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGALQTRKWQ DQSGQERYTTEVVVNVGGTMQMLGGRQGGGAPAGGSQQDGGAQGG WGQPQQPQGGNQFSGGQTSRPAQSAPAAQPQGGNEPPMDFDDDIP F	
121	ssDNA-binding protein [Klebsiella pneumoniae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVSGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
122	ssDNA-binding protein [Cronobacter condimenti]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANLRLATSESWRDKQ TGEMKEVTEWHSVVLYGKLAEVAGEYLRKGSQIYIEGQLRTRKWQ DQSGQDRYSTEVVVNVGGTMQMLGGRQGGGAPAGGNMGGGQQQGG WGQPQQPQQQSGGAQFSGGAQSRPQQQAPAPSNEPPMDFDDDIPF	
123	ssDNA-binding protein [Klebsiella sp. 10982]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGSQFSGGAQSRPQQQAPAAPSNEPPMDFDDDIPF	
124	single-stranded DNA-binding protein [Klebsiella pneumoniae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQDKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQQAPAAPSNETPMDFDDDIPFMASRGV NKVILVGNLGQDPEVRYMPSGGAVANFTLATSESWRDKQTGEMKE QTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWTDQSGQD KYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQPQQPQGG NQFSGGAQSRPQQQAPAAPSNETPMDFDDDIPFAEVAGEYLRKGS QVYIEGQLRTRKWTDQSGQDKYTTEVVVNVGGTMQMLGGRQGGGA PAGGGQQQGGWGQPQQPQGGNQFSGGAQSRPQQQAPAAPSNETPM DFDDDIPF	
125	ssDNA-binding protein [Trabulsiella guamensis]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQQGAGAPAGGGQQQQGGWG QPQQPQGGAQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
126	ssDNA-binding protein [Enterobacter cloacae]	MASKGVNKVILVGNLGQDPEVRYLPSGGAVCSVTLATSESWRDKA TGELKEQTEWHRIVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQSQQHGGWG QYQHPQVGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
127	ssDNA-binding protein [Trabulsiella odontotermitis]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQQGAGAPAGGGQPQQQGGW GQPQQPQGGAQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
128	ssDNA-binding protein [Trabulsiella odontotermitis]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVASEYLRKGSQVYIEGQLRTRKWT DQSGVEKYTTEVVVNVGGTMQMLGGRQQGAGAPAGGGQQQGGWGQ PQQPQQQGGAQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
129	ssDNA-binding protein [Kosakonia radicincitans]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKQ TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGQEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQGGWGQP QQPQGGNQFSGGAQSRPQQSSAPAPSNEPPMDFDDDIPF	
130	single-stranded DNA-binding protein [Serratia marcescens]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEQKEKTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGSLQTRKWT DQAGVEKYTTEVVVNVGGTMQMLGGRQGGGAPAGQSAGGQGGWGQ PQQPQGGNQFSGGQQQSRPAQNSAPAASSNEPPMDFDDDIPF	
131	ssDNA-binding protein [Kluyvera]	MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGAEKYTTEVVVNVGGTMQMLGGRQGGGAPAGGGQQQQGGWGQ PQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
132	ssDNA-binding protein [Enterobacter asburiae]	MASRGVNKVILVGNLGQDPEVRYMPSGGAVANITLATSESWRDKA TGEMKEQTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGQLRTRKWT DQSGAEKYTTEVVVNVGGTMQMLGGRQGGGTPAGGGQQQQGGWGQ PQQPQGGNQFSGGAQSRPQQQSAPAPSNEPPMDFDDDIPF	
133	c2 NLS-SSB fusion protein	MQPSLKRMKIQPSSQPASRGVNKVILVGNLGQDPEVRYMPNGGAV ANITLATSESWRDKATGEMKEQTEWHRVVLFGKLAEVASEYLRKG SQVYIEGQLRTRKWTDQSGQDRYTTEVVVNVGGTMQMLGGRQGGG APAGGNIGGGQPQGGWGQPQQPQGGNQFSGGAQSRPQQSAPAAPS NEPPMDFDDDIPF	
134	c2 NLS-Bacteriophage Lambda Red beta SSAP-fusion protein	MQPSLKRMKIQPSSQPMSTALATLAGKLAERVGMDSVDPQELITT LRQTAFKGDASDAQFIALLIVANQYGLNPWTKEIYAFPDKQNGIV PVVGVDGWSRIINENQQFDGMDFEQDNESCTCRIYRKDRNHPICV TEWMDECRREPFKTREGREITGPWQSHPKRMLRHKAMIQCARLAF GFAGIYDKDEAERIVENTAYTAERQPERDITPVNDETMQEINTLL IALDKTWDDDLLPLCSQIFRRDIRASSELTQAEAVKALGFLKQKA AEQKVAA	
135	c2 NLS-Bacteriophage Lambda Red Exonuclease-fusion protein	MQPSLKRMKIQPSSQPTPDIILQRTGIDVRAVEQGDDAWHKLRLG VITASEVHNVIAKPRSGKKWPDMKMSYFHTLLAEVCTGVAPEVNA KALAWGKQYENDARTLFEFTSGVNVTESPIIYRDESMRTACSPDG LCSDGNGLELKCPFTSRDFMKFRLGGFEAIKSAYMAQVQYSMWVT RKNAWYFANYDPRMKREGLHYVVIERDEKYMASFDEIVPEFIEKM DEALAEIGFVFGEQWR	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
136	Artemis	MSSFEGQMAEYPTISIDRFDRENLRARAYFLSHCHKDHMKGLRAP TLKRRLECSLKVYLYCSPVTKELLLTSPKYRFWKKRIISIEIETP TQISLVDEASGEKEEIVVTLLPAGHCPGSVMFLFQGNNGTVLYTG DFRLAQGEAARMELLHSGGRVKDIQSVYLDTTFCDPRFYQIPSRE ECLSGVLELVRSWITRSPYHVVWLNCKAAYGYEYLFTNLSEELGV QVHVNKLDMFRNMPEILHHLTTDRNTQIHACRHPKAEEYFQWSKL PCGITSRNRIPLHIISIKPSTMWFGERSRKTNVIVRTGESSYRAC FSFHSSYSEIKDFLSYLCPVNAYPNVIPVGTTMDK VVEILKPLCRSSQSTEPKYKPLGKLKRARTVHRDSEEEDDYLFDD PLPIPLRHKVPYPETFHPEVFSMTAVSEKQPEKLRQTPGCCRAEC MQSSRFTNFVDCEESNSESEEEVGIPASLQGDLGSVLHLQKADGD VPQWEVFFKRNDEITDESLENFPSSTVAGGSQSPKLFSDSDGEST HISSQNSSQSTHITEQGSQGWDSQSDTVLLSSQERNSGDITSLDK ADYRPTIKENIPASLMEQNVICPKDTYSDLKSRDKDVTIVPSTGE PTTLSSETHIPEEKSLLNLSTNADSQSSSDFEVPSTPEAELPKRE HLQYLYEKLATGESIAVKKRKCSLLDT	NCBI Reference Sequence: NP_001029027.1
137	Apollo (Actinidia chinensis var. chinensis)	MGIQGLLPLLKSIMVPIHIKDLEDCCVAIDTYSWLHKGALSCSKD LCKGQSTSKHIDYCMNRVNLLQHYGIRPILVFDGGPLPMKSEQES KRARSRKENLACAIENESNGNNASAYKCYQKAVVISPSVAYELIQ VLKKENVYYVVAPYEADAQMTFLAVSKQVDAVITEDSDLIAFGCP RIIYKMDKLEQGVEFRYSMLQQNKELNFTGFTKRMLLEMCILSGC DYLQSLPGIGLKKAHALVKKFKSYDKVIKHLKYSTASVSSSYEES FRKAIMTFQHQRVYDPTIEDIVHLSDLPQYVGDDLDFLGPAILQH IAKGIARGDLDPFTKMPIQGVNNGAGLVDEGMYKLNNFKSEGFAS LEAKRRFMAPRSTPKHRNPITETCSTVEHITEDADACKTNCSLES LLDSRYFDVASPSEGYVKHGVAAKSPESKSPSHGSHDKEEILGEG DNRSPQDPLLQQFKHSIPKLCMTLQKERAKSVADSGQDKTRKENT KVIVRSSYFQHKLVKENDKENIKEDVTTDKGENINPKREHKSASD GGEAKTRIKNRKTIVRSSYFLHKSVNENDQDNRHEKLIINDDFTT HTHENGIPESASGDGYFNNSIVKRKVSPVDSVQMEKTNYKCMRMD ASLPIESSSISTLNNTTMETKAEGGKFGSNISHLKNYSDIAEKSI ERFVSVISSFKCSSSGSSASGLRAPLRNTEHMY	GenBank: PSS29025.1

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
138	DNA2 exonuclease (Mus musculus)	MEPLDELDLLLLEEDGGAEAVPRVELLRKKADALFPETVLSRGVD NRYLVLAVETSQNERGAEEKRLHVTASQDREHEVLCILRNGWSSV PVEPGDIVHLEGDCTSEPWIIDDDFGYFILYPDMMISGTSVASSI RCLRRAVLSETFRGSDPATRQMLIGTILHEVFQKAISESFAPERL QELALQTLREVRHLKEMYRLNLSQDEILCEVEEYLPSFSKWAEDF MRKGPSSEFPQMQLSLPSDGSNRSSPCNIEVVKSLDIEESIWSPR FGLKGKIDVTVGVKIHRDCKMKYKVMPLELKTGKESNSIEHRSQV VLYTLLSQERREDPEAGWLLYLKTGQMYPVPANHLDKRELLKLRN WLAASLLHRVSRAAPGEEARLSALPQIIEEEKTCKYCSQIGNCAL YSRAVEEQGDDASIPEAMLSKIQEETRHLQLAHLKYFSLWCLMLT LESQSKDNRKTHQSIWLTPASELEESGNCVGNLVRTEPVSRVCDG QYLHNFQRKNGPMPATNLMAGDRIILSGEERKLFALSKGYVKKMN KAAVTCLLDRNLSTLPATTVFRLDREERHGDISTPLGNLSKLMES TDPSKRLRELIIDFREPQFIAYLSSVLPHDAKDTVANILKGLNKP QRQAMKRVLLSKDYTLIVGMPGTGKTTTICALVRILSACGFSVLL TSYTHSAVDNILLKLAKFKVGFLRLGQSHKVHPDIQKFTEEEICR SRSIASLAHLEELYNSHPIVATTCMGINHPIFSRKTFDFCIVDEA SQISQPVCLGPLFFSRRFVLVGDHQQLPPLVVNREARALGMSESL FKRLERNESAVVQLTVQYRMNRKIMSLSNKLTYAGKLECGSDRVA NAVLALPNLKDARLSLQLYADYSDSPWLAGVLEPDNPVCFLNTDK VPAPEQVENGGVSNVTEARLIVFLTSTFIKAGCSPSDIGVIAPYR QQLRIISDLLARSSVGMVEVNTVDKYQGRDKSLILVSFVRSNEDG TLGELLKDWRRLNVALTRAKHKLILLGSVSSLKRFPPLGTLFDHL NAEQLILDLPSREHESLSHILGDCQRD	NCBI Reference Sequence: NP_796346.2
139	Exo1 exonuclease (Saccharomyces cerevisiae)	MGIQGLLPQLKPIQNAVSLRRYEGEVLAIDGYAWLHRAACSCAYE LAMGKPTDKYLQFFIKRFSLLKTFKVEPYLVFDGDAIPVKKSTES KRRDKRKENKAIAERLWACGEKKNAMDYFQKCVDITPEMAKCIIC YCKLNGIRYIVAPFEADSQMVYLEQKNIVQGIISEDSDLLVFGCR RLITKLNDYGECLEICRDNFIKLPKKFPLGSLTNEEIITMVCLSG CDYTNGIPKVGLITAMKLVRRFNTIERIILSIQREGKLMIPDTYI NEYEAAVLAFQFQRVFCPIRKKIVSLNEIPLYLKDTESKRKRLYA CIGFVIHRETQKKQIVHFDDDIDHHLHLKIAQGDLNPYDFHQPLA NREHKLQLASKSNIEFGKTNSTNSEAKVKPIESFFQKMTKLDHYP KVANNIHSLRQAEDKLTMAIKRRKLSNANVVQETLKDTRSKFFNK PSMTVVENFKEKGDSTQDFKEDTNSQSLEEPVSESQLSTQIPSSF ITTNLEDDDNLSEEVSEVVSDTEEDRKNSEGKIIGNEIYNTDDDG DGDTSEDYSETAESRVPTSSTTSFPGSSQRSISGCTKVLQKFRYS SSFSGVNANRQPLFPRHVNQKSRGMVYVNQNRDDDCDDNDGKNQI MQRPLLRKSLIGARSQRIVIDMKSVDERKSFNSSPILHEESKKRD IETTKSSQARPAVRSISLLSQFVYKGK	GenBank: KZV07919.1
140	SOX (herpesvirus)	MEATPTPADLFSEDYLVDTLDGLTVDDQQAVLASLSFSKFLKHAK VRDWCAQAKIQPSMPALRMAYNYFLFSKVGEFIGSEDVCNFFVDR VFGGVRLLDVASVYAACSQMNAHQRHHICCLVERATSSQSLNPVW DALRDGIISSSKFHWAVKQQNTSKKIFSPWPITNNHFVAGPLAFG LRCEEVVKTLLATLLHPDEANCLDYGFMQSPQNGIFGVSLDFAAN VKTDTEGRLQFDPNCKVYEIKCRFKYTFAKMECDPIYAAYQRLYE APGKLALKDFFYSISKPAVEYVGLGKLPSESDYLVAYDQEWEACP RKKRKLTPLHNLIRECILHNSTTESDVYVLTDPQDTRGQISIKAR FKANLFVNVRHSYFYQVLLQSSIVEEYIGLDSGIPRLGSPKYYIA TGFFRKRGYQDPVNCTIGGDALDPHVEIPTLLIVTPVYFPRGAKH RLLHQAANFWSRSAKDTFPYIKWDFSYLSANVPHSP	UniProtKB/Swiss-Prot: Q2HR95.1

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
141	UL12 exonuclease	MELEPVGKKYRPEREDSSKGRKILTVSVNSQLQGASPTLGTRAHP PHSELTDYTFSRYILYHLAPSELKEAIHPLYHRLNYIADVIKRGT SEGRWLGYPYSCILDTEDELRNESRRNTSSPSDHALRWCLLVESF TIEQANCDLWHIFRQSLLTASSVKWTDDGKLDTVGIMSDNSTAYV ETCSVAFGKHNEPLAKSLVTMFCLNHSRHVHNTSPRRENVFVFED VSDRTIQSESDYSCGLMIDTRTGMVGASLDMLVCERDPFGLLQPD SENQAIETYEIKCRAKYAFCPDKRSELSQCYERLLNVRTMGSLRL FISAIQRPCVDYFQPGNVPRSKEALITSNEEWKVGNSAYHAAQSR IRCNAFDKCHLELNSNVQSRVWLFGEPDLETDTIYPLPWDTGKLS LDVPIFSNPRHPNFKQIYLQTYVAAGYFGERRTTPFLVTFIGRWR KRREFGKKFSLIADSGLGKPISTVHADQAIPVLLIVTPVIVDEAF YGEIESAGCRAFGELVKQLWAKQPHT	GenBank: AAG30051.1
142	E. coli exonuclease VIII	MSKVFICAAIPDELATREEGAVAVATAIEAGDERRARAKFHWQFL EHYPAAQDCAYKFIVCEDKPGIPRPALDSWDAEYMQENRWDEESA SFVPVETESDPMNVTFDKLAPEVQNAVMVKFDTCENITVDMVISA QELLQEDMATFDGHIVEALMKMPEVNAMYPELKLHAIGWVKHKCI PGAKWPEIQAEMRIWKKRREGERKETGKYTSVVDLARARANQQYT ENSTGKISPVIAAIHREYKQTWKTLDDELAYALWPGDVDAGNIDG SIHRWAKKEVIDNDREDWKRISASMRKQPDALRYDRQTIFGLVRE RPIDIHKDPIALNKYICEYLTTKGVFENEETDLGTVDVLQSSETQ TDAVETEVSDIPKNETAPEAEPSVEREGPFYFLFADKDGEKYGRA NKLSGLDKALAAGATEITKEEYFARKNGTYTGLPQNVDTAEDSEQ PEPIKVTADEVNKIMQAANISQPDADKLLAASRGEFVEEISDPND PKWVKGIQTRDSVNQNQHESERNYQKAEQNSTNALQNEPETKQPE PVAQQEVEKVCTACGQTGGGNCPDCGAVMGDATYQETFDEEYQVE VQEDDPEEMEGAEHPHKENTGGNQHHNSDNETGETADHSIKVNGH HEITSTSRAGIHLMIDLETMGKNPDAPIICNRLI	NCBI Reference Sequence: WP_077887717.1
143	T7 phage exonuclease (Enterobacteria phage T7)	MALLDLKQFYELREGCDDKGILVMDGDWLVFQAMSAAEFDASWEE EIWHRCCDHAKARQILEDSIKSYETRKKAWAGAPIVLAFTDSVNW RKELVDPNYKANRKAVKKPVGYFEFLDALFEREEFYCIREPMLEG DDVMGVIASNPSAFGARKAVIISCDKDFKTIPNCDFLWCTTGNIL TQTEESADWWHLFQTIKGDITDGYSGIAGWGDTAEDFLNNPFITE PKTSVLKSGKNKGQEVTKWVKRDPEPHETLWDCIKSIGAKAGMTE EDIIKQGQMARILRFNEYNFIDKEIYLWRP	NCBI Reference Sequence: NP_041988.1
144	Exonuclease III (E.coli)	MKFVSFNINGLRARPHQLEAIVEKHQPDVIGLQETKVHDDMFPLE EVAKLGYNVFYHGQKGHYGVALLTKETPIAVRRGFPGDDEEAQRR IIMAEIPSLLGNVTVINGYFPQGESRDHPIKFPAKAQFYQNLQNY LETELKRDNPVLIMGDMNISPTDLDIGIGEENRKRWLRTGKCSFL PEEREWMDRLMSWGLVDTFRHANPQTADRFSWFDYRSKGFDDNRG LRIDLLLASQPLAECCETGIDYEIRSMEKPSDHAPVWATFRR	GenBank: BAA15540.1
145	Trex2 exonuclease (mouse)	MSEPPRAETFVFLDLEATGLPNMDPEIAEISLFAVHRSSLENPER DDSGSLVLPRVLDKLTLCMCPERPFTAKASEITGLSSESLMHCGK AGFNGAVVRTLQGFLSRQEGPICLVAHNGFDYDFPLLCTELQRLG AHLPQDTVCLDTLPALRGLDRAHSHGTRAQGRKSYSLASLFHRYF QAEPSAAHSAEGDVHTLLLIFLHRAPELLAWADEQARSWAHIEPM YVPPDGPSLEA	NCBI Reference Sequence: NP_036037.1
146	Hammerhead ribozyme	AAATTACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
147	Hepatitis delta virus (HDV) ribozyme	GGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACA TGCTTCGGCATGGCGAATGGGAC	
148	Amino acid linker	MAPKKKRKVGGSGS	For linking SV40 NLS to HDR promoting agent proteins in human cells

SEQ ID NO:	DESCRIPTION	SEQUENCE	COMMENTS
149	Tomato SlUBI10 promoter	atcgtatccagtgcaccatatttttggcgattaccactcatatt attgtgtttagtagatattttaggtgcataattgatctcttcttt aaaactaggggcacttattattatacatccacttgacacttgctt tagttggctatttttttattttttatttttttgtcaactacccca atttaaattttatttgattaagatatttttatggacctactttat aattaaaaatattttctatttgaaaaggaaggacaaaaatcatac aattttggtccaactactcctctctttttttttttggctttataa aaaaggaaagtgattagtaataaataattaaataatgaaaaaagg aggaaataaaattttcgaattaaaatgtaaaagagaaaaaggaga gggagtaatcattgtttaactttatctaaagtaccccaattcgat tttacatgtatatcaaattatacaaatattttattaaaatataga tattgaataatttttattattcttgaacatgtaaataaaaattatc tattatttcaatttttatataaactattatttgaaatctcaatta tgatttttaatatcactttctatccatgataatttcagcttaaa aagttttgtcaataattacattaattttgttgatgaggatgacaa gatttcggtcatcaattacatatacacaaattgaaatagtaagca acttgatttttttttctcataatgataatgacaaagacacgaaaag acaattcaatattcacattgatttatttttatatgataataatta caataataatattcttataaagaaagagatcaattttgactgatc caaaaatttatttatttttactataccaacgtcactaattatatc taataatgtaaaacaattcaatcttacttaaatattaatttgaaa taaactatttttataacgaaattactaaatttatccaataacaaa aaggtcttaagaagacataaattctttttttgtaatgctcaaata aatttgagtaaaaaagaatgaaattgagtgatttttttttaatca taagaaaataaataattaatttcaatataataaaacagtaatata atttcataaatggaattcaatacttacctcttagatataaaaaat aaatataaaaataaagtgtttctaataaacccgcaatttaaataa aatatttaatattttcaatcaaatttaaataattatattaaaata tcgtagaaaaagagcaatatataatacaagaaagaagatttaagt acaattatcaactattattatactctaattttgttatatttaatt tcttacggttaaggtcatgttcacgataaactcaaaatacgctgt atgaggacatattttaaattttaaccaataataaaactaagttat ttttagtatatttttttgtttaacgtgacttaatttttctttttct agaggagcgtgtaagtgtcaacctcattctcctaatttttcccaac cacataaaaaaaaaataaaggtagcttttgcgtgttgatttggta cactacacgtcattattacacgtgttttcgtatgattggttaatc catgaggcggtttcctctagagtcggccataccatctataaaata aagctttctgcagctcatttttttcatcttctatctgatttctatt ataatttctctgaattgccttcaaatttctctttcaaggttagaa ttttttctctatttttttggtttttgtttgtttagattctgagttta gttaatcaggtgctgttaaagccctaaattttgagtttttttcgg ttgttttgatggaaaatacctaacaattgagtttttttcatgttgt tttgtcggagaatgcctacaattggagttcctttcgttgttttga tgagaaagcccctaatttgagtgtttttccgtcgatttgatttta aaggtttatattcgagttttttttcgtcggtttaatgagaaggcct aaaataggagtttttctggttgatttgactaaaaaagccatggaa ttttgtgtttttgatgtcgctttggttctcaaggcctaagatctg agtttctccggttgttttgatgaaaaagccctaaaattggagttt ttatcttgtgttttaggttgttttaatccttataatttgagtttt ttcgttgttctgattgttgtttttatgaatttcctgca	

Example 5. Genome Editing in Tomato Protoplasts

[0240] The following example describes experiments assessing gene editing in tomato protoplasts using a Cas nuclease in the presence and absence of HDR promoting agents (*i.e.*, an exonuclease, SSB protein, and SSAP). Specifically, experiments to test the effects of modifying the form and delivery method of the template donor DNA, HDR promoting agents, and nuclease reagents on genome editing were performed.

Materials and Methods

[0241] Tomato protoplasts were isolated, cultivated, and transfected as described in Example 2. Genome editing was assessed using amplicon sequencing, as described in Example 2.

Design of plasmids for transfection

[0242] Plasmids were constructed comprising either all the components as part of a single vector (plasmid, see **FIG. 3**), or with components separated on two different plasmids for co-transfection (see **FIGS. 4-5**). In particular, a first vector encoded CasS nuclease and its corresponding guide RNA, and a second vector all three HDR promoting agents (*i.e.*, the SSB protein, exonuclease, and SSAP). In addition, the donor template flanked by endonuclease recognition sequences was present in either the first or second vector.

[0243] DNA donor templates to target the promoter region of the tomato Ant1 gene for insertion of a 42 base pair heterologous sequence and deletion of 3 base pairs by HDR were constructed.

Linearized donor DNA

[0244] Donor template DNA was added either as a linear double stranded DNA molecule, or as part of a circular vector flanked by specific nuclease recognition sequences.

Presence of gRNA recognition sites on DNA template

[0245] The effect of the presence of the gRNA-recognized cut sites that flanked the donor DNA template was tested by eliminating them from a transfection vector.

Results

[0246] Tomato protoplasts were transformed with one or two plasmid vectors encoding a Cas nuclease, a guide RNA, and a donor DNA in the presence and absence of HDR

promoting agents (*i.e.*, an exonuclease, a SSB protein, and a SSAP) (see **FIGS. 3-5**). **Tables 4A-4C**, below, provide a summary of data from tomato protoplast gene editing experiments.

[0247] Co-transformation of two vectors consistently showed a significant increase in precise genome editing attributable to HDR, and a decrease in insertion and deletion (indel) editing attributable to non-homologous end joining (NHEJ), as shown in **Table 4A**, below. There was a high proportion (*e.g.* ~70-80%) of precise to indel edits in the presence of HDR promoting agents (*i.e.*, the SSB, the exonuclease, and the SSAP). When the donor template DNA and Cas nuclease were co-transformed on separate vectors (**FIGS. 4-5**), inclusion of the donor template in the absence of HDR promoting agents significantly decreased NHEJ editing without significantly promoting precise editing. When the donor template DNA and Cas nuclease were on a single vector (**FIG. 3**), the presence of the HDR promoting agents decreased NHEJ editing to a lesser extent. When the gRNA-recognized cut sites flanking the donor template DNA were eliminated, the presence of the HDR promoting agents did not decrease the level of NHEJ editing. Co-transformation of components on different vectors did not significantly improve the HDR efficiency over the efficiency described in Example 2.

Table 4A: Tomato protoplast gene editing with one vs. two vectors (Experiment LR-16)

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
Lambda RED, CasS, gRNA, donor DNA template plasmid **(all – 1 vector)**	4.37	13.22	0.72	1.71
CasS, gRNA, donor DNA template plasmid + Lambda Red plasmid **(all – 2 vectors)**	1.92	7.98	0.84	1.57
CasS, gRNA plasmid + Lambda RED, donor DNA template plasmid **(all – 2 vectors)**	4.60	2.91	0.57	0.13
CasS, gRNA	6.31	0.48	0.52	0.17

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
plasmid + donor DNA template plasmid **(no Lambda Red)**				
CasS, gRNA plasmid **(CasS only)**	32.89	0.00	1.37	0.00
Donor DNA template plasmid **(donor only)**	0.27	0.16	0.13	0.09
Lambda Red plasmid **(Lambda Red only)**	0.14	0.00	0.11	0.00
GFP plasmid	0.12	0.00	0.04	0.00

[0248] The linear template DNA was as effective in promoting precise (HDR) editing and decreased indel (NHEJ) editing as the circular vector flanked by specific nuclease recognition sequences, as used in Example 2 (**Table 4B**).

Table 4B: Tomato protoplast gene editing with linear vs. circular donor DNA template (Experiment LR-18)

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
Lambda RED, CasS, gRNA, donor DNA template plasmid **(all – 1 vector)**	2.46	8.74	0.19	0.75
CasS, gRNA, donor DNA template plasmid + Lambda Red plasmid **(all – 2 vectors)**	1.15	3.12	0.08	0.07
CasS, gRNA plasmid + Lambda RED, donor DNA	6.95	4.24	0.36	0.31

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
template plasmid **(all – 2 vectors)**				
CasS, gRNA plasmid + Lambda Red plasmid + Linear donor DNA template **(linear donor)**	0.47	2.75	0.11	0.31
CasS, gRNA plasmid + donor DNA template plasmid **(no Lambda Red – 2 vectors)**	6.64	0.21	0.24	0.11
CasS, gRNA, donor DNA template plasmid **(no Lambda Red – 1 vector)**	12.21	0.09	0.16	0.05
CasS, gRNA plasmid **(CasS only)**	25.64	0.00	0.50	0.00
Donor DNA template plasmid **(donor only)**	0.08	0.22	0.07	0.06
Lambda Red plasmid **(Lambda Red only)**	0.01	0.00	0.01	0.00
GFP plasmid	0.00	0.00	0.00	0.00
no transfection	0.01	0.00	0.02	0.00

[0249] The effect of the DNA template flanking cut sites was tested by eliminating them from a transfection vector. The number and percentage of precise edits was greater than that of negative controls that had no HDR promoting agents, but were less than that of positive controls having the DNA template flanking cut sites as in Example 2 (**Table 4C**). Similarly, the indel frequency was less than that of negative controls, and slightly higher than positive controls.

Table 4C: Tomato protoplast gene editing with donor template with or without flanking cut sites (FCS) (Experiment LR-21)

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
Lambda RED, CasS, gRNA, donor DNA template with FCS plasmid **(all – FCS)**	4.03	17.30	0.27	0.82
Lambda RED, CasS, gRNA, donor DNA template without FCS plasmid **(all – no FCS)**	6.06	3.86	0.16	0.18
Lambda RED, donor DNA template with FCS plasmid **(no nuclease - FCS)**	0.00	0.01	0.00	0.01
Lambda RED, donor DNA template without FCS plasmid **(no nuclease – no FCS)**	0.02	0.18	0.02	0.09
CasS, gRNA, donor DNA template with FCS plasmid **(no Lambda Red – FCS)**	27.99	0.24	1.90	0.12
CasS, gRNA, donor DNA template without FCS plasmid **(no Lambda Red – no FCS)**	39.46	0.27	0.88	0.04
CasS, gRNA plasmid **(CasS only)**	36.57	0.00	1.27	0.00
Donor DNA template with FCS plasmid **(donor only - FCS)**	0.02	0.42	0.02	0.16

Transfection Components	% indel (NHEJ)	% precise (HDR)	SD indel	SD precise
Donor DNA template with FCS plasmid **(donor only – no FCS)**	0.02	0.55	0.01	0.06
no transfection	0.00	0.00	0.01	0.00

Example 6. Genomic Replacement of *SPX* in Maize

[0250] The following example describes editing of a miRNA binding site at the *SPX* locus in maize protoplasts using HDR promoting agents (*i.e.*, the exonuclease, lambda beta SSAP, and *E. coli* SSB protein).

Materials and Methods

Design of plasmid constructs

[0251] Two gRNAs are used to target regions surrounding the miRNA binding site at the *SPX* locus in maize for CasS-mediated cleavage, to thereby mediate replacement of the site. A donor DNA fragment is used as a template for HDR repair/editing mediated by HDR promoting agents.

[0252] Plasmid constructs are designed to replace the miRNA binding site at the *SPX* locus in maize and its flanking regions with a fragment containing SNPs every three base pairs within the miRNA binding site. In addition, SNPs are introduced to mutate the two PAM sites, and thereby prevent cutting of the locus after editing has occurred. One of the SNPs introduced into the miRNA binding site acts as a SNP for both the miRNA binding site and one of the PAM sequences.

[0253] A system with a CasS nuclease with two gRNAs specific to the target, the HDR promoting agents (exonuclease, lambda beta SSAP, and the *E. coli* SSB protein), and a donor template with the replacement fragment and ~700 base pair homology arms which are homologous to the target editing site is used. The vectors expressing Cas9 and the HDR promoting agents were designed as described in Example 6. The homology arms were designed to be ~700 base pairs, because previous experiments have shown that ~500-750 base pair arms are functional (see Example 6). In addition, GC content of the homology arms was also considered and maximized, which, without wishing to be bound by theory, may help with annealing and promoting precise editing. Each of the two gRNA target sequences were also present at the ends of the donor in order for the donor to be cleaved and released from

the plasmid for subsequent editing mediated by HDR promoting agents. A single plasmid expressed all necessary components for editing (see **FIG. 6**). Each expressed component was driven by its own promoter.

Maize cultivation and transfection, and amplicon sequencing

[0254] Each individual plasmid is transfected into maize protoplasts in four separate replicates. Cells are incubated for 48 hours. Genomic DNA is then extracted, and of amplicon sequencing libraries are prepared. Insertion and deletion (indel) frequencies and replacement efficiency are quantified from the amplicon sequencing data as described in Example 2, above.

Results

[0255] The miRNA binding site at the *SPX* locus in maize is edited using a CasS nuclease targeted by two gRNAs in the presence or absence of HDR promoting agents. In addition to this experimental sample, baseline controls as well as several other controls are included in the experiment. As shown in **Table 5**, vectors encoding CasS with the two gRNAs and the donor, CasS with the two gRNAs, CasS with the individual gRNAs, and the donor only serve as controls.

Table 5: Summary of samples in maize protoplast *SPX* locus editing experiment

Transfection Components
CasS + Lambda Red + 2 gRNAs + donor DNA
CasS + 2 gRNAs + donor DNA
CasS + 2 gRNAs
CasS + 1 gRNA
CasS + 1 gRNA
Donor DNA
CasS + 2 gRNAs + Lambda Red
CasS + 1st gRNA + Lambda Red + donor
CasS + 2nd gRNA + Lambda Red + donor
CasS + 1st gRNA
CasS + 2nd gRNA
Lambda Red only control
GFP control
No transfection control

[0256] Precise editing and indels are measured by sequencing and compared between the different samples.

Example 7. Enhanced HDR in *Nicotiana benthamiana*

[0257] The following example describes genome editing in *Nicotiana benthamiana* leaves. In particular, the efficiency of editing *in planta* is measured by repairing the coding sequence of GFP in a *N. benthamiana* reporter line with a mutant allele of GFP, in the presence or absence of HDR promoting agents (*i.e.*, the exonuclease, lambda beta SSAP, and the *E. coli* SSB protein).

Materials and Methods

N. benthamiana cultivation and transfection

[0258] Seeds of *N. benthamiana* with a loss-of-function allele of GFP are germinated on kanamycin selection media (50mg/mL) for two weeks before being transferred to soil and grown in a Conviron growth chamber (12h/12h/75µmol/m^2s^{-1}, day:night:light) for two weeks. *N. benthamiana* leaves are syringe-infiltrated with *Agrobacterium tumefaciens* (strain GV3101) expressing a T-DNA vector that contains the CasS and HDR promoting agents expression cassettes, as well as a donor template that has the GFP-repair template (see **FIG. 7**). Leaf samples are then taken for genotyping to confirm the presence of the reporter transgene via PCR. Plants are incubated with the growth lid on for 3 days before being evaluated and harvested. Treated leaves are transferred to tissue culture and whole plants are regenerated from tissue culture. All samples are tested in triplicate.

Assessment of GFP coding sequence repair

[0259] The repair of the GFP coding sequence is assessed using one of a number of methods. The proportion and number of leaf cells containing the targeted insertion is quantified by the visualization of GFP signal using fluorescence microscopy 3 days after infiltration.

[0260] The frequency of target insertion within infiltrated leaves is quantified using amplicon sequencing, as described in Example 2, of the right genome/donor border to estimate the overall efficiency of precise editing.

[0261] Regenerated whole plants are qualitatively compared to confirm stable expression of the targeted insertion by visualization of GFP signal using fluorescence microscopy.

[0262] The frequency of targeted insertion within regenerated whole plants is quantified by Sanger sequencing of the right-hand genome/donor border to estimate the overall efficiency of precise editing.

Results

[0263] *N. benthamiana* leaves are transformed to express a CasS system for genetically modifying a mutant GFP gene, with and without HDR promoting agents. **Table 6**, below, provides a summary of the components transformed into *N. benthamiana* leaves. "Lambda RED" refers to all three HDR promoting agents (the exonuclease, lambda beta SSAP protein, and the SSB).

Table 6: Summary of samples in *N. benthamiana* GFP reporter editing experiment

Transfection Components
CasS + Lambda Red + gRNA + donor DNA
CasS + gRNA + donor DNA
CasS + gRNA
GFP (positive infiltration control)
GUS (negative infiltration control)
No treatment

[0264] Repair of the mutant GFP is measured and compared between the samples.

Example 8. Enhanced HDR in Dividing Tomato and Maize Tissue

[0265] The following example describes experiments testing gene editing mediated by HDR promoting agents in dividing plant tissues. In particular, tomato cotyledon explants were editing using a Cas nuclease in the presence and absence of HDR promoting agents. In addition, maize embryo explants are edited using a Cas nuclease in the presence and absence of HDR promoting agents.

Maize Explant Transformation

Materials and Methods

Design of plasmid for maize transformation

[0266] This example describes the construction of plant expression vectors for *Agrobacterium* mediated maize transformation. Two plant gene expression vectors were prepared. Plant expression cassettes for expressing a Bacteriophage lambda exonuclease (SEQ ID NO:8), a bacteriophage lambda beta SSAP protein (SEQ ID NO: 1), and an *E.coli* SSB (SEQ ID NO:31) were constructed. A DNA sequence encoding a tobacco c2 nuclear localization signal (NLS) of SEQ ID NO:15 was fused to the DNA sequences encoding the exonuclease, the bacteriophage lambda beta SSAP protein, and the *E.coli* SSB to provide a

DNA sequence encoding the c2 NLS-Exo, c2 NLS lambda beta SSAP, and c2 NLS-SSB fusion proteins that are set forth in SEQ ID NO: 135, SEQ ID NO: 134, and SEQ ID NO: 133, respectively. DNA sequences encoding the c2 NLS-Exo, c2 NLS lambda beta SSAP, and c2NLS-SSB fusion proteins were operably linked to a OsUBI1, SlUBI1, OsACT promoter and a pea3A, pea rbcs E9, NtEXT polyadenylation site respectively, to provide the exonuclease, SSAP, and SSB plant expression cassettes.

[0267] A DNA donor sequence that targets the promoter region of the maize gln1-3 gene for insertion of a 36 base pair heterologous sequence by HDR was constructed. The DNA donor sequence includes a replacement template with desired insertion region (36 base pairs long) flanked on both sides by homology arms about 500-635 bp in length. The homology arms match (i.e., are homologous to) gDNA (genomic DNA) regions flanking the target gDNA insertion site. The replacement template region comprising the donor DNA is flanked at each end by DNA sequences identical to the gln1-3 gene sequence recognized by an RNA-guided nuclease.

[0268] A plant expression cassette that provides for expression of the RNA-guided sequence-specific (CasB cutting type) endonuclease was constructed. A plant expression cassette that provides for expression of a guide RNA complementary to sequences adjacent to the insertion site was constructed. An *Agrobacterium* superbinary plasmid transformation vector containing a cassette that provides for the expression of the phosphinothricin N-acetyltransferasesynthase (PAT) protein was constructed. Once the cassettes, donor sequence and *Agrobacterium* superbinary plasmid transformation vector are constructed, they were combined to generate two maize transformation plasmids.

[0269] Maize transformation plasmid pIN1757 was constructed with the PAT cassette, the RNA-guided sequence-specific endonuclease cassette, the guide RNA cassette, and the gln1-3 DNA donor sequence into the *Agrobacterium* superbinary plasmid transformation vector (**FIG. 8**).

[0270] Maize transformation plasmid pIN1756 was constructed with the PAT cassette, the RNA-guided sequence-specific endonuclease cassette, the guide RNA cassette, , the SSB cassette, the lambda beta SSAP cassette, the Exo cassette, and the gln1-3 DNA donor sequence into the *Agrobacterium* superbinary plasmid transformation vector (**FIG. 8**).

Maize transformation

[0271] All constructs were delivered from superbinary vectors in *Agrobacterium* strain LBA4404.

[0272] Maize transformations were performed based on published methods (Ishida *et. al*, Nature Protocols 2007; 2, 1614-1621). Briefly, immature embryos from inbred line GIBE0104, approximately 1.8-2.2 mm in size, were isolated from surface sterilized ears 10-14 days after pollination. Embryos were placed in an *Agrobacterium* suspension made with infection medium at a concentration of OD_{600} = 1.0. Acetosyringone (200 μM) was added to the infection medium at the time of use. Embryos and *Agrobacterium* were placed on a rocker shaker at slow speed for 15 minutes. Embryos were then poured onto the surface of a plate of co-culture medium. Excess liquid media was removed by tilting the plate and drawing off all liquid with a pipette. Embryos were flipped as necessary to maintain a scutelum up orientation. Co-culture plates were placed in a box with a lid and cultured in the dark at 22°C for 3 days. Embryos were then transferred to resting medium, maintaining the scutellum up orientation. Embryos remain on resting medium for 7 days at 27-28°C. Embryos that produced callus were transferred to Selection 1 medium with 7.5 mg/L phosphinothricin (PPT) and cultured for an additional 7 days. Callused embryos were placed on Selection 2 medium with 10 mg/L PPT and cultured for 14 days at 27-28°C. Growing calli resistant to the selection agent were transferred to Pre-Regeneration media with 10 mg/L PPT to initiate shoot development. Calli remained on Pre-Regeneration media for 7 days. Calli beginning to initiate shoots were transferred to Regeneration medium with 7.5 mg/L PPT in Phytatrays and cultured in light at 27-28°C. Shoots that reached the top of the Phytatray with intact roots were isolated into Shoot Elongation medium prior to transplant into soil and gradual acclimatization to greenhouse conditions.

Results

[0273] The number of explants in each experimental condition is provided in **Table 7A**, below. Regenerated shoots were sampled and gDNA was extracted from 45 regenerated plants from 16 embryos ("events") for pIN1757 and from 201 regenerated plants from 53 embryos for pIN1756. The ZmGln1.3 locus was amplified from gDNA using primers designed to generate an amplicon of about 835 base pairs; the forward primer is about 130 bp 5' of the endonuclease cut site, and the reverse primer is outside of the 3' homology arm, so that only the endogenous locus is amplified. After bead clean-up, the amplicons were analyzed by next-generation sequencing.

[0274] The numbers reported in **Table 7A**, # Indel and # HDR columns, represent samples with at least 5,000 mapped reads to the target sequence and at least 50% full alignment to the amplicon. After filtering for samples with at least 5,000 reads mapping to

the target sequence and at least 50% full alignment to the amplicon, 2 independent events (5 plants) were identified out of 53 events (201 plants) with targeted insertion (3.77%) when the HDR promoting agents were present, compared to 0 out of 16 events when the HDR promoting agents were not present.

Table 7A: Summary of transformed maize embryos

Construct	# embryos treated	Shoots recovered/ events	# Indel	# HDR
pIN1757	397	45/16	40/43	0/43
pIN1756	472	201/53	112/137	105/137

Tomato Explant Transformation

Materials and Methods

Design of plasmids for tomato transformation

[0275] Plant expression cassettes for expressing a Bacteriophage lambda exonuclease (SEQ ID NO:8), a bacteriophage lambda beta SSAP protein (SEQ ID NO: 1), and an *E.coli* SSB (SEQ ID NO:31) were constructed. A DNA sequence encoding a tobacco c2 nuclear localization signal (NLS) of SEQ ID NO:15 was operably linked to the DNA sequences encoding the exonuclease, the bacteriophage lambda beta SSAP protein, and the *E.coli* SSB to provide a DNA sequence encoding the c2 NLS-Exo, c2 NLS lambda beta SSAP, and c2 NLS-SSB fusion proteins that are set forth in SEQ ID NO: 135, SEQ ID NO: 134, and SEQ ID NO: 133, respectively. DNA sequences encoding the c2 NLS-Exo, c2 NLS lambda beta SSAP, and c2NLS-SSB fusion proteins were operably linked to a 2x35S, SlUBI10, PcUBI4 promoter and a 35S, AtHSP, pea3A polyadenylation site respectively, to provide the exonuclease, SSAP, and SSB plant expression cassettes.

[0276] In addition, a DNA donor sequence that targeted the promoter region of the tomato Ant1 gene (SlAnt1) for insertion of a 42 base pair heterologous sequence by HDR was constructed. The DNA donor sequences included a replacement template with desired insertion region (42 base pairs long) flanked on both sides by homology arms about 600-800 bp in length. The homology arms matched (*i.e.*, were homologous to) endogenous DNA regions flanking the target gDNA insertion site. The replacement template region comprising the donor DNA was flanked at each end by DNA sequences identical to the endogeneous target editing site sequence recognized by an RNA-guided nuclease.

[0277] Further, a plant expression cassette that provides for expression of the RNA-guided sequence-specific endonuclease was constructed. A plant expression cassette that provides for expression of a guide RNA complementary to sequences adjacent to the insertion site was constructed. A plant expression cassette that provides for expression of the green fluorescent protein (GFP) was constructed. An *Agrobacterium* binary plasmid transformation vector containing a cassette that provides for the expression of the 5-enolpyruvylshikimate-3-phosphate (EPSPS) synthase was constructed.

[0278] Once the cassettes, donor sequence and *Agrobacterium* transformation plasmid vector were constructed, they were combined to generate three tomato transformation plasmids.

[0279] Tomato transformation plasmid pIN1703 was constructed with the RNA-guided sequence-specific endonuclease cassette, the guide RNA cassette and the GFP cassette cloned into the *Agrobacterium* transformation plasmid vector (**FIG. 9B**). Tomato transformation plasmid pIN1704 was constructed with the RNA-guided sequence-specific endonuclease cassette, the guide RNA cassette and Ant1 DNA donor sequence cloned into the *Agrobacterium* transformation plasmid vector (**FIG. 9B**). Tomato transformation plasmid pIN1705 was constructed with the RNA-guided sequence-specific endonuclease cassette, the guide RNA cassette, the SSB cassette, the lambda beta SSAP cassette, the exonuclease cassette and Ant1 DNA donor sequence cloned into the *Agrobacterium* transformation plasmid vector (**FIGS. 9A-9B**).

[0280] All vectors were delivered to tomato using the *Agrobacterium* strain EHA105.

Tomato explant transformation

[0281] The vectors described above were used to transform tomato (cv. Moneymaker) explants to regenerated stably transformed transgenic shoots with the above mentioned components. Tomato transformations were performed based on previously published methods (Van Eck J., Keen P., Tjahjadi M. (2019) *Agrobacterium tumefaciens-Mediated Transformation of Tomato*. In: Kumar S., Barone P., Smith M. (eds) *Transgenic Plants. Methods in Molecular Biology*, vol 1864. Humana Press, New York, NY). Briefly, tomato seeds were sterilized with 50% commercial bleach for 10 minutes and germinated on ½ strength MSO media. Before the true leaf has emerged, cotyledonary leaves were dissected to collect the middle 3-5 mm section of the leaves. These leaves were transformed with *Agrobacterium* and then placed on resting regeneration media for two weeks. After two

weeks, explants were moved to regeneration media supplemented with 2 mg/L glyphosate as a selection agent. Explants were subcultured every two weeks. In about 6-7 weeks, shoots began regenerating from these explants.

[0282] Samples were collected from well-elongated shoots, and shoots were moved to rooting media supplemented with 2 mg/L glyphosate. For small shoots, entire shoot masses were collected (*i.e.*, destructive sampling) for molecular analysis.

Assessment of tomato explant transformation

[0283] Regenerated shoots were first identified as transgene positive by a TaqMan qPCR assay to detect the presence of the nuclease sequence. Further, the qPCR assay was used to estimate whether the transgene insertion occurred in low (1-2 copies) or high (>2 copies) copy numbers, as shown in **Table 7B**, below. To assess the level of HDR-mediated editing events, the SlAnt1 locus was amplified from the same gDNA source extracted from the previously confirmed nuclease sequence positive explants, and analyzed via next generation sequencing.

Results

[0284] A system was designed with a CRISPR endonuclease (CasS), a guide RNA for site-specific cleavage and the HDR promoting agents (exonuclease, lambda beta SSAP protein, and *E. coli* SSB), as described above. A donor DNA molecule featuring the sequence to be integrated flanked by homology arms that matched the targeted genomic locus was also included. The donor DNA was flanked by a cut site matching the guide RNA on either side so that the donor molecule can be excised, and released from the genomic insertion site in which the transgene was inserted. To test the effectiveness of this system in improving targeted integrations into the genome of dividing plant tissues, the full system described above was delivered via *Agrobacterium* to explants of tomato.

[0285] The system's effectiveness was measured by comparing the efficiency of precise targeted integration from the HDR promoting agents system (**FIG. 9A**) compared to a baseline experimental condition composed of just the CasS nuclease, guide RNA, and DNA donor (see pIN1704 in **FIG. 9B**). Efficiency of precise targeted integration was calculated based on DNA sequencing of shoots regenerated from the transformed explants. The percentage of tomato shoots that contained the integrated donor sequence out of the total number of regenerated shoots is shown in **Table 7B**, below, for each construct. The sampled tissues were chimeric rather than genetically uniform due to the nature of tomato

transformation system, and the sequencing results reflected some independent editing occurrences within individual plants. In **Table 7B**, indel refers to both NHEJ-type and HDR-type of mutation at the target location in the SlAnt1 promoter. HDR mutations were considered likely heritable when more than 30% of the sequencing reads from an individual sample were precise edits, *i.e.* insertions of the template DNA. The the level of precise editing did not correlate with number of transgene copies. The percentage of heritable HDR-mediated editing events was highest in the shoots transformed with the vector encoding the HDR promoting agents (pIN1705). A few edited plants were further characterized by long read sequencing. Of six pIN1704-transformed plant samples, some scarless editing was detected in only one. Of fifteen pIN1705-transformed plant samples, some scarless editing was detected in ten, of which at least four had biallelic 100% scarless editing. As a result of the targeted sequence insertion, edited plants showed different levels of anthocyanin accumulation. Altogether, the vector encoding the HDR promoting agents significantly improved the HDR-mediated precise editing.

Table 7B: Summary of gene editing in tomato explants

Construct	Number of low copy (1-2 copy) events	Number of high copy (>2) events	% mutation freq. (% Indel >30 %)	% heritable HDR (>30 % HDR) events	Normalized % heritable HDR (>30 % HDR) events
pIN1703	20	10	100% (30/30)	0 % (0/30)	0%
pIN1704	124	6	75.3% (98/130)	0.7% (1/130)	0.93%
pIN1705	190	10	74% (148/200)	4% (8/200)	5.4%

[0286] Tomato editing experiments as described above were repeated, and the results are shown in **Table 7C**. Again, the percentage of heritable HDR-mediated editing events was highest in the shoots transformed with the vector encoding the HDR promoting agents (pIN1705); the same trend was observed.

Table 7C: Summary of gene editing in tomato explants

Construct	% mutation freq. (% Indel >30 %)	% heritable HDR (>30 % HDR) events	Normalized % heritable HDR (>30 % HDR) events
pIN1704	54% (54/100)	2% (2/100)	3.7%

pIN1705	75.6% (189/250)	6.8% (17/250)	8.9%

Example 9. Enhanced HDR in Mammalian Cells

[0287] The following example describes the precise editing of loci in human embryonic kidney 293 (HEK-293) cells in the presence or absence of HDR promoting agents. An FRT site and a minimal AAVS1 site are inserted into the EMX1 and GRIN2b genes, respectively. Plasmids expressing the editing machinery are transfected into cell lines in order to induce targeted insertions at specific target editing sites in these genes.

Materials and Methods

Design of plasmid for transfection

[0288] A single plasmid is generated encoding a CasS nuclease with a gRNA specific to the EMX1 or GRIN2b target locus, the HDR promoting agents (exonuclease, lambda beta SSAP, and the *E. coli* SSB protein), and a donor template with the insertion sequence and ~700 base pair homology arms that are homologous to the target editing site. Each component is driven by a separate promoter. The gene cassettes are first synthesized in three separate intermediary plasmids called module A, B and C and then assembled into a single expression plasmid.

[0289] The amino acid sequences of CasS and the HDR promoting agents are as described in Example 1, except for the NLS for the HDR promoting agents. In particular, the HDR promoting agents are fused to the SV40 NLS with an amino acid linker (SEQ ID NO: 148, MAPKKKRKVGGSGS). All coding-sequences are codon-optimized for expression in humans. As shown in **FIG. 10**, CasB is under control of the CAG promoter and the rabbit beta-globin terminator (CAGp- CasS -rb_globin_t), the gRNA is under control of the *H. sapiens* U6 promoter (HsU6p-gRNA), the SSB protein is under control of the *H. sapiens* EF1a promoter and the human growth hormone (hGH) terminator (HsEF1ap-SSB-hGHt), the SSAP is under control of the *H. sapiens* ACTB promoter and the bovine growth hormone (bGH) terminator (HsACTB-Beta-bGHt), and the exonuclease is under control of the CMV promoter and the SV40 terminator (CMVp-Exo-SV40t).

[0290] In addition, the donor is also flanked by the same gRNA target sequence as the one present in the genomic target, thus leading to the release of the donor from the delivered plasmid, and subsequent editing mediated by HDR promoting agents (see **FIG. 10**).

[0291] A separate plasmid is constructed for each sample shown in **Table 8**, below.

Transfection of HEK-293 cells

[0292] The plasmid is transfected into HEK-293 cells. Three separate transfections per plasmid serve as replicates.

[0293] After transfections, the cells are incubated for 48-72 hours, after which genomic DNA is extracted from all samples for subsequent preparation of amplicon sequencing libraries.

Amplicon sequencing

[0294] The targets are amplified with a primer annealing to the sequence directly adjacent to the insertion site and a primer annealing to the genomic sequence outside of the homology region present in the donor (to prevent amplification of the donor from the plasmid). The insertion efficiencies at the target loci are then quantified using the amplicon sequencing data from the read coming from the primer adjacent to the insertion sequence.

[0295] HEK-293 cells are edited in the presence or absence of HDR promoting agents. In particular, a 34 base pair FRT site is inserted into the EMX1 locus, and a 33 base pair minimal AAVS1 site is inserted into GRIN2b locus using the plasmids described above.

[0296] In addition to the sample containing CasS, all three HDR promoting agents ("Lambda Red"), a gRNA, and a donor DNA, several controls are included in order to compare the editing efficiency of the samples with HDR promoting agents to baseline controls, as shown in **Table 8**. "Lambda RED" refers to all three HDR promoting agents (the exonuclease, lambda beta SSAP protein, and the SSB).

Table 8: Summary of samples in HEK-293 cells gene editing experiment

Transfection Components
CasS + Lambda Red + gRNA + donor DNA
CasS + gRNA + donor DNA
CasS + gRNA
Donor DNA
No transfection

[0297] In particular, samples containing CasS with the gRNA and donor (the baseline control without HDR promoting agents), the Lambda Red genes and the donor (no nuclease control to confirm the nuclease-mediated cleavage of target DNA is important), the donor only, and CasS with the gRNA (cleavage control to make sure we are getting efficient cleavage of the target) are transfected individually as controls. The sample with CasS with

the gRNA and donor is the baseline sample that the samples with the HDR promoting agents are compared to. In addition, no transfection controls are also evaluated.

[0298] The breadth and scope of the present disclosure should not be limited by any of the above-described Examples, but should be defined only in accordance with the preceding embodiments, the following claims, and their equivalents.

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