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(71) Applicant: **INARI AGRICULTURE TECHNOLOGY, INC.** [US/US]; One Kendall Square, Building 600/700, Suite 7-501, Cambridge, Massachusetts 02139 (US).

(72) Inventors: **KOCK, Michael Andreas**; c/o Inari Agriculture Technology, Inc., One Kendall Square, Building 600/700, Suite 7-501, Cambridge, Massachusetts 02139 (US). **NUCCIO, Michael Lee**; c/o Inari Agriculture Technology, Inc., One Kendall Square, Building 600/700, Suite 7-501, Cambridge, Massachusetts 02139 (US). **VAN EX, Frédéric**; c/o Inari Agriculture Technology, Inc., One Kendall Square, Building 600/700, Suite 7-501, Cambridge, Massachusetts 02139 (US). **ELATA, Alexandra**; c/o Inari Agriculture Technology, Inc., One Kendall Square, Building 600/700, Suite 7-501, Cambridge, Massachusetts 02139 (US). **RODRIGUEZ LEAL, Daniel**; c/o Inari Agriculture Technology, Inc., One Kendall Square, Building 600/700, Suite 7-501, Cambridge, Massachusetts 02139 (US). **PRICE, Joshua L.**; c/o Inari Agriculture Technol-

(54) Title: EXPEDITED BREEDING OF TRANSGENIC CROP PLANTS BY GENOME EDITING

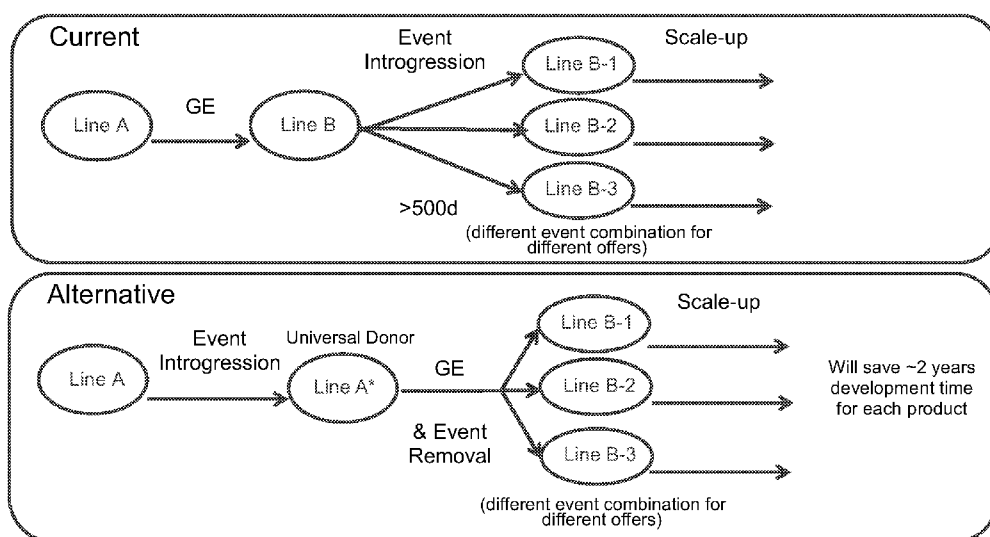


FIG. 11

(57) Abstract: Methods of selectively excising transgenic loci from transgenic plants and use of such methods to facilitate plant breeding are disclosed.

ogy, Inc., One Kendall Square, Building 600/700, Suite 7-501, Cambridge, Massachusetts 02139 (US).

(74) **Agent: ROMANO, Charles P.** et al.; McKee, Voorhees & Sease, P.L.C., 801 Grand Avenue, Suite 3200, Des Moines, Iowa 50309-2721 (US).

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EXPEDITED BREEDING OF TRANSGENIC CROP PLANTS BY GENOME EDITING

Inventors: Michael A. Kock, Michael Nuccio, Frédéric Van Ex,
Alexandra Elata, Daniel Rodriguez Leal, Joshua L. Price

BIOLOGICAL SEQUENCES

[0001] The sequence listing contained in the file named “10076WO1_ST25.txt”, which is 475,315 bytes as measured in the Windows operating system and which was created on July 26, 2021 and electronically filed on July 26, 2021, is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Transgenes which are placed into different positions in the plant genome through non-site specific integration can exhibit different levels of expression (Weising et al., 1988, Ann. Rev. Genet. 22:421-477). Such transgene insertion sites can also contain various undesirable rearrangements of the foreign DNA elements that include deletions and/or duplications. Furthermore, many transgene insertion sites can also comprise selectable or scoreable marker genes which in some instances are no longer required once a transgenic plant event containing the linked transgenes which confer desirable traits are selected.

[0003] Commercial transgenic plants typically comprise one or more independent insertions of transgenes at specific locations in the host plant genome that have been selected for features that include expression of the transgene(s) of interest and the transgene-conferred trait(s), absence or minimization of rearrangements, and normal Mendelian transmission of the trait(s) to progeny. Examples of selected transgenic maize, soybean, cotton, and canola plant events which confer traits such as herbicide tolerance and/or pest tolerance are disclosed in US Patent Nos. 7323556; 8575434; 6040497; 10316330; 8618358; 8212113; 9428765; 8455720; 7897748; 8273959; 8093453; 8901378; 8466346; RE44962; 9540655; 9738904; 8680363; 8049071; 9447428; 9944945; 8592650; 10184134; 7179965; 7371940; 9133473; 8735661; 7381861; 8048632; and 9738903.

[0004] Methods for removing selectable marker genes and/or duplicated transgenes in transgene insertion sites in plant genomes involving use of site-specific recombinase systems (e.g., cre-lox) as well as for insertion of new genes into transgene insertion sites have been disclosed (Srivastava and Ow; Methods Mol Biol, 2015,1287:95-103; Dale and Ow, 1991, *Proc. Natl Acad. Sci. USA* 88, 10558–10562; Srivastava and Thomson, Plant Biotechnol J, 2016;14(2):471-82). Such methods typically require incorporation of the recombination site sequences recognized by the recombinase at particular locations within the transgene.

SUMMARY

[0005] Methods of producing an elite crop plant comprising a targeted genetic change and at least one transgenic locus comprising steps of: (i) inducing at least one targeted genetic change in the genome of the crop plant with one or more genome editing molecules in an elite crop plant comprising a first transgenic loci and a second transgenic loci; (ii) excising a DNA segment comprising all or most of the first transgenic locus with genome editing molecules by (a) contacting genomic DNA of said plant with: (i) at least a first and at least a second guide RNA directed to genomic DNA adjacent to two PAM sites, wherein the PAM sites are operably linked to a 5' and a 3' DNA junction polynucleotide of the first transgenic locus; and (ii) one or more RNA dependent DNA endonucleases (RdDe) which recognize the PAM sites; and (iii) selecting an elite crop plant wherein the first transgenic locus is excised, the second transgenic locus is present, and the targeted genetic change is present are provided.

[0006] Elite crop plants or parts thereof comprising at least one first transgenic locus and a transgenic locus excision site wherein all of a second transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second transgenic locus is excised are provided.

[0007] Methods for obtaining elite crop plants disclosed herein comprising the steps of: (a) obtaining a crop plant comprising at least the first transgenic locus and a second transgenic locus; (b) introgressing the first transgenic locus and a second transgenic locus into the germplasm of the elite crop plant; (c) excising a DNA segment comprising the second transgenic locus from the elite crop plant of step (b) with genome editing molecules and optionally inducing at least one targeted genetic change in the genome of the crop plant of step (b) with one or more genome editing molecules; and (d) selecting an elite crop plant comprising: (i) the first

transgenic locus and a transgenic locus excision site wherein all of the second transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second transgenic locus is excised; and optionally (ii) the targeted genetic change are provided.

[0008] DNA comprising a transgenic locus excision site wherein all of a transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the transgenic locus is excised.

[0009] Nucleic acid markers adapted for detection of genomic DNA or fragments thereof comprising a transgenic locus excision site wherein all of a transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the transgenic locus is excised and wherein the marker does not detect a transgenic locus which has not been excised are provided.

[0010] Biological sample comprising plant genomic DNA or fragments thereof, said genomic DNA or fragments comprising a transgenic locus excision site wherein all of a transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the transgenic locus is excised.

[0011] Methods of identifying any one the aforementioned plants, DNA, or biological samples, comprising detecting a polynucleotide comprising a transgenic locus excision site wherein all of a transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the transgenic locus is excised with a nucleic acid detection assay are provided.

[0012] Elite crop plants or parts thereof comprising at least one first transgenic locus and a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of a second transgenic locus is excised are provided.

[0013] Methods for obtaining the aforementioned elite crop plants comprising the steps of: (a) obtaining a crop plant comprising at least the first transgenic locus and a second transgenic locus; (b) introgressing the first transgenic locus and a second transgenic locus into the germplasm of the elite crop plant; (c) excising a DNA segment comprising the second transgenic locus from the elite crop plant of step (b) with genome editing molecules and optionally inducing at least one targeted genetic change in the genome of the crop plant of step (b) with one or more genome editing molecules; and (d) selecting an elite crop plant comprising:

(i) the first transgenic locus and a transgenic locus excision site wherein all of the second transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second transgenic locus is excised; and optionally (ii) the targeted genetic change are provided.

[0014] Methods for obtaining a bulked population of inbred seed for commercial seed production comprising selfing any one of the aforementioned elite crop plants and harvesting seed from the selfed elite crop plants are provided.

[0015] Methods for obtaining hybrid crop seed comprising crossing a first crop plant comprising any one of the aforementioned elite crop plants to a second crop plant and harvesting seed from the cross are provided.

[0016] DNA comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of a transgenic locus is excised is provided.

[0017] Nucleic acid markers adapted for detection of genomic DNA or fragments comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of a transgenic locus is excised and wherein the marker does not detect a transgenic locus which has not been excised are provided.

[0018] Biological samples comprising the plant genomic DNA or fragments thereof, said genomic DNA or fragments comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of a transgenic locus is excised are provided.

[0019] Methods of identifying the any of the aforementioned plants, DNA, or biological samples comprising detecting a polynucleotide comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of a transgenic locus is excised with a nucleic acid detection assay.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0020] Figure 1 shows of a diagram of transgene expression cassettes and selectable markers in the DAS-59122-7 transgenic locus set forth in SEQ ID NO: 1.

[0021] Figure 2 shows a diagram of transgene expression cassettes and selectable markers in the DP-4114 transgenic locus set forth in SEQ ID NO: 2.

- [0022] Figure 3 shows a diagram of transgene expression cassettes and selectable markers in the MON87411 transgenic locus set forth in SEQ ID NO: 3.
- [0023] Figure 4 shows a diagram of transgene expression cassettes and selectable markers in the MON89034 transgenic locus.
- [0024] Figure 5 shows a diagram of transgene expression cassettes and selectable markers in the MIR162 transgenic locus.
- [0025] Figure 6 shows a diagram of transgene expression cassettes and selectable markers in the MIR604 transgenic locus set forth in SEQ ID NO: 6.
- [0026] Figure 7 shows a diagram of transgene expression cassettes and selectable markers in the NK603 transgenic locus set forth in SEQ ID NO: 7.
- [0027] Figure 8 shows a diagram of transgene expression cassettes and selectable markers in the SYN-E3272-5 transgenic locus set forth in SEQ ID NO: 8.
- [0028] Figure 9 shows a diagram of transgene expression cassettes and selectable markers in the transgenic locus set forth in SEQ ID NO: 8.
- [0029] Figure 10 shows a diagram of transgene expression cassettes and selectable markers in the TC1507 transgenic locus set forth in SEQ ID NO: 10.
- [0030] Figure 11 shows a schematic diagram which compares current breeding strategies for introgression of transgenic events (*i.e.*, transgenic loci) to alternative breeding strategies for introgression of transgenic events where the transgenic events (*i.e.*, transgenic loci) can be removed following introgression to provide different combinations of transgenic traits.
- [0031] Figure 12 shows a diagram of transgene expression cassettes and selectable markers in the DAS68416-4 transgenic locus set forth in SEQ ID NO: 12.
- [0032] Figure 13 shows a diagram of transgene expression cassettes and selectable markers in the MON87701 transgenic locus set forth in SEQ ID NO: 14.
- [0033] Figure 14 shows a diagram of transgene expression cassettes and selectable markers in the MON89788 transgenic locus set forth in SEQ ID NO: 16.
- [0034] Figure 15 shows a diagram of transgene expression cassettes and selectable markers in the COT102 transgenic locus set forth in SEQ ID NO: 19.
- [0035] Figure 16 shows a diagram of transgene expression cassettes and selectable markers in the MON88302 transgenic locus set forth in SEQ ID NO: 21.

DETAILED DESCRIPTION

[0036] 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.

[0037] Where a term is provided in the singular, the inventors also contemplate embodiments described by the plural of that term.

[0038] 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.

[0039] The term “about” as used herein means a value or range of values which would be understood as an equivalent of a stated value and can be greater or lesser than the value or range of values stated by 10 percent. Each value or range of values preceded by the term “about” is also intended to encompass the embodiment of the stated absolute value or range of values.

[0040] 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).

[0041] As used herein, the phrase “approved transgenic locus” is a genetically modified plant event which has been authorized, approved, and/or de-regulated for any one of field testing, cultivation, human consumption, animal consumption, and/or import by a governmental body. Illustrative and non-limiting examples of governmental bodies which provide such approvals include the Ministry of Agriculture of Argentina, Food Standards Australia New Zealand, National Biosafety Technical Committee (CTNBio) of Brazil, Canadian Food Inspection Agency, China Ministry of Agriculture Biosafety Network, European Food Safety Authority, US Department of Agriculture, US Department of Environmental Protection, and US Food and Drug Administration.

[0042] The term “backcross”, as used herein, refers to crossing an F1 plant or plants with one of the original parents. A backcross is used to maintain or establish the identity of one parent

(species) and to incorporate a particular trait from a second parent (species). The term “backcross generation”, as used herein, refers to the offspring of a backcross.

[0043] As used herein, the phrase “biological sample” refers to either intact or non-intact (e.g. milled seed or plant tissue, chopped plant tissue, lyophilized tissue) plant tissue. It may also be an extract comprising intact or non-intact seed or plant tissue. The biological sample can comprise flour, meal, syrup, oil, starch, and cereals manufactured in whole or in part to contain crop plant by-products. In certain embodiments, the biological sample is “non-regenerable” (*i.e.*, incapable of being regenerated into a plant or plant part). In certain embodiments, the biological sample refers to a homogenate, an extract, or any fraction thereof containing genomic DNA of the organism from which the biological sample was obtained, wherein the biological sample does not comprising living cells.

[0044] As used herein, the terms “correspond,” “corresponding,” and the like, when used in the context of an nucleotide position, mutation, and/or substitution in any given polynucleotide (e.g., an allelic variant of SEQ ID NO: 1-34) with respect to the reference polynucleotide sequence (e.g., SEQ ID NO: 1-34) all refer to the position of the polynucleotide residue in the given sequence that has identity to the residue in the reference nucleotide sequence when the given polynucleotide is aligned to the reference polynucleotide sequence using a pairwise alignment algorithm (e.g., CLUSTAL O 1.2.4 with default parameters).

[0045] As used herein, the terms “Cpf1” and “Cas12a” are used interchangeably to refer to the same RNA dependent DNA endonuclease (RdDe). Cas12a proteins include the protein provided herein as SEQ ID NO: 85.

[0046] The term “crossing” as used herein refers to the fertilization of female plants (or gametes) by male plants (or gametes). The term “gamete” refers to the haploid reproductive cell (egg or pollen) produced in plants by meiosis from a gametophyte and involved in sexual reproduction, during which two gametes of opposite sex fuse to form a diploid zygote. The term generally includes reference to a pollen (including the sperm cell) and an ovule (including the ovum). When referring to crossing in the context of achieving the introgression of a genomic region or segment, the skilled person will understand that in order to achieve the introgression of only a part of a chromosome of one plant into the chromosome of another plant, random portions of the genomes of both parental lines recombine during the cross due to the occurrence of crossing-over events in the production of the gametes in the parent lines. Therefore, the genomes

of both parents must be combined in a single cell by a cross, where after the production of gametes from the cell and their fusion in fertilization will result in an introgression event.

[0047] As used herein, the phrases “DNA junction polynucleotide” and “junction polynucleotide” refers to a polynucleotide of about 18 to about 500 base pairs in length comprised of both endogenous chromosomal DNA of the plant genome and heterologous transgenic DNA which is inserted in the plant genome. A junction polynucleotide can thus comprise about 8, 10, 20, 50, 100, 200, or 250 base pairs of endogenous chromosomal DNA of the plant genome and about 8, 10, 20, 50, 100, 200, or 250 base pairs of heterologous transgenic DNA which span the one end of the transgene insertion site in the plant chromosomal DNA. Transgene insertion sites in chromosomes will typically contain both a 5’ junction polynucleotide and a 3’ junction polynucleotide. In embodiments set forth herein in SEQ ID NO: 1-34, the 5’ junction polynucleotide is located at the 5’ end of the sequence and the 3’ junction polynucleotide is located at the 3’ end of the sequence. In a non-limiting and illustrative example, a 5’ junction polynucleotide of a transgenic locus is telomere proximal in a chromosome arm and the 3’ junction polynucleotide of the transgenic locus is centromere proximal in the same chromosome arm. In another non-limiting and illustrative example, a 5’ junction polynucleotide of a transgenic locus is centromere proximal in a chromosome arm and the 3’ junction polynucleotide of the transgenic locus is telomere proximal in the same chromosome arm.

[0048] The term “donor,” as used herein in the context of a plant, refers to the plant or plant line from which the trait, transgenic event, or genomic segment originates, wherein the donor can have the trait, introgression, or genomic segment in either a heterozygous or homozygous state.

[0049] As used herein, the terms “excise” and “delete,” when used in the context of a DNA molecule, are used interchangeably to refer to the removal of a given DNA segment or element (*e.g.*, transgene element or transgenic locus) of the DNA molecule.

[0050] As used herein, the phrase “elite crop plant” refers to a plant which has undergone breeding to provide one or more trait improvements. Elite crop plant lines include plants which are an essentially homozygous, *e.g.* inbred or doubled haploid. Elite crop plants can include inbred lines used as is or used as pollen donors or pollen recipients in hybrid seed production (*e.g.* used to produce F1 plants). Elite crop plants can include inbred lines which are selfed to produce non-hybrid cultivars or varieties or to produce (*e.g.*, bulk up) pollen donor or recipient

lines for hybrid seed production. Elite crop plants can include hybrid F1 progeny of a cross between two distinct elite inbred or doubled haploid plant lines.

[0051] As used herein, an “event,” “a transgenic event,” “a transgenic locus” and related phrases refer to an insertion of one or more transgenes at a unique site in the genome of a plant as well as to DNA fragments, plant cells, plants, and plant parts (*e.g.*, seeds) comprising genomic DNA containing the transgene insertion. Such events typically comprise both a 5’ and a 3’ DNA junction polynucleotide and confer one or more useful traits including herbicide tolerance, insect resistance, male sterility, and the like.

[0052] As used herein, the phrases “endogenous sequence,” “endogenous gene,” “endogenous DNA” and the like refer to the native form of a polynucleotide, gene or polypeptide in its natural location in the organism or in the genome of an organism.

[0053] The term “exogenous DNA sequence” as used herein is any nucleic acid sequence that has been removed from its native location and inserted into a new location altering the sequences that flank the nucleic acid sequence that has been moved. For example, an exogenous DNA sequence may comprise a sequence from another species.

[0054] As used herein, the term “F1” refers to any offspring of a cross between two genetically unlike individuals.

[0055] The term “gene,” as used herein, refers to a hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and that contains the genetic instruction for a particular characteristics or trait in an organism. The term “gene” thus includes a nucleic acid (for example, DNA or RNA) sequence that comprises coding sequences necessary for the production of an RNA, or a polypeptide or its precursor. A functional polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence as long as the desired activity or functional properties (*e.g.*, enzymatic activity, pesticidal activity, ligand binding, and/or signal transduction) of the RNA or polypeptide are retained.

[0056] The term “identifying,” as used herein with respect to a plant, refers to a process of establishing the identity or distinguishing character of a plant, including exhibiting a certain trait, containing one or more transgenes, and/or containing one or more molecular markers.

[0057] The term “isolated” as used herein means having been removed from its natural environment.

[0058] 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.

[0059] As used herein, the phrase “introduced transgene” is a transgene not present in the original transgenic locus in the genome of an initial transgenic event or in the genome of a progeny line obtained from the initial transgenic event. Examples of introduced transgenes include exogenous transgenes which are inserted in a resident original transgenic locus.

[0060] As used herein, the terms “introgression”, “introgressed” and “introgressing” refer to both a natural and artificial process, and the resulting plants, whereby traits, genes or DNA sequences of one species, variety or cultivar are moved into the genome of another species, variety or cultivar, by crossing those species. The process may optionally be completed by backcrossing to the recurrent parent. Examples of introgression include entry or introduction of a gene, a transgene, a regulatory element, a marker, a trait, a trait locus, or a chromosomal segment from the genome of one plant into the genome of another plant.

[0061] The phrase “marker-assisted selection”, as used herein, refers to the diagnostic process of identifying, optionally followed by selecting a plant from a group of plants using the presence of a molecular marker as the diagnostic characteristic or selection criterion. The process usually involves detecting the presence of a certain nucleic acid sequence or polymorphism in the genome of a plant.

[0062] The phrase “molecular marker”, as used herein, refers to an indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), microsatellite markers (e.g. SSRs), sequence-characterized amplified region (SCAR) markers, Next Generation Sequencing (NGS) of a molecular marker, cleaved amplified polymorphic sequence (CAPS) markers or isozyme markers or combinations of the markers described herein which defines a specific genetic and chromosomal location.

[0063] As used herein the terms “native” or “natural” define a condition found in nature. A “native DNA sequence” is a DNA sequence present in nature that was produced by natural means or traditional breeding techniques but not generated by genetic engineering (*e.g.*, using molecular biology/transformation techniques).

[0064] The term “offspring”, as used herein, refers to any progeny generation resulting from crossing, selfing, or other propagation technique.

[0065] The phrase "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. When the phrase “operably linked” is used in the context of a PAM site and a DNA junction polynucleotide, it refers to a PAM site which permits cleavage of at least one strand of DNA in the junction polynucleotide with an RNA dependent DNA endonuclease, RNA dependent DNA binding protein, or RNA dependent DNA nickase which recognizes the PAM site when a guide RNA complementary to sequences adjacent to the PAM site is present.

[0066] As used herein, the term “plant” includes a whole plant and any descendant, cell, tissue, or part of a plant. The term “plant parts” include any part(s) of a plant, including, for example and without limitation: seed (including mature seed and immature seed); a plant cutting; a plant cell; a plant cell culture; or a plant organ (e.g., pollen, embryos, flowers, fruits, shoots, leaves, roots, stems, and explants). A plant tissue or plant organ may be a seed, protoplast, callus, or any other group of plant cells that is organized into a structural or functional unit. A plant cell or tissue culture may be capable of regenerating a plant having the physiological and morphological characteristics of the plant from which the cell or tissue was obtained, and of regenerating a plant having substantially the same genotype as the plant. Regenerable cells in a plant cell or tissue culture may be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, or stalks. In contrast, some plant cells are not capable of being regenerated to produce plants and are referred to herein as “non-regenerable” plant cells.

[0067] The term “purified,” as used herein defines an isolation of a molecule or compound in a form that is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment and means having been increased in purity as a result of being separated from other components of the original composition. The term “purified nucleic acid” is used herein to describe a nucleic acid sequence which has been separated from other compounds including, but not limited to polypeptides, lipids and carbohydrates.

[0068] The term “recipient”, as used herein, refers to the plant or plant line receiving the trait, transgenic event or genomic segment from a donor, and which recipient may or may not

have the have trait, transgenic event or genomic segment itself either in a heterozygous or homozygous state.

[0069] As used herein the term “recurrent parent” or “recurrent plant” describes an elite line that is the recipient plant line in a cross and which will be used as the parent line for successive backcrosses to produce the final desired line.

[0070] As used herein the term “recurrent parent percentage” relates to the percentage that a backcross progeny plant is identical to the recurrent parent plant used in the backcross. The percent identity to the recurrent parent can be determined experimentally by measuring genetic markers such as SNPs and/or RFLPs or can be calculated theoretically based on a mathematical formula.

[0071] The terms “selfed,” “selfing,” and “self,” as used herein, refer to any process used to obtain progeny from the same plant or plant line as well as to plants resulting from the process. As used herein, the terms thus include any fertilization process wherein both the ovule and pollen are from the same plant or plant line and plants resulting therefrom. Typically, the terms refer to self-pollination processes and progeny plants resulting from self-pollination.

[0072] The term “selecting”, as used herein, refers to a process of picking out a certain individual plant from a group of individuals, usually based on a certain identity, trait, characteristic, and/or molecular marker of that individual.

[0073] As used herein, the phrase “a transgenic locus excision site” refers to the DNA which remains in the genome of a plant or in a DNA molecule (*e.g.*, an isolated or purified DNA molecule) wherein a segment comprising, consisting essentially of, or consisting of a transgenic locus has been deleted. In a non-limiting and illustrative example, a transgenic locus excision site can thus comprise a contiguous segment of DNA comprising at least 10 base pairs of DNA that is telomere proximal to the deleted transgenic locus or to the deleted segment of the transgenic locus and at least 10 base pairs of DNA that is centromere proximal to the deleted transgenic locus or to the deleted segment of the transgenic locus.

[0074] As used herein, the phrase “transgene element” refers to a segment of DNA comprising, consisting essentially of, or consisting of a promoter, a 5' UTR, an intron, a coding region, a 3'UTR, or a polyadenylation signal. Polyadenylation signals include transgene elements referred to as “terminators” (*e.g.*, NOS, pinII, rbcS, Hsp17, TubA).

[0075] 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.

[0076] Genome editing molecules can permit introduction of targeted genetic change conferring desirable traits in a variety of crop plants (Zhang et al. *Genome Biol.* 2018; 19: 210; Schindele et al. *FEBS Lett.* 2018;592(12):1954). Desirable traits introduced into crop plants such as maize and soybean include herbicide tolerance, improved food and/or feed characteristics, male-sterility, and drought stress tolerance. Nonetheless, full realization of the potential of genome editing methods for crop improvement will entail efficient incorporation of the targeted genetic changes in germplasm of different elite crop plants adapted for distinct growing conditions. Such elite crop plants will also desirably comprise useful transgenic loci which confer various traits including herbicide tolerance, pest resistance (*e.g.*; insect, nematode, fungal disease, and bacterial disease resistance), conditional male sterility systems for hybrid seed production, abiotic stress tolerance (*e.g.*, drought tolerance), improved food and/or feed quality, and improved industrial use (*e.g.*, biofuel). Provided herein are methods whereby targeted genetic changes are efficiently combined with desired subsets of transgenic loci in elite progeny plant lines (*e.g.*, elite inbreds used for hybrid seed production or for inbred varietal production). Also provided are plant genomes containing unique transgenic locus excision sites, DNA molecules comprising the unique transgenic locus excision sites and/or plants comprising the same, biological samples containing the DNA, nucleic acid markers adapted for detecting the DNA molecules, and related methods of identifying the elite crop plants comprising unique transgenic locus excision sites.

[0077] Provided herein are methods for the directed excision of transgenic loci in transgenic plants. In certain embodiments, methods for the excision of the transgenic loci include targeted excision of a given transgenic locus in certain breeding lines to facilitate recovery of germplasm with subsets of transgenic traits tailored for specific geographic locations and/or grower preferences. Other useful applications of the methods for the excision of the transgenic loci include removal of transgenic traits from certain breeding lines when it is desirable to replace the trait in the breeding line without disrupting other transgenic loci and/or non-transgenic loci. In certain embodiments, excision of transgenic loci can be accompanied or followed by insertion of new transgenes that confer a replacement or other desirable trait at the genomic location of the excised transgenic locus (*i.e.*, the transgenic locus excision site which remains in the genome following excision of the transgenic locus).

[0078] Methods provided herein can be used to excise any transgenic locus where the 5' and 3' junction sequences comprising the endogenous non-transgenic genomic DNA and the heterologous transgenic DNA which are joined at the site of transgene insertion in the plant genome are known or have been determined. In certain embodiments provided herein, transgenic loci can be removed from crop plant lines to obtain crop plant lines with tailored combinations of transgenic loci and optionally targeted genetic changes. Such 5' and 3' junction sequences are readily identified in new transgenic events by inverse PCR techniques using primers which are complementary the inserted transgenic sequences. In certain embodiments, the 5' and 3' junction sequences are published. Examples of transgenic loci which can be improved and used in the methods provided herein include the maize, soybean, cotton, and canola transgenic loci set forth in Tables 1, 2, 3, and 4, respectively. Transgenic junction sequences for certain events are also depicted in the drawings. Such transgenic loci set forth in Tables 1-4 are found in crop plants which have in some instances been cultivated, been placed in commerce, and/or have been described in a variety of publications by various governmental bodies. Databases which have compiled descriptions of approved transgenic loci including the loci set forth in Tables 1-4 include the International Service for the Acquisition of Agri-biotech Applications (ISAAA) database (available on the world wide web internet site "isaaa.org/gmapprovaldatabase/event"), the GenBit LLC database (available on the world wide web internet site "genbitgroup.com/en/gmo/gmodatabase"), and the Biosafety Clearing-House (BCH) database (available on the http internet site "bch.cbd.int/database/organisms").

[0079] Table 1. Maize Events (transgenic loci)

Event Name (traits) ¹	Patent or Patent Application Number(s) ²	ATCC or NCIMB Deposit Designation	Trait expression cassette(s)	SEQ ID NO
BVLA430101 (Q)	CN2013103194381A		phyA2	
Bt10 (IR, HT)			Cry1Ab, PAT	
Bt11 (IR, HT)	US 6,342,660; US 6,403,865; US 6,943,282	ATCC 209671	Cry1Ab and PAT	
Bt176			Cry1Ab, PAT	
CBH-351 (HT, IR)	JP 2006197926 A		PAT, Cry9c	

Event Name (traits) ¹	Patent or Patent Application Number(s) ²	ATCC or NCIMB Deposit Designation	Trait expression cassette(s)	SEQ ID NO
DAS-59122-7 (IR, HT)	US 6127180; US 6340593; US 6548291; US 6624145; US 6893872; US 6900371; US 7323556 (Event); US 7695914 (Event); US 7696341; US 7956246 (Event); US 8592653 (Event); US 8952223 (Event); RE 43,373; US 9878321 (Event)	PTA-11384	cry34Ab1, cry35Ab1, PAT	SEQ ID NO: 1
DAS-40278 (HT)	US 20120244533	PTA-10244	aad-1	SEQ ID NO: 22
DBT418 (IR, HT)			Cry1Ac, PAT, pinII	
DP-4114 (IR, HT)	US 8,575,434; US 10,190,179; US 20190136331	PTA-11506	Cry1Ab, cry34Ab1, cry35Ab1, PAT	SEQ ID NO: 2 (Fig. 2)
DP-32138 (MS, MSR)	US 20130031674 US 20090038026 US 20060288440	PTA-9158	Zm Ms45, Zm aal gene, DsRed2	SEQ ID NO: 24
DP-33121 (IR, HT)	US 20150361446	PTA-13392	Cry2A.127, Cry1A.88, VIP3Aa20, PAT	SEQ ID NO: 23
GA21 (HT)	US 2005086719; US 6,040,497; US 6,762,344; US 7,314,970	ATCC 209033	EPSPS	
HCEM485 (HT)	US 8759618 B2	PTA-12014	zmEPSPS	SEQ ID NO: 25

Event Name (traits) ¹	Patent or Patent Application Number(s) ²	ATCC or NCIMB Deposit Designation	Trait expression cassette(s)	SEQ ID NO
LY038 (Q)	US 7157281	PTA-5623	cordapA	SEQ ID NO: 26
MON810 (IR, HT, AR)	US 6,852,915	PTA-6260	Cry1Ab, goxv247, cp4epsps	
MON832 (HT)			Goxv247, cp4 epsps, nptII	
MON863 (IR)	US 7705216	PTA-2605	Cry3Bb1	
MON87403 (YG)	US 20170088904	PTA-13584	athb17	SEQ ID NO: 27
MON87411 (IR, HT)	US 10,316,330	PTA-12669	cry3Bb1, cp4epsps, dvsnf7	SEQ ID NO: 3
MON87419 (HT)	US 2015/0267221	PTA-120860	DMO, PAT	SEQ ID NO: 28
MON87427 (HT/MS) ³	US 8,618,358	PTA-7899	cp4epsps	
MON87460 (AST)	US 8450561	PTA-8910	cspB	SEQ ID NO: 29
MON88017 (IR, HT)	US 8,212,113; US 8,686,230	PTA-5582	cry3Bb1, cp4epsps	
MON89034 (IR) ⁴	US 9,428,765	PTA-7455	cry2Ab2, cry1A.105	SEQ ID NO: 4
MIR162 (IR, MU)	US 8,455,720	PTA-8166	VIP3Aa20	SEQ ID NO: 5
MIR604 (IR, MU)	US 7,897,748	none	cry3A055	SEQ ID NO: 6
MS3			Barnase, PAT	
MS6			barnase	

Event Name (traits) ¹	Patent or Patent Application Number(s) ²	ATCC or NCIMB Deposit Designation	Trait expression cassette(s)	SEQ ID NO
MZHG0JG (HT)	US_201662346688_P WO 2017214074	PTA-122835	ZmEPSPS, PAT	SEQ ID NO: 30
MZIR098 (IR, HT)	US 20200190533	PTA-124143	ecry3.1Ab, mcry3A, PAT	SEQ ID NO: 31
MYDT09Y				
DP-E29				
NK603 (HT)	US 8,273,959	PTA-2478	cp4epsps	SEQ ID NO: 7
SYN-E3272-5 (BF, MU)	US 8,093,453	PTA-9972	amy797E	SEQ ID NO: 8
T14 (HT)			PAT	
T25 (HT)			PAT	
TC1507 (IR, HT)	US 8,901,378; US 8,502,047	PTA-5448 (Inbred BE1146BMR); PTA-8519 (LLD06BM)	cry1Fa2, PAT	SEQ ID NO: 9
TC6275 (IR, HT)			PAT, moCry1F	
VCO-Ø1981-5 (HT)	US 9,994,863	NCIMB 41842	EPSPS	SEQ ID NO: 32
676 (MS, HT)			dam, PAT	
678 (MS, HT)			dam, PAT	
680 (MS, HT)			dam, PAT	
98140 (HT)	US 7,928,296	PTA-8296	zm-hra, GAT	SEQ ID NO: 33
5307 (IR, MU)	US 8,466,346	PTA-9561	ecry3.1Ab	SEQ ID NO: 10

¹ Traits: IR=Insect Resistance; HT=Herbicide Tolerance; AR=Antibiotic Resistance; MU=mannose utilization; BF=Biofuel; MS=Male Sterility; MSR=Male Sterility Restoration; Q=Food and/or Feed Quality; AST=Abiotic Stress Tolerance; YG=Yield/Growth.

² Each US Patent or Patent Application Publication is incorporated herein by reference in its entirety.

³ A single transgene confers vegetative tolerance to glyphosate and exhibits glyphosate-induced male sterility.

⁴ Resistance to coleopteran and lepidopteran insect pests.

[0080] Table 2. Soybean Events (transgenic loci)

Event Name (traits)¹	Patent or Patent Application Number(s)²	ATCC;³ NCIMB⁴ Deposit Number; or Commercial Source	Trait expression cassette(s)	SEQ ID NO
A5547-127 (HT)	US 20080196127 RE44962	NCIMB 41660	PAT	
DAS44406-6 (HT) ⁵	US 9,540,655 US 10,400,250	PTA-11336	Aad-12, 2mepsps, PAT	SEQ ID NO: 11
DAS68416-4 (IR, HT) ⁶	US 9,738,904	PTA-10442 PTA-12006	Aad-12, PAT	SEQ ID NO: 12
DAS81419-2 (IR, HT)	US 8680363 US 8632978 US 9695441 US 9738904	PTA-12006	cry1Ac, cry1F, PAT	SEQ ID NO: 13
GTS 40-3-2 (HT)	US 20070136836	M690GT 0.9 RM Soybean ⁷	cp4epsps	
MON87701 (IR)	US 8049071	PTA-8194	cry1Ac	SEQ ID NO: 14
MON87708 (HT) ⁸	US 9447428	PTA-9670	DMO	SEQ ID NO: 15
MON89788 (HT)	US 9944945	PTA-6708	cp4epsps	SEQ ID NO: 16
MST-FGØ72-3 (HT) ⁹	US 8592650	NCIMB 41659	hppdPF W336, 2mepsps	SEQ ID NO: 34
SYHT0H2 ¹⁰	US 10,184,134	PTA-11226	cAvHPPD-03	

¹ Traits: IR=Insect Resistance; HT=Herbicide Tolerance; AR=Antibiotic Resistance; MU=mannose utilization; BF=Biofuel; MS=Male Sterility.

² Each US Patent or Patent Application Publication is incorporated herein by reference in its entirety.

³ ATCC is the American Type Culture Collection, 10801 University Boulevard Manassas, VA 20110 USA (for “PTA-XXXXX” deposits).

⁴ NCIMB is the National Collection of Industrial, Food and Marine Bacteria, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB9YA, Scotland.

⁵ HT to 2,4-D; glyphosate, and glufosinate; also referred to as pDAB8264.44.06.1.

⁶ Independent IR/HT and HT events combined by breeding. IR/HT event (Cry1F, Cry1Ac synpro (Cry1Ac), and PAT) is DAS81419-2, deposited with ATCC under PTA-12006, also referred to as DAS81419-2.

⁷ Elk Mound Seed, 308 Railroad Street Elk Mound, WI, USA 54739.

⁸ HT to dicamba.

⁹ HT to both glyphosate and isoxaflutole herbicides.

¹⁰ HT to glufosinate and mesotrione herbicides.

[0081] Table 3. Cotton Events (transgenic loci)

Event Name (traits) ¹	Patent Number(s)	ATCC Deposit	Trait expression cassette(s)	SEQ ID NO
DAS-21023-5 (IR, HT) ¹	US 7,179,965	PTA-6233	Cry1Ac, PAT	SEQ ID NO: 17
DAS-24236-5(IR, HT) ¹	US 7,179,965	PTA-6233	Cry1F, PAT	SEQ ID NO: 18
COT102 (IR, AR) ²	US 7,371,940		Vip3A(a),	SEQ ID NO: 19
LLcotton25 (HT)	US 20030097687	PTA-3343	PAT	
MON15985 (IR, AR, SM) ³	US 9,133,473	PTA-2516	cry1Ac, cry2Ab2	
MON88701 (HT) ⁴	US 8,735,661	PTA-11754	DMO, PAT	SEQ ID NO: 20
MON88913 (HT)	US 7,381,861	PTA-4854	cp4 epsps	

¹ Traits: IR=Insect Resistance; HT=Herbicide Tolerance; AR=Antibiotic Resistance; SM=Screenable Marker.

² Both cry1Ac cotton event 3006-210-23 and cry1F cotton event 281-24-236 described in US 7,179,965; seed comprising both events deposited with ATCC as PTA-6233.

³ Contains both the MON531 chimeric Cry1A and MON15985X Cry2Ab insertions.

⁴ Tolerance to dicamba and glufosinate herbicides.

[0082] Table 4. Canola Events (transgenic loci)

Event Name (traits) ¹	Patent or Patent Application Publication Number(s)	ATCC Deposit	Trait Expression cassette	SEQ ID NO (Figure Number)
GT73 (HT)	US 8,048,632 US 9,474,223	PTA- 121409	cp4 epsps	
HCN28/T45 (HT)				

MON88302 (HT)	US 9,738,903	PTA-10955	cp4 epsps	SEQ ID NO: 21
MS8 (MS)	US2003188347	PTA-730		
RF3 (HT)	US2003188347	PTA-730		

¹ Traits: HT=Herbicide Tolerance; MS=Male Sterility.

[0083] Sequences of the 5' and 3' junction polynucleotides as well as the transgenic insert(s) of certain transgenic loci which can be excised by the methods provided herein are set forth in Tables 1-4 (*e.g.*, SEQ ID NO: 1-34), the patent references set forth therein and incorporated herein by reference in their entirety, and elsewhere in this disclosure. Allelic or other variant sequences corresponding to the sequences set forth in Tables 1-4 and elsewhere in this disclosure which may be present in certain variant transgenic plant loci can also be improved by identifying sequences in the variants that correspond to the sequences of Tables 1-4 (*e.g.*, SEQ ID NO: 1-34), the patent references set forth therein and incorporated herein by reference and incorporated herein by reference in their entirety, and elsewhere in this disclosure by performing a pairwise alignment (*e.g.*, using CLUSTAL O 1.2.4 with default parameters) and making corresponding changes in the allelic or other variant sequences. Such allelic or other variant sequences include sequences having at least 85%, 90%, 95%, 98%, or 99% sequence identity across the entire length or at least 20, 40, 100, or 500, 1,000, 2,000, 4,000, 8,000, 10,000, or 12,000 nucleotides of the sequences set forth in Tables 1-4 (*e.g.*, SEQ ID NO: 1-34), the patent references set forth therein and incorporated herein by reference in their entirety, and elsewhere in this disclosure. Also provided are plants, genomic DNA, and/or DNA obtained from plants set forth in Tables 1-4 which comprise one or more transgenic loci excision sites wherein a segment comprising, consisting essentially of, or consisting of a transgenic locus is deleted. Also provided herein are methods of detecting plants, genomic DNA, and/or DNA obtained from plants set forth in Tables 1-4 comprising a transgenic locus excision site.

[0084] Methods provided herein can be used in a variety of breeding schemes to obtain elite crop plants comprising subsets of desired transgenic loci and transgenic loci excision sites where undesired transgenic loci have been removed. Such methods are useful at least insofar as they allow for production of distinct useful donor plant lines each having unique sets of transgenic loci and, in some instances, targeted genetic changes that are tailored for distinct geographies and/or product offerings. In an illustrative and non-limiting example, a different product lines comprising transgenic loci conferring only two of three types of herbicide tolerance (*e.g.*, glyphosate, glufosinate, and dicamba) can be obtained from a single donor line comprising

three distinct transgenic loci conferring resistance to all three herbicides. In certain aspects, plants comprising the subsets of undesired transgenic loci and transgenic loci excision sites can further comprise targeted genetic changes. Such elite crop plants can be inbred plant lines or can be hybrid plant lines. In certain embodiments, at least two transgenic loci (*e.g.*, transgenic loci in Tables 1-4 or modifications thereof wherein a selectable marker gene and/or non-essential DNA are deleted) are introgressed into a desired donor line comprising elite crop plant germplasm and then subjected to genome editing molecules to recover plants comprising one of the two introgressed transgenic loci as well as a transgenic loci excision site introduced by excision of the other transgenic locus by the genome editing molecules. In certain embodiments, the genome editing molecules can be used to remove a transgenic locus and introduce targeted genetic changes in the crop plant genome. Introgression can be achieved by backcrossing plants comprising the transgenic loci to a recurrent parent comprising the desired elite germplasm and selecting progeny with the transgenic loci and recurrent parent germplasm. Such backcrosses can be repeated and/or supplemented by molecular assisted breeding techniques using SNP or other nucleic acid markers to select for recurrent parent germplasm until a desired recurrent parent percentage is obtained (*e.g.*, at least about 95%, 96%, 97%, 98%, or 99% recurrent parent percentage). A non-limiting, illustrative depiction of a scheme for obtaining plants with both subsets of transgenic loci and the targeted genetic changes is shown in the Figure 11 (bottom “Alternative” panel), where two or more of the transgenic loci (“Event” in Figure 11) are provided in Line A and then moved into elite crop plant germplasm by introgression. In the non-limiting Figure 11 illustration, introgression can be achieved by crossing a “Line A” comprising two or more of the modified or unmodified transgenic loci to the elite germplasm and then backcrossing progeny of the cross comprising the transgenic loci to the elite germplasm as the recurrent parent) to obtain a “Universal Donor” (*e.g.* Line A+ in Figure 11) comprising two or more of the modified or unmodified transgenic loci. This elite germplasm containing the modified or unmodified transgenic loci (*e.g.* “Universal Donor” of Figure 11) can then be subjected to genome editing molecules which can excise at least one of the modified or unmodified transgenic loci (“Event Removal” in Figure 11) and introduce other targeted genetic changes (“GE” in Figure 11) in the genomes of the elite crop plants containing one of the transgenic loci and a transgenic locus excision site corresponding to the removal site of one of the transgenic loci. Such selective excision of transgenic loci can be effected by contacting the genome of the plant comprising two transgenic loci with gene editing molecules (*e.g.*, RdDe and

gRNAs, TALENS, and/or ZFN) which recognize one transgenic loci but not another transgenic loci. Distinct plant lines with different subsets of transgenic loci and desired targeted genetic changes are thus recovered (*e.g.*, “Line B-1,” “Line B-2,” and “Line B-3” in Figure 11). In certain embodiments, it is also desirable to bulk up populations of inbred elite crop plants or their seed comprising the subset of transgenic loci and a transgenic locus excision site by selfing. Such inbred progeny of the selfed plants can be used either as is for commercial sales where the crop can be grown a varietal, non-hybrid crop (*e.g.*, commonly though not always in soybean, cotton, or canola) comprising the subset of desired transgenic loci and one or more transgenic loci excision sites. In certain embodiments, inbred progeny of the selfed plants can be used as a pollen donor or recipient for hybrid seed production (*e.g.*, most commonly in maize but also in cotton, soybean, and canola). Such hybrid seed and the progeny grown therefrom can comprise a subset of desired transgenic loci and a transgenic loci excision site.

[0085] Hybrid plant lines comprising elite crop plant germplasm, at least one transgenic locus and at least one transgenic locus excision site, and in certain aspects, additional targeted genetic changes are also provided herein. Methods for production of such hybrid seed can comprise crossing elite crop plant lines where at least one of the pollen donor or recipient comprises at least the transgenic locus and a transgenic locus excision site and/or additional targeted genetic changes. In certain embodiments, the pollen donor and recipient will comprise germplasm of distinct heterotic groups and provide hybrid seed and plants exhibiting heterosis. In certain embodiments, the pollen donor and recipient can each comprise a distinct transgenic locus which confers either a distinct trait (*e.g.*, herbicide tolerance or insect resistance), a different type of trait (*e.g.*, tolerance to distinct herbicides or to distinct insects such as coleopteran or lepidopteran insects), or a different mode-of-action for the same trait (*e.g.*, resistance to coleopteran insects by two distinct modes-of-action or resistance to lepidopteran insects by two distinct modes-of-action). In certain embodiments, the pollen recipient will be rendered male sterile or conditionally male sterile. Methods for inducing male sterility or conditional male sterility include emasculation (*e.g.*, detasseling), cytoplasmic male sterility, chemical hybridizing agents or systems, a transgenes or transgene systems, and/or mutation(s) in one or more endogenous plant genes. Descriptions of various male sterility systems that can be adapted for use with the elite crop plants provided herein are described in Wan et al. *Molecular Plant*; 12, 3, (2019):321-342 as well as in US 8,618,358; US 20130031674; and US 2003188347.

[0086] In certain embodiments, it will be desirable to use genome editing molecules to excise transgenic loci and/or make targeted genetic changes in elite crop plant or other germplasm. Techniques for effecting genome editing in crop plants (e.g., maize,) include use of morphogenic factors such as Wuschel (WUS), Ovule Development Protein (ODP), and/or Babyboom (BBM) which can improve the efficiency of recovering plants with desired genome edits. In some aspects, the morphogenic factor comprises WUS1, WUS2, WUS3, WOX2A, WOX4, WOX5, WOX9, BBM2, BMN2, BMN3, and/or ODP2. In certain embodiments, compositions and methods for using WUS, BBM, and/or ODP, as well as other techniques which can be adapted for effecting genome edits in elite crop plant and other germplasm, are set forth in US 20030082813, US 20080134353, US 20090328252, US 20100100981, US 20110165679, US 20140157453, US 20140173775, and US 20170240911, which are each incorporated by reference in their entireties. In certain embodiments, the genome edits can be effected in regenerable plant parts (e.g.; plant embryos) of elite crop plants by transient provision of gene editing molecules or polynucleotides encoding the same and do not necessarily require incorporating a selectable marker gene into the plant genome (e.g., US 20160208271 and US 20180273960, both incorporated herein by reference in their entireties; Svtashev et al. Nat Commun. 2016; 7:13274).

[0087] In certain embodiments, edited transgenic plant genomes, transgenic plant cells, parts, or plants containing those genomes, and DNA molecules obtained therefrom, can comprise a desired subset of transgenic loci and/or comprise at least one transgenic locus excision site. In a non-limiting and illustrative example where a segment comprising an original transgenic locus has been deleted, the transgenic locus excision site can comprise a contiguous segment of DNA comprising at least 10 base pairs of DNA that is telomere proximal to the deleted segment of the transgenic locus and at least 10 base pairs of DNA that is centromere proximal to the deleted segment of the transgenic locus wherein the transgenic DNA (*i.e.*, the heterologous DNA) that has been inserted into the crop plant genome has been deleted. In certain embodiments where a segment comprising a transgenic locus has been deleted, the transgenic locus excision site can comprise a contiguous segment of DNA comprising at least 10 base pairs DNA that is telomere proximal to the deleted segment of the transgenic locus and at least 10 base pairs of DNA that is centromere proximal DNA to the deleted segment of the transgenic locus wherein the heterologous transgenic DNA and at least 1, 2, 5, 10, 20, 50, or more base pairs of endogenous DNA located in a 5' junction sequence and/or in a 3' junction sequence of the original transgenic

locus that has been deleted. In such embodiments where DNA comprising the transgenic locus is deleted, a transgenic locus excision site can comprise at least 10 base pairs of DNA that is telomere proximal to the deleted segment of the transgenic locus and at least 10 base pairs of DNA that is centromere proximal to the deleted segment of the transgenic locus wherein all of the transgenic DNA is absent and either all or less than all of the endogenous DNA flanking the transgenic DNA sequences are present. In certain embodiments where a segment consisting essentially of an original transgenic locus has been deleted, the transgenic locus excision site can be a contiguous segment of at least 10 base pairs of DNA that is telomere proximal to the deleted segment of the transgenic locus and at least 10 base pairs of DNA that is centromere proximal to the deleted segment of the transgenic locus wherein less than all of the heterologous transgenic DNA that has been inserted into the crop plant genome is excised. In certain aforementioned embodiments where a segment consisting essentially of an original transgenic locus has been deleted, the transgenic locus excision site can thus contain at least 1 base pair of DNA or 1 to about 2 or 5, 8, 10, 20, or 50 base pairs of DNA comprising the telomere proximal and/or centromere proximal heterologous transgenic DNA that has been inserted into the crop plant genome. In certain embodiments where a segment consisting of an original transgenic locus has been deleted, the transgenic locus excision site can contain a contiguous segment of DNA comprising at least 10 base pairs of DNA that is telomere proximal to the deleted segment of the transgenic locus and at least 10 base pairs of DNA that is centromere proximal to the deleted segment of the transgenic locus wherein the heterologous transgenic DNA that has been inserted into the crop plant genome is deleted. In certain embodiments where DNA consisting of the transgenic locus is deleted, a transgenic locus excision site can comprise at least 10 base pairs of DNA that is telomere proximal to the deleted segment of the transgenic locus and at least 10 base pairs of DNA that is centromere proximal to the deleted segment of the transgenic locus wherein all of the heterologous transgenic DNA that has been inserted into the crop plant genome is deleted and all of the endogenous DNA flanking the heterologous sequences of the transgenic locus is present. In any of the aforementioned embodiments or in other embodiments, the continuous segment of DNA comprising the transgenic locus excision site can further comprise an insertion of 1 to about 2, 5, 10, 20, or more nucleotides between the DNA that is telomere proximal to the deleted segment of the transgenic locus and the DNA that is centromere proximal to the deleted segment of the transgenic locus. Such insertions can result either from endogenous DNA repair and/or recombination activities at the double stranded breaks introduced at the

excision site and/or from deliberate insertion of an oligonucleotide. Plants, edited plant genomes, biological samples, and DNA molecules (*e.g.*, including isolated or purified DNA molecules) comprising the transgenic loci excision sites are provided herein. Nucleic acid markers adapted for detecting the transgenic loci excision sites as well as methods for detecting the presence of DNA molecules comprising the transgenic loci excision sites are also provided herein.

[0088] In certain embodiments provided herein, a modified version of an approved transgenic locus which in its unmodified form (in certain embodiments, the “unmodified form” is the “original form,” “original transgenic locus,” etc.) comprises at least one selectable marker gene. In the modified version, at least one selectable marker has been deleted with genome editing molecules as described elsewhere herein from the unmodified approved transgenic locus. In certain embodiments, the deletion of the selectable marker gene does not affect any other functionality of the approved transgenic locus. In certain embodiments, the selectable marker gene that is deleted confers resistance to an antibiotic, tolerance to an herbicide, or an ability to grow on a specific carbon source, for example, mannose. In certain embodiments, the selectable marker gene comprises a DNA encoding a phosphinothricin acetyl transferase (PAT), a glyphosate tolerant 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS), a glyphosate oxidase (GOX), neomycin phosphotransferase (*npt*), a hygromycin phosphotransferase (*hyg*), an aminoglycoside adenyl transferase, or a phosphomannose isomerase (*pmi*). In certain embodiments, the modified locus does not contain a site-specific recombination system DNA recognition site, for example, in certain embodiments, the modified locus does not contain a *lox* or FRT site. In certain embodiments, the selectable marker gene to be deleted is flanked by operably linked protospacer adjacent motif (PAM) sites in the unmodified form of the approved transgenic locus. Thus, in certain embodiments of the modified locus, PAM sites flank the excision site of the deleted selectable marker gene. In certain embodiments, the PAM sites are recognized by an RNA dependent DNA endonuclease (RdDe); for example, a class 2 type II or class 2 type V RdDe. In certain embodiments, the deleted selectable marker gene is replaced in the modified approved transgenic locus by an introduced DNA sequence as discussed in further detail elsewhere herein. For example, in certain embodiments, the introduced DNA sequence comprises a trait expression cassette such as a trait expression cassette of another transgenic locus. In addition to the deletion of a selectable marker gene, in certain embodiments at least one copy of a repetitive sequence has also been deleted with genome editing molecules from an unmodified approved transgenic locus. In certain embodiments, deletion of the repetitive

sequence enhances the functionality of the modified approved transgenic locus. In certain embodiments, the approved transgenic locus which is modified is: (i) a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 transgenic locus in a transgenic maize plant genome; (ii) an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus in a transgenic soybean plant genome; (iii) a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus in a transgenic cotton plant genome; or (iv) a GT73, HCN28, MON88302, and/or MS8 transgenic locus in a transgenic canola plant genome. Also provided herein are plants comprising any of aforementioned modified transgenic loci.

[0089] In certain embodiments, edited transgenic plant genomes and transgenic plant cells, plant parts, or plants containing those edited genomes, comprising a modification of an original transgenic locus, where the modification comprises a deletion of a segment of the original transgenic locus are provided. In certain embodiments, the modification comprises two or more separate deletions and/or there is a modification in two or more original transgenic plant loci. In certain embodiments, the deleted segment comprises, consists essentially of, or consists of a segment of non-essential DNA in the transgenic locus. Illustrative examples of non-essential DNA include but are not limited to synthetic cloning site sequences, duplications of transgene sequences; fragments of transgene sequences, and *Agrobacterium* right and/or left border sequences. In certain embodiments, the non-essential DNA is a duplication and/or fragment of a promoter sequence and/or is not the promoter sequence operably linked in the cassette to drive expression of a transgene. In certain embodiments, excision of the non-essential DNA improves a characteristic, functionality, and/or expression of a transgene of the transgenic locus or otherwise confers a recognized improvement in a transgenic plant comprising the edited transgenic plant genome. In certain embodiments, the non-essential DNA does not comprise DNA encoding a selectable marker gene. In certain embodiments of an edited transgenic plant genome, the modification comprises a deletion of the non-essential DNA and a deletion of a selectable marker gene. The modification producing the edited transgenic plant genome could occur by excising both the non-essential DNA and the selectable marker gene at the same time, e.g., in the same modification step, or the modification could occur step-wise. For example, an edited transgenic

plant genome in which a selectable marker gene has previously been removed from the transgenic locus can comprise an original transgenic locus from which a non-essential DNA is further excised and vice versa. In certain embodiments, the modification comprising deletion of the non-essential DNA and deletion of the selectable marker gene comprises excising a single segment of the original transgenic locus that comprises both the non-essential DNA and the selectable marker gene. Such modification would result in one excision site in the edited transgenic genome corresponding to the deletion of both the non-essential DNA and the selectable marker gene. In certain embodiments, the modification comprising deletion of the non-essential DNA and deletion of the selectable marker gene comprises excising two or more segments of the original transgenic locus to achieve deletion of both the non-essential DNA and the selectable marker gene. Such modification would result in at least two excision sites in the edited transgenic genome corresponding to the deletion of both the non-essential DNA and the selectable marker gene. In certain embodiments of an edited transgenic plant genome, prior to excision, the segment to be deleted is flanked by operably linked protospacer adjacent motif (PAM) sites in the original or unmodified transgenic locus and/or the segment to be deleted encompasses an operably linked PAM site in the original or unmodified transgenic locus. In certain embodiments, following excision of the segment, the resulting edited transgenic plant genome comprises PAM sites flanking the deletion site in the modified transgenic locus. In certain embodiments of an edited transgenic plant genome, the modification comprises a modification of a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 original transgenic locus in a transgenic corn plant genome. In certain embodiments of an edited transgenic plant genome, the modification comprises a modification of an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 original transgenic locus in a transgenic soybean plant genome. In certain embodiments of an edited transgenic plant genome, the modification comprises a modification of a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 original transgenic locus in a transgenic cotton plant genome. In certain embodiments of an edited transgenic plant genome, the modification comprises a modification of

an GT73, HCN28, MON88302, and/or MS8 original transgenic locus in a transgenic canola plant genome.

[0090] Methods and reagents (*e.g.*, nucleic acid markers including nucleic acid probes and/or primers) for detecting plants, edited plant genomes, and biological samples containing DNA molecules comprising the transgenic loci excision sites and/or non-essential DNA deletions are also provided herein. Detection of the DNA molecules can be achieved by any combination of nucleic acid amplification (*e.g.*, PCR amplification), hybridization, sequencing, and/or mass-spectrometry based techniques. Methods set forth for detecting junction nucleic acids in unmodified transgenic loci set forth in US 20190136331 and US 9,738,904, both incorporated herein by reference in their entireties, can be adapted for use in detection of the nucleic acids provided herein. In certain embodiments, such detection is achieved by amplification and/or hybridization-based detection methods using a method (*e.g.*, selective amplification primers) and/or probe (*e.g.*, capable of selective hybridization or generation of a specific primer extension product) which specifically recognizes the target DNA molecule (*e.g.*, transgenic locus excision site) but does not recognize DNA from an unmodified transgenic locus. In certain embodiments, the hybridization probes can comprise detectable labels (*e.g.*, fluorescent, radioactive, epitope, and chemiluminescent labels). In certain embodiments, a single nucleotide polymorphism detection assay can be adapted for detection of the target DNA molecule (*e.g.*, transgenic locus excision site).

[0091] Excision of transgenic plant loci and production of transgenic loci excision sites can be obtained by using suitable gene editing molecules which can introduce blunt or staggered double stranded DNA breaks in 5' and 3' junction polynucleotides of transgenic loci. Such blunt or staggered dsDNA breaks can be introduced in non-transgenic plant genomic DNA of the junction polynucleotide, in the inserted transgenic DNA of the junction polynucleotide, or can span the junction comprising both non-transgenic plant genomic DNA and inserted transgenic DNA of the junction polynucleotide. In certain embodiments, the gene editing molecules can comprise zinc finger nucleases, zinc finger nickases, TALENs, and/or TALE nickases which introduce double stranded breaks in junction polynucleotides. In certain embodiments, the gene editing molecules comprise RdDe and guide RNAs directed to DNA targets in the junction polynucleotides comprising pre-existing PAM sites which are operably linked to the DNA junction polynucleotides of the transgenic locus in the transgenic plant genome. Such PAM sites can be recognized by RdDe and suitable guide RNAs directed to DNA sequences adjacent to the

PAM to provide for cleavage within or near the two junction polynucleotides. In certain embodiments, the PAMs are recognized by the same class and/or type of RdDe (*e.g.*, Class 2 type II or Class 2 type V) or by the same RdDe (*e.g.*, both PAMs recognized by the same Cas9 or Cas 12 RdDe). Guide RNAs can be directed to the junction polynucleotides by using a pre-existing PAM site located within or adjacent to a junction polynucleotide of the transgenic locus. Non-limiting examples of such pre-existing PAM sites present in junction polynucleotides which can be used by suitable guide RNAs to direct RdDe or RNA dependent nickases in a 5' or 3' junction polynucleotide are set forth in Table 5 of the Examples.

[0092] In certain embodiments, edited transgenic plant genomes provided herein can lack one or more selectable and/or scoreable markers found in an original event (transgenic locus). Original transgenic loci (events), including those set forth in Tables 1-4 (*e.g.*, SEQ ID NO: 1-34), the patent references set forth therein and incorporated herein by reference in their entireties, and depicted in the drawings, can contain selectable transgenes markers conferring herbicide tolerance, antibiotic resistance, or an ability to grow on a carbon source. Selectable marker transgenes which can confer herbicide tolerance include genes encoding a phosphinothricin acetyl transferase (PAT), a glyphosate tolerant 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS), an a glyphosate oxidase (GOX). Selectable marker transgenes which can confer antibiotic resistance include genes encoding a neomycin phosphotransferase (npt), a hygromycin phosphotransferase, an aminoglycoside adenyl transferase. Transgenes encoding a phosphomannose isomerase (pmi) can confer the ability to grow on mannose. Original transgenic loci (events), including those set forth in Tables 1-4, can contain scoreable transgenic markers which can be detected by enzymatic, histochemical, or other assays. Scoreable markers can include genes encoding beta-glucuronidase (uid) or fluorescent proteins (*e.g.*, a GFP, RFP, or YFP). Such selectable or scoreable marker transgenes can be excised from an original transgenic locus by contacting the transgenic locus with one or more gene editing molecules which introduce double stranded breaks in the transgenic locus at the 5' and 3' end of the expression cassette comprising the selectable marker transgene (*e.g.*, an RdDe and guide RNAs directed to PAM sites located at the 5' and 3' end of the expression cassette comprising the selectable marker transgenes) and selecting for plant cells, plant parts, or plants wherein the selectable or scoreable marker has been excised. In certain embodiments, the selectable or scoreable marker transgene can be inactivated. Inactivation can be achieved by modifications including insertion, deletion, and/or substitution of one or more nucleotides in a promoter element, 5' or 3'

untranslated region (UTRs), intron, coding region, and/or 3' terminator and/or polyadenylation signal of the selectable marker transgene. Such modifications can inactivate the selectable or scoreable marker transgene by eliminating or reducing promoter activity, introducing a missense mutation, and/or introducing a pre-mature stop codon. In certain embodiments, the selectable and/or scoreable marker transgene can be replaced by an introduced transgene. In certain embodiments, an original transgenic locus that was contacted with gene editing molecules which introduce double stranded breaks in the transgenic locus at the 5' and 3' end of the expression cassette comprising the selectable marker and/or scoreable transgene can also be contacted with a suitable donor DNA template comprising an expression cassette flanked by DNA homologous to remaining DNA in the transgenic locus located 5' and 3' to the selectable marker excision site. In certain embodiments, a coding region of the selectable and/or scoreable marker transgene can be replaced with another coding region such that the replacement coding region is operably linked to the promoter and 3' terminator or polyadenylation signal of the selectable and/or scoreable marker transgene.

[0093] In certain embodiments, edited transgenic plant genomes provided herein can comprise additional new introduced transgenes (*e.g.*, expression cassettes) inserted into the transgenic locus of a given event. Introduced transgenes inserted at the transgenic locus of an event subsequent to the event's original isolation can be obtained by inducing a double stranded break at a site within an original transgenic locus (*e.g.*, with genome editing molecules including an RdDe and suitable guide RNA(s); a suitable engineered zinc-finger nuclease; a TALEN protein and the like) and providing an exogenous transgene in a donor DNA template which can be integrated at the site of the double stranded break (*e.g.* by homology-directed repair (HDR) or by non-homologous end-joining (NHEJ). In certain embodiments, introduced transgenes can be integrated in a 5' junction polynucleotide or a 3' junction polynucleotide using a suitable RdDe, guide RNA, and either a pre-existing PAM site. In other embodiments, pre-existing PAM sites located in both the a 5' junction polynucleotide or a 3' junction polynucleotide can be used to delete the transgenic locus and replace it with one or more new expression cassettes. In certain embodiments, such deletions and replacements are effected by introducing dsDNA breaks in both junction polynucleotides and providing the new expression cassettes on a donor DNA template or other DNA template suitable for integration by NHEJ or MMEJ (microhomology mediated end joining). Suitable expression cassettes for insertion include DNA molecules comprising promoters which are operably linked to DNA encoding proteins and/or RNA molecules which

confer useful traits which are in turn operably linked to polyadenylation signal or terminator elements. In certain embodiments, such expression cassettes can also comprise 5' UTRs, 3' UTRs, and/or introns. Useful traits include biotic stress tolerance (*e.g.*, insect resistance, nematode resistance, or disease resistance), abiotic stress tolerance (*e.g.*, heat, cold, drought, and/or salt tolerance), herbicide tolerance, and quality traits (*e.g.*, improved fatty acid compositions, protein content, starch content, and the like). Suitable expression cassettes for insertion include expression cassettes contained in any of the events (transgenic loci) listed in Tables 1-4 (*e.g.*, SEQ ID NO: 1-34), the patent references set forth therein which are incorporated in their entirety, or set forth in the drawings which confer insect resistance, herbicide tolerance, biofuel use, male sterility, or other useful traits.

[0094] In certain embodiments, plants provided herein, including plants with one or more transgenic loci and/or comprising transgenic loci excision sites can further comprise one or more targeted genetic changes introduced by one or more of gene editing molecules or systems. Also provided are methods where the targeted genetic changes and one or more transgenic loci excision sites are removed from plants either in series or in parallel (*e.g.*, as set forth in the non-limiting illustration in Figure 11, bottom "Alternative" panel, where "GE" can represent targeted genetic changes induced by gene editing molecules and "Event Removal" represents excision of one or more transgenic loci with gene editing molecules). Such targeted genetic changes include those conferring traits such as improved yield, improved food and/or feed characteristics (*e.g.*, improved oil, starch, protein, or amino acid quality or quantity), improved nitrogen use efficiency, improved biofuel use characteristics (*e.g.*, improved ethanol production), male sterility/conditional male sterility systems (*e.g.*, by targeting endogenous MS26, MS45 and MSCA1 genes), herbicide tolerance (*e.g.*, by targeting endogenous ALS, EPSPS, HPPD, or other herbicide target genes), delayed flowering, non-flowering, increased biotic stress resistance (*e.g.*, resistance to insect, nematode, bacterial, or fungal damage), increased abiotic stress resistance (*e.g.*, resistance to drought, cold, heat, metal, or salt), enhanced lodging resistance, enhanced growth rate, enhanced biomass, enhanced tillering, enhanced branching, delayed flowering time, delayed senescence, increased flower number, improved architecture for high density planting, improved photosynthesis, increased root mass, increased cell number, improved seedling vigor, improved seedling size, increased rate of cell division, improved metabolic efficiency, and increased meristem size in comparison to a control plant lacking the targeted genetic change. Types of targeted genetic changes that can be introduced include insertions, deletions, and

substitutions of one or more nucleotides in the crop plant genome. Sites in endogenous plant genes for the targeted genetic changes include promoter, coding, and non-coding regions (*e.g.*, 5' UTRs, introns, splice donor and acceptor sites and 3' UTRs). In certain embodiments, the targeted genetic change comprises an insertion of a regulatory or other DNA sequence in an endogenous plant gene. Non-limiting examples of regulatory sequences which can be inserted into endogenous plant genes with gene editing molecules to effect targeted genetic changes which confer useful phenotypes include those set forth in US Patent Application Publication 20190352655, which is incorporated herein by example, such as: (a) auxin response element (AuxRE) sequence; (b) at least one D1-4 sequence (Ulmasov et al. (1997) *Plant Cell*, 9:1963-1971), (c) at least one DR5 sequence (Ulmasov et al. (1997) *Plant Cell*, 9:1963-1971); (d) at least one m5-DR5 sequence (Ulmasov et al. (1997) *Plant Cell*, 9:1963-1971); (e) at least one P3 sequence; (f) a small RNA recognition site sequence bound by a corresponding small RNA (*e.g.*, an siRNA, a microRNA (miRNA), a trans-acting siRNA as described in U.S. Patent No. 8,030,473, or a phased sRNA as described in U.S. Patent No. 8,404,928; both of these cited patents are incorporated by reference herein); (g) a microRNA (miRNA) recognition site sequence; (h) the sequence recognizable by a specific binding agent includes a microRNA (miRNA) recognition sequence for an engineered miRNA wherein the specific binding agent is the corresponding engineered mature miRNA; (i) a transposon recognition sequence; (j) a sequence recognized by an ethylene-responsive element binding-factor-associated amphiphilic repression (EAR) motif; (k) a splice site sequence (*e.g.*, a donor site, a branching site, or an acceptor site; see, for example, the splice sites and splicing signals set forth in the internet site [lemur\[dot\]amu\[dot\]edu\[dot\]pl/share/ERISdb/home.html](http://lemur[dot]amu[dot]edu[dot]pl/share/ERISdb/home.html)); (l) a recombinase recognition site sequence that is recognized by a site-specific recombinase; (m) a sequence encoding an RNA or amino acid aptamer or an RNA riboswitch, the specific binding agent is the corresponding ligand, and the change in expression is upregulation or downregulation; (n) a hormone responsive element recognized by a nuclear receptor or a hormone-binding domain thereof; (o) a transcription factor binding sequence; and (p) a polycomb response element (see Xiao et al. (2017) *Nature Genetics*, 49:1546-1552, doi: 10.1038/ng.3937). Non limiting examples of target maize genes that can be subjected to targeted gene edits to confer useful traits include: (a) ZmIPK1 (herbicide tolerant and phytate reduced maize; Shukla et al., *Nature*. 2009;459:437–41); (b) ZmGL2 (reduced epicuticular wax in leaves; Char et al. *Plant Biotechnol J*. 2015;13:1002); (c) ZmMTL (induction of haploid plants; Kelliher et al. *Nature*. 2017;542:105); (d) Wx1 (high

amylopectin content; US 20190032070; incorporated herein by reference in its entirety); (e) TMS5 (thermosensitive male sterile; Li et al. J Genet Genomics. 2017;44:465–8); (f) ALS (herbicide tolerance; Svitashv et al.; Plant Physiol. 2015;169:931–45); and (g) ARGOS8 (drought stress tolerance; Shi et al., Plant Biotechnol J. 2017;15:207–16). Non-limiting examples of target soybean genes that can be subjected to targeted gene edits to confer useful traits include: (a) FAD2-1A, FAD2-1B (increased oleic acid content; Haun et al.; Plant Biotechnol J. 2014;12:934–40); (b) FAD2-1A, FAD2-1B, FAD3A (increased oleic acid and decreased linolenic content; Demorest et al., BMC Plant Biol. 2016;16:225); and (c) ALS (herbicide tolerance; Svitashv et al.; Plant Physiol. 2015;169:931–45). A non-limiting examples of target *Brassica* genes that can be subjected to targeted gene edits to confer useful traits include: (a) the FRIGIDA gene to confer early flowering (Sun Z, et al.. J Integr Plant Biol. 2013;55:1092–103); and (b) ALS (herbicide tolerance; US 20160138040, incorporated herein by reference in its entirety). Non-limiting examples of target genes in crop plants including corn and soybean which can be subjected to targeted genetic changes which confer useful phenotypes include those set forth in US Patent Application Nos. 20190352655, 20200199609, 20200157554, and 20200231982, which are each incorporated herein in their entireties; and Zhang et al. (Genome Biol. 2018; 19: 210).

[0095] Gene editing molecules of use in methods provided herein include molecules capable of introducing a double-strand break (“DSB”) or single-strand break (“SSB”) 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 DNA template polynucleotides. Examples of such gene editing molecules include: (a) a nuclease comprising an RNA-guided nuclease, an RNA-guided DNA endonuclease or RNA directed DNA endonuclease (RdDe), a class 1 CRISPR type nuclease system, a type II Cas nuclease, a Cas9, a nCas9 nickase, a type V Cas nuclease, a Cas12a nuclease, a nCas12a nickase, a Cas12d (CasY), a Cas12e (CasX), a Cas12b (C2c1), a Cas12c (C2c3), a Cas12i, a Cas12j, a 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 TALEN) or nickase (TALE-nickase), an Argonaute, and a meganuclease or engineered meganuclease; (b) a polynucleotide encoding one or more nucleases capable of effectuating site-specific alteration (including introduction of a DSB or SSB) of a target nucleotide sequence; (c) a guide RNA (gRNA) for an RNA-guided nuclease, or a DNA encoding a gRNA for an RNA-guided nuclease; (d) donor DNA template polynucleotides; and (e) other DNA templates (dsDNA, ssDNA, or combinations thereof) suitable for insertion at a

break in genomic DNA (*e.g.*, by non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ).

[0096] CRISPR-type genome editing can be adapted for use in the plant cells and methods provided herein in several ways. CRISPR elements, *e.g.*, gene editing molecules comprising CRISPR endonucleases and CRISPR guide RNAs including single guide RNAs or guide RNAs in combination with tracrRNAs or scoutRNA, 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 methods 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 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. Examples of PAM sequences include 5'-NGG (*Streptococcus pyogenes*), 5'-NNAGAA (*Streptococcus thermophilus* CRISPR1), 5'-NGGNG (*Streptococcus thermophilus* CRISPR3), 5'-NNGRRT or 5'-NNGRR (*Staphylococcus aureus* Cas9, SaCas9), and 5'-NNNGATT (*Neisseria meningitidis*). T-rich PAM sites (*e.g.*, 5'-TTN or 5'-TTTV, where "V" is A, C, or G) are typically targeted for design of crRNAs or sgRNAs used with Cas12a proteins. In some instances, Cas12a can also recognize a 5'-CTA PAM motif. Other examples of potential Cas12a PAM sequences include TTN, CTN, TCN, CCN, TTTN, TCTN, TTCN, CTTN, ATTN, TCCN, TTGN, GTTN, CCCN, CCTN, TTAN, TCGN, CTCN, ACTN, GCTN, TCAN, GCCN, and CCGN (wherein N is defined as any nucleotide). 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 site editing.

[0097] 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; Harrington et al. (2020) Molecular Cell doi:10.1016/j.molcel.2020.06.022) and CasX and CasY (see Burstein et al. (2016) Nature, doi:10.1038/nature21059; Harrington et al. (2020) Molecular Cell doi:10.1016/j.molcel.2020.06.022), or Cas12j (Pausch et al, (2020) Science 10.1126/science.abb1400). 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 site is used. Blunt-end cutting RNA-guided endonucleases include Cas9, Cas12c, and Cas 12h (Yan et al., 2019). In certain embodiments, an RNA-guided endonuclease that leaves a staggered single stranded DNA overhanging end following cleavage of the target site following cleavage of the target site is used. Staggered-end cutting RNA-guided endonucleases include Cas12a, Cas12b, and Cas12e.

[0098] The methods can also use sequence-specific endonucleases or sequence-specific endonucleases and guide RNAs that cleave a single DNA strand in a dsDNA target site. Such cleavage of a single DNA strand in a dsDNA target 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 be 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). 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.

[0099] Genomic DNA may also be modified via base editing. Both adenine base editors (ABE) which convert A/T base pairs to G/C base pairs in genomic DNA as well as cytosine base pair editors (CBE) which effect C to T substitutions can be used in certain embodiments of the methods provided herein. In certain embodiments, useful ABE and CBE can comprise genome site specific DNA binding elements (*e.g.*, RNA-dependent DNA binding proteins including catalytically inactive Cas9 and Cas12 proteins or Cas9 and Cas12 nickases) operably linked to adenine or cytidine deaminases and used with guide RNAs which position the protein near the nucleotide targeted for substitution. Suitable ABE and CBE disclosed in the literature (Kim, *Nat Plants*, 2018 Mar;4(3):148-151) can be adapted for use in the methods set forth herein. In certain embodiments, a CBE can comprise a fusion between a catalytically inactive Cas9 (dCas9) RNA dependent DNA binding protein fused to a cytidine deaminase which converts cytosine (C) to uridine (U) and selected guide RNAs, thereby effecting a C to T substitution; see Komor *et al.* (2016) *Nature*, 533:420 – 424. In other embodiments, C to T substitutions are effected with Cas9 nickase [Cas9n(D10A)] fused to an improved cytidine deaminase and optionally a bacteriophage Mu dsDNA (double-stranded DNA) end-binding protein Gam; see Komor *et al.*, *Sci Adv.* 2017 Aug; 3(8):eaao4774. In other embodiments, adenine base editors (ABEs) comprising an adenine deaminase fused to catalytically inactive Cas9 (dCas9) or a Cas9 D10A nickase can be used to convert A/T base pairs to G/C base pairs in genomic DNA (Gaudelli *et al.*, (2017) *Nature* 551(7681):464-471.

[00100] In certain embodiments, zinc finger nucleases or zinc finger nickases can also be used in the methods provided herein. Zinc-finger nucleases are site-specific endonucleases

comprising two protein domains: a DNA-binding domain, comprising a plurality of individual zinc finger repeats that each recognize between 9 and 18 base pairs, and a DNA-cleavage domain that comprises a nuclease domain (typically FokI). The cleavage domain dimerizes in order to cleave DNA; therefore, a pair of ZFNs are required to target non-palindromic target polynucleotides. In certain embodiments, zinc finger nuclease and zinc finger nickase design methods which have been described (Urnov *et al.* (2010) *Nature Rev. Genet.*, 11:636 – 646; Mohanta *et al.* (2017) *Genes* vol. 8,12: 399; Ramirez *et al.* *Nucleic Acids Res.* (2012); 40(12): 5560–5568; Liu *et al.* (2013) *Nature Communications*, 4: 2565) can be adapted for use in the methods set forth herein. The zinc finger binding domains of the zinc finger nuclease or nickase provide specificity and can be engineered to specifically recognize any desired target DNA sequence. 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) can be adapted for use in the methods described herein. 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 and can be adapted for use in the methods described herein; see, *e.g.*, Guo *et al.* (2010) *J. Mol. Biol.*, 400:96 - 107.

[00101] 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 genome editing proteins, referred to as TAL-effector nucleases or TALENs. As in the case of ZFNs, a restriction endonuclease, such as FokI, can be conveniently used. Methods for use of TALENs in plants have been described and can be adapted for use in the methods described herein, see Mahfouz *et al.* (2011) *Proc. Natl. Acad. Sci. USA*, 108:2623 – 2628; Mahfouz (2011) *GM Crops*, 2:99 – 103; and Mohanta *et al.* (2017) *Genes* vol. 8,12: 399). TALE nickases have also been described and can be adapted for use in methods described herein (Wu *et al.*; *Biochem Biophys Res Commun.* (2014);446(1):261-6; Luo *et al.*; *Scientific Reports* 6, Article number: 20657 (2016)).

[00102] Embodiments of the donor DNA template molecule having a sequence that is integrated at the site of at least one double-strand break (DSB) in a genome include double-stranded DNA, a single-stranded DNA, a single-stranded DNA/RNA hybrid, and a double-stranded DNA/RNA hybrid. In embodiments, a donor DNA template molecule that is a double-

stranded (e. g., a dsDNA or dsDNA/RNA hybrid) molecule is provided directly to the plant protoplast or plant cell in the form of a double-stranded DNA or a double-stranded DNA/RNA hybrid, or as two single-stranded DNA (ssDNA) molecules that are capable of hybridizing to form dsDNA, or as a single-stranded DNA molecule and a single-stranded RNA (ssRNA) molecule that are capable of hybridizing to form a double-stranded DNA/RNA hybrid; that is to say, the double-stranded polynucleotide molecule is not provided indirectly, for example, by expression in the cell of a dsDNA encoded by a plasmid or other vector. In various non-limiting embodiments of the method, the donor DNA template molecule that is integrated (or that has a sequence that is integrated) at the site of at least one double-strand break (DSB) in a genome is double-stranded and blunt-ended; in other embodiments the donor DNA template molecule is double-stranded and has an overhang or "sticky end" consisting of unpaired nucleotides (e. g., 1, 2, 3, 4, 5, or 6 unpaired nucleotides) at one terminus or both termini. In an embodiment, the DSB in the genome has no unpaired nucleotides at the cleavage site, and the donor DNA template molecule that is integrated (or that has a sequence that is integrated) at the site of the DSB is a blunt-ended double-stranded DNA or blunt-ended double-stranded DNA/RNA hybrid molecule, or alternatively is a single-stranded DNA or a single-stranded DNA/RNA hybrid molecule. In another embodiment, the DSB in the genome has one or more unpaired nucleotides at one or both sides of the cleavage site, and the donor DNA template molecule that is integrated (or that has a sequence that is integrated) at the site of the DSB is a double-stranded DNA or double-stranded DNA/RNA hybrid molecule with an overhang or "sticky end" consisting of unpaired nucleotides at one or both termini, or alternatively is a single-stranded DNA or a single-stranded DNA/RNA hybrid molecule; in embodiments, the donor DNA template molecule DSB is a double-stranded DNA or double-stranded DNA/RNA hybrid molecule that includes an overhang at one or at both termini, wherein the overhang consists of the same number of unpaired nucleotides as the number of unpaired nucleotides created at the site of a DSB by a nuclease that cuts in an off-set fashion (e.g., where a Cas12 nuclease effects an off-set DSB with 5-nucleotide overhangs in the genomic sequence, the donor DNA template molecule that is to be integrated (or that has a sequence that is to be integrated) at the site of the DSB is double-stranded and has 5 unpaired nucleotides at one or both termini). In certain embodiments, one or both termini of the donor DNA template molecule contain no regions of sequence homology (identity or complementarity) to genomic regions flanking the DSB; that is to say, one or both termini of the donor DNA template molecule contain no regions of sequence that is sufficiently complementary to permit

hybridization to genomic regions immediately adjacent to the location of the DSB. In embodiments, the donor DNA template molecule contains no homology to the locus of the DSB, that is to say, the donor DNA template molecule contains no nucleotide sequence that is sufficiently complementary to permit hybridization to genomic regions immediately adjacent to the location of the DSB. In embodiments, the donor DNA template molecule is at least partially double-stranded and includes 2-20 base-pairs, e. g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 base-pairs; in embodiments, the donor DNA template molecule is double-stranded and blunt-ended and consists of 2-20 base-pairs, e. g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 base-pairs; in other embodiments, the donor DNA template molecule is double-stranded and includes 2-20 base-pairs, e. g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 base-pairs and in addition has at least one overhang or "sticky end" consisting of at least one additional, unpaired nucleotide at one or at both termini. In an embodiment, the donor DNA template molecule that is integrated (or that has a sequence that is integrated) at the site of at least one double-strand break (DSB) in a genome is a blunt-ended double-stranded DNA or a blunt-ended double-stranded DNA/RNA hybrid molecule of about 18 to about 300 base-pairs, or about 20 to about 200 base-pairs, or about 30 to about 100 base-pairs, and having at least one phosphorothioate bond between adjacent nucleotides at a 5' end, 3' end, or both 5' and 3' ends. In embodiments, the donor DNA template molecule includes single strands of at least 11, at least 18, at least 20, at least 30, at least 40, at least 60, at least 80, at least 100, at least 120, at least 140, at least 160, at least 180, at least 200, at least 240, at about 280, or at least 320 nucleotides. In embodiments, the donor DNA template molecule has a length of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least 11 base-pairs if double-stranded (or nucleotides if single-stranded), or between about 2 to about 320 base-pairs if double-stranded (or nucleotides if single-stranded), or between about 2 to about 500 base-pairs if double-stranded (or nucleotides if single-stranded), or between about 5 to about 500 base-pairs if double-stranded (or nucleotides if single-stranded), or between about 5 to about 300 base-pairs if double-stranded (or nucleotides if single-stranded), or between about 11 to about 300 base-pairs if double-stranded (or nucleotides if single-stranded), or about 18 to about 300 base-pairs if double-stranded (or nucleotides if single-stranded), or between about 30 to about 100 base-pairs if double-stranded (or nucleotides if single-stranded). In embodiments, the donor DNA template molecule includes chemically modified nucleotides (see, *e.g.*, the various modifications of internucleotide linkages, bases, and sugars described in Verma and Eckstein

(1998) *Annu. Rev. Biochem.*, 67:99-134); in embodiments, the naturally occurring phosphodiester backbone of the donor DNA template molecule is partially or completely modified with phosphorothioate, phosphorodithioate, or methylphosphonate internucleotide linkage modifications, or the donor DNA template molecule includes modified nucleoside bases or modified sugars, or the donor DNA template molecule is labelled with a fluorescent moiety (*e.g.*, fluorescein or rhodamine or a fluorescent nucleoside analogue) or other detectable label (*e.g.*, biotin or an isotope). In another embodiment, the donor DNA template molecule contains secondary structure that provides stability or acts as an aptamer. Other related embodiments include double-stranded DNA/RNA hybrid molecules, single-stranded DNA/RNA hybrid donor molecules, and single-stranded DNA donor molecules (including single-stranded, chemically modified DNA donor molecules), which in analogous procedures are integrated (or have a sequence that is integrated) at the site of a double-strand break.

[00103] Donor DNA template molecules used in the methods 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 a target site-specific endonuclease cleavage site in the gDNA. In certain embodiments, the replacement DNA can comprise an insertion, deletion, or substitution of 1 or more DNA base pairs relative to the target gDNA. In an embodiment, the donor DNA template molecule is double-stranded and perfectly base-paired through all or most of its length, with the possible exception of any unpaired nucleotides at either terminus or both termini. In another embodiment, the donor DNA template molecule is double-stranded and includes one or more non-terminal mismatches or non-terminal unpaired nucleotides within the otherwise double-stranded duplex. In an embodiment, the donor DNA template molecule that is integrated at the site of at least one double-strand break (DSB) includes between 2-20 nucleotides in one (if single-stranded) or in both strands (if double-stranded), *e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides on one or on both strands, each of which can be base-paired to a nucleotide on the opposite strand (in the case of a perfectly base-paired double-stranded polynucleotide molecule). Such donor DNA templates can be integrated in genomic DNA containing blunt and/or staggered double stranded DNA breaks by homology-directed repair (HDR). 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 DNA template molecule can be delivered to 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 DNA template molecule comprising, from 5' to 3', a first copy of the target sequence-specific endonuclease cleavage site sequence, the first homology arm, the replacement DNA, the second homology arm, and a second copy of the target sequence-specific endonuclease cleavage site sequence. Without seeking to be limited by theory, such modified donor DNA template molecules can be cleaved by the same sequence-specific endonuclease that is used to cleave the target site gDNA of the eukaryotic cell to release a donor DNA template molecule that can participate in HDR-mediated genome modification of the target editing site in the plant cell genome. In certain embodiments, the donor DNA template can comprise a linear DNA molecule comprising, from 5' to 3', a cleaved target sequence-specific endonuclease cleavage site sequence, the first homology arm, the replacement DNA, the second homology arm, and a cleaved target sequence-specific endonuclease cleavage site sequence. In certain embodiments, the cleaved target sequence-specific 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 target sequence-specific endonuclease sequence comprises a DNA end having a single-stranded 5' or 3' DNA overhang. Such cleaved target sequence-specific endonuclease cleavage site sequences can be produced by either cleaving an intact target sequence-specific endonuclease cleavage site sequence or by synthesizing a copy of the cleaved target sequence-specific endonuclease cleavage site sequence. Donor DNA templates can be synthesized either chemically or enzymatically (*e.g.*, in a polymerase chain reaction (PCR)).

[00104] Various treatments are useful in delivery of gene editing molecules and/or other molecules to a plant cell. In certain embodiments, one or more treatments is employed to deliver the gene editing or other molecules (*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 molecules are delivered directly, for example by direct contact of the composition with a plant 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 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 genome editing molecule-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 genome editing molecules (*e.g.*, RNA dependent DNA endonuclease, RNA dependent DNA binding protein, RNA dependent nickase, ABE, or CBE, 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.

[00105] In some embodiments, one or more polynucleotides or vectors driving expression of one or more genome editing molecules or trait-conferring genes (*e.g.*; herbicide tolerance, insect resistance, and/or male sterility) are introduced into a plant cell. In certain embodiments, a polynucleotide vector comprises a regulatory element such as a promoter operably linked to one

or more polynucleotides encoding genome editing molecules and/or trait-conferring genes. 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 RNA polymerase III promoter is a plant U6 spliceosomal RNA promoter, which can be native to the genome of the plant cell or from a different species, *e.g.*, a U6 promoter from maize, tomato, or soybean such as those disclosed US Patent Application Publication 2017/0166912, or a homologue thereof; in an example, such a promoter is operably linked to DNA sequence encoding a first RNA molecule including a Cas12a gRNA followed by an operably linked and suitable 3' element such as a U6 poly-T terminator. In another embodiment, the RNA polymerase III promoter is a plant U3, 7SL (signal recognition particle RNA), U2, or U5 promoter, or chimerics thereof, *e.g.*, as described in US Patent Application Publication 20170166912. 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.

[00106] 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 "terminator" 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 US Patent 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. All of the patent publications referenced in this paragraph are incorporated herein by reference in their entirety..

[00107] 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](http://www.openwetware.org/images/d/d3/Haploid_Arabidopsis_protocol.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 or for producing hybrid plants that are offspring of at least one doubled-haploid plant.

[00108] In certain embodiments, the plant cells used in the methods provided herein can include non-dividing cells. Such non-dividing cells can include plant cell protoplasts, plant cells subjected to one or more of a genetic and/or pharmaceutically-induced cell-cycle blockage, and the like.

[00109] In certain embodiments, the plant cells in used in the methods 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.

[00110] 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 RdDe), 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 or homogeneous populations of such plants or parts thereof (*e.g.*, seeds), 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. 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. In other embodiments, processed products are made from the plant or its seeds, including: (a) maize, soy, cotton, or canola seed meal (defatted or non-defatted); (b) extracted proteins, oils, sugars, and starches; (c) fermentation products; (d) animal feed or human food products (*e.g.*, feed and food comprising maize, soy, cotton, or canola seed meal (defatted or non-defatted) and other ingredients (*e.g.*, other cereal grains, other seed meal, other protein meal, other oil, other starch, other sugar, a binder, a preservative, a humectant, a vitamin, and/or mineral; (e) a pharmaceutical; (f) raw or processed biomass (*e.g.*, cellulosic and/or lignocellulosic material); and (g) various industrial products.

Embodiments

[00111] Various embodiments of the plants, genomes, methods, biological samples, and other compositions described herein are set forth in the following sets of numbered embodiments.

[00112] Embodiment List One

[00113] 1. A method of obtaining a transgenic plant cell containing an edited transgenic plant genome comprising: (a) contacting a transgenic plant genome comprising a first transgenic locus and a second transgenic locus with: (i) at least a first and at least a second guide RNA directed to genomic DNA adjacent to two PAM sites, wherein the PAM sites are operably linked to a 5' and a 3' DNA junction polynucleotide of the first transgenic locus; and (ii) one or more RNA dependent DNA endonucleases (RdDe) which recognize the PAM sites; wherein the unedited transgenic plant genome is in a transgenic plant cell; and (b) selecting a transgenic plant cell, transgenic plant part, or transgenic plant comprising the edited transgenic plant genome, wherein the first transgenic locus has been excised and the second transgenic locus is present.

[00114] 2. A method for obtaining a transgenic plant cell containing an edited transgenic plant genome comprising: (a) contacting the transgenic plant genome of the donor inbred parent plant line with gene editing molecules which introduce a blunt or staggered double stranded

DNA break in a 5' and a 3' DNA junction polynucleotide of the first transgenic locus; and (b) selecting a transgenic plant cell, transgenic plant part, or transgenic plant comprising the edited transgenic plant genome, wherein the first transgenic locus has been excised and the second transgenic locus is present.

[00115] 3. The method of embodiment 2, wherein the gene editing molecules comprise one or more TALEN, TALE-nickases, Zinc Finger Nucleases, or Zinc Finger nickases which cleave a 5' and a 3' DNA junction polynucleotide of the first transgenic locus.

[00116] 4. The method of embodiment 1, wherein the transgenic plant genome is contacted in step (a) by introducing one or more compositions comprising or encoding the RdDe(s) and gRNAs into a transgenic plant cell comprising the transgenic plant genome.

[00117] 5. The method of any one of embodiments 1 to 4, wherein the transgenic plant genome in step (a) further comprises a third transgenic plant locus.

[00118] 6. The method of any one of embodiments 1 to 4, wherein the transgenic plant genome in step (a) is further contacted in step (a) with a donor DNA template molecule comprising an introduced transgene and a transgenic plant cell comprising an edited transgenic plant genome comprising an insertion of the introduced transgene in genomic DNA comprising the excision site of the first transgenic locus is selected in step (b).

[00119] 7. The method of any one of embodiments 1 to 4, wherein the transgenic plant genome in step (a) is further contacted in step (a) with: (i) a donor DNA template molecule comprising an introduced transgene; and (ii) one or more DNA editing molecules which introduce a double stranded DNA break in the second transgenic locus; and a transgenic plant cell comprising an edited transgenic plant genome comprising an insertion of the introduced transgene in the second transgenic locus is selected in step (b).

[00120] 8. The method of any one of embodiments 1 to 7 further comprising contacting the transgenic plant genome in step (a) with one or more gene editing molecules that provide for excision or inactivation of a selectable marker transgene of the second transgenic locus and selecting for a transgenic plant cell, transgenic plant part, or transgenic plant wherein the selectable marker transgene has been excised or inactivated.

[00121] 9. The method of any one of embodiments 1 to 8, wherein the gene editing molecules include a donor DNA template containing an expression cassette or coding region which confers a useful trait and the transgenic plant cell, transgenic plant part, or transgenic plant

is selected for integration of the expression cassette at the site of the selectable marker transgene excision or inactivation.

[00122] 10. The method of any one of embodiments 1 to 9, further comprising inducing at least one targeted genetic change in the transgenic plant genome with one or more genome editing molecules.

[00123] 11. The method of any one of embodiments 1 to 10, further comprising: (c) contacting the edited transgenic plant genome in the selected transgenic plant cell of step (b) with: (i) a donor DNA template molecule comprising an introduced transgene; and (ii) one or more DNA editing molecules which introduce a double stranded DNA break in or near genomic DNA comprising the excision site of the first transgenic locus or in the second transgenic locus; and, (d) selecting a transgenic plant cell, transgenic plant part, or transgenic plant comprising a further edited transgenic plant genome comprising an insertion of the introduced transgene in or near the excision site of the first transgenic locus or in the second transgenic locus.

[00124] 12. The method of any one of embodiments 1 to 11, wherein the transgenic plant cell is a transgenic maize plant cell and wherein the first, second, and/or third transgenic locus comprises Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.

[00125] 13. The method of any one of embodiments 1 to 11, wherein the transgenic plant cell is a transgenic soybean plant cell and wherein the first, second, and/or third transgenic locus comprises an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, SYHT0H2 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic soybean plant genome.

[00126] 14. The method of any one of embodiments 1 to 11, wherein the transgenic plant cell is a transgenic cotton plant cell and wherein the first, second, and/or third transgenic locus comprises a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, MON88913 transgenic locus, , or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic cotton plant genome.

[00127] 15. The method of any one of embodiments 1 to 11, wherein the transgenic plant cell is a transgenic canola plant cell and wherein the first, second, and/or third transgenic locus comprises a GT73, HCN28, MON88302, MS8 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic canola plant genome.

[00128] 16. A method for obtaining inbred transgenic plant germplasm containing different transgenic traits comprising: (a) introgressing at least a first transgenic locus and a second transgenic locus into inbred germplasm to obtain a donor inbred parent plant line comprising the first and second transgenic loci; (b) contacting the transgenic plant genome of the donor inbred parent plant line with: (i) at least a first guide RNA directed to genomic DNA adjacent to two PAM sites, wherein the PAM sites are operably linked to a 5' and a 3' DNA junction polynucleotide of the first transgenic locus; and (ii) one or more RNA dependent DNA endonucleases (RdDe) which recognize the PAM sites; and (c) selecting a transgenic plant cell, transgenic plant part, or transgenic plant comprising an edited transgenic plant genome in the inbred germplasm, wherein the first transgenic locus has been excised and the second transgenic locus is present in the inbred germplasm.

[00129] 17. A method for obtaining inbred transgenic plant germplasm containing different transgenic traits comprising: (a) introgressing at least a first transgenic locus and a second transgenic locus into inbred germplasm to obtain a donor inbred parent plant line comprising the first and second transgenic loci; (b) contacting the transgenic plant genome of the donor inbred parent plant line with gene editing molecules which introduce a blunt or staggered double stranded DNA break in a 5' and a 3' DNA junction polynucleotide of the first transgenic locus; and (c) selecting a transgenic plant cell, transgenic plant part, or transgenic plant comprising an edited transgenic plant genome in the inbred germplasm, wherein the first transgenic locus has been excised and the second transgenic locus is present in the inbred germplasm.

[00130] 18. The method of embodiment 17, further comprising inducing at least one targeted genetic change in the transgenic plant genome with one or more genome editing molecules.

[00131] 19. The method of embodiment 17, wherein the gene editing molecules comprise one or more TALEN, TALE-nickases, Zinc Finger Nucleases, or Zinc Finger nickases which cleave a 5' and a 3' DNA junction polynucleotide of the first transgenic locus.

[00132] 20. The method of embodiment 17, further comprising contacting the transgenic plant genome in step (b) with one or more gene editing molecules that provide for excision or inactivation of a selectable marker transgene of the second transgenic locus and selecting for a transgenic plant cell, transgenic plant part, or transgenic plant wherein the selectable marker transgene has been excised or inactivated.

[00133] 21. The method of embodiment 20, wherein the gene editing molecules include a donor DNA template containing an expression cassette or coding region which confers a useful trait and the transgenic plant cell, transgenic plant part, or transgenic plant is selected for integration of the expression cassette at the site of the selectable marker transgene excision or inactivation.

[00134] 22. The method of embodiment 17, wherein a third transgenic locus is introgressed or introduced into the inbred germplasm to obtain a donor inbred parent plant line comprising the first, second, and third transgenic loci.

[00135] 23. The method of embodiment 17, further comprising contacting the transgenic plant genome with a second guide RNA directed to genomic DNA adjacent to two PAM sites, wherein the PAM sites are operably linked to a 5' and a 3' DNA junction polynucleotide of the second or third transgenic locus; and (ii) one or more RNA dependent DNA endonucleases (RdDe) which recognize the PAM sites in step (b); and selecting a transgenic plant cell, transgenic plant part, or transgenic plant wherein the second or third transgenic locus has been excised in step (c).

[00136] 24. The method of embodiment 17, wherein the transgenic plant genome is contacted in step (b) by introducing one or more compositions comprising or encoding the RdDe(s) and gRNAs into a transgenic plant cell comprising the transgenic plant genome.

[00137] 25. The method of embodiment 17, wherein the transgenic plant genome of step (b) further comprises a third transgenic plant locus.

[00138] 26. The method of embodiment 17, wherein the transgenic plant genome is further contacted in step (b) with a donor DNA template molecule comprising an introduced transgene and a transgenic plant cell comprising an edited transgenic plant genome comprising an insertion of the introduced transgene in genomic DNA comprising the excision site of the first transgenic locus is selected in step (c).

[00139] 27. The method of embodiment 17, wherein the transgenic plant genome is further contacted in step (b) with: (i) a donor DNA template molecule comprising an introduced

transgene; and (ii) one or more DNA editing molecules which introduce a double stranded DNA break in the second transgenic locus; and a transgenic plant cell comprising an edited transgenic plant genome comprising an insertion of the introduced transgene in genomic DNA comprising the excision site of the second transgenic locus is selected in step (b).

[00140] 28. The method of embodiment 17, further comprising: (d) contacting the edited transgenic plant genome in the selected transgenic plant cell of step (c) with: (i) a donor DNA template molecule comprising an introduced transgene; and (ii) one or more DNA editing molecules which introduce a double stranded DNA break in or near the excision site of the first transgenic locus or in the second transgenic locus; and, (e) selecting a transgenic plant cell, transgenic plant part, or transgenic plant comprising a further edited transgenic plant genome comprising an insertion of the introduced transgene in or near the excision site of the first transgenic locus or in the second transgenic locus.

[00141] 29. The method of any one of embodiments 17 to 28, wherein the transgenic plant germplasm is transgenic maize plant germplasm and wherein the first, second, and/or third transgenic locus comprises a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.

[00142] 30. The method of any one of embodiments 17 to 28, wherein the transgenic plant germplasm is transgenic soybean plant germplasm and wherein the first, second, and/or third transgenic locus comprises an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, SYHT0H2 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic soybean plant genome.

[00143] 31. The method of any one of embodiments 17 to 28, wherein the transgenic plant germplasm is transgenic cotton plant germplasm and wherein the first, second, and/or third transgenic locus comprises a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, MON88913 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic cotton plant genome.

[00144] 32. The method of any one of embodiments 17 to 28, wherein the transgenic plant germplasm is transgenic canola plant germplasm and wherein the first, second, and/or third transgenic locus comprises a GT73, HCN28, MON88302, MS8 transgenic locus in a transgenic canola plant genome, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA.

[00145] 33. The method of embodiment 1, wherein the RdDe is a class II or class V RdDe.

[00146] 34. The method of embodiment 17, wherein the introgression comprises crossing germplasm comprising the first and/or second transgenic plant locus with the inbred germplasm, selecting progeny comprising the first or second transgenic plant locus, and crossing the selected progeny with the inbred germplasm as a recurrent parent.

[00147] Embodiment List Two

[00148] 1. A method of producing an elite crop plant comprising a targeted genetic change and at least one approved transgenic locus comprising steps of: (i) inducing at least one targeted genetic change in the genome of the crop plant with one or more genome editing molecules in an elite crop plant comprising a first approved transgenic locus or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, and a second approved transgenic locus or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA; (ii) excising a DNA segment comprising all or most of the first approved transgenic locus or modification thereof with genome editing molecules by (a) contacting genomic DNA of said plant with: (i) at least a first and at least a second guide RNA directed to genomic DNA adjacent to two PAM sites, wherein the PAM sites are operably linked to a 5' and a 3' DNA junction polynucleotide of the first approved transgenic locus; and (ii) one or more RNA dependent DNA endonucleases (RdDe) which recognize the PAM sites; and (iii) selecting an elite crop plant wherein the first approved transgenic locus or modification thereof is excised, the second approved transgenic locus or modification thereof is present, and the targeted genetic change is present.

[00149] 2. The method of embodiment 1, wherein steps (i) and (ii) are performed sequentially.

[00150] 3. The method of embodiment 1, wherein steps (i) and (ii) are performed simultaneously.

- [00151] 4. The method of embodiment 1, wherein the targeted genetic change confers a desirable agronomic or quality trait.
- [00152] 5. The method of embodiment 1, wherein all of the first approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide is excised.
- [00153] 6. The method of embodiment 1, wherein all but at least one nucleotide of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of the first approved transgenic locus is excised
- [00154] 7. The method of embodiment 1, further comprising the step of obtaining the elite crop plant comprising a first approved transgenic loci and a second approved transgenic loci of step (i).
- [00155] 8. The method of any one of embodiments 1 to 7, wherein the first approved transgenic locus comprises a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.
- [00156] 9. The method of any one of embodiments 1 to 7, wherein the second approved transgenic locus comprises a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.
- [00157] 10. The method of any one of embodiments 1 to 7, wherein the first approved transgenic locus comprises a A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, SYHT0H2 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic soybean plant genome.
- [00158] 11. The method of any one of embodiments 1 to 7, wherein the second approved transgenic locus comprises a A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-

2, MON87701, MON87708, MON89788, MST-FGØ72-3, SYHT0H2 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic soybean plant genome.

[00159] 12. The method of any one of embodiments 1 to 7, wherein the first approved transgenic locus comprises a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, MON88913 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic cotton plant genome.

[00160] 13. The method of any one of embodiments 1 to 7, wherein the second approved transgenic locus comprises a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, MON88913 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic cotton plant genome.

[00161] 14. The method of any one of embodiments 1 to 7, wherein the first approved transgenic locus comprises a GT73, HCN28, MON88302, MS8 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic canola plant genome.

[00162] 15. The method of any one of embodiments 1 to 7, wherein the second approved transgenic locus comprises a GT73, HCN28, MON88302, MS8 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic canola plant genome.

[00163] 16. An elite crop plant or part thereof comprising at least one approved first transgenic locus and a transgenic locus excision site wherein all of a second approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second approved transgenic locus is excised.

[00164] 17. The elite crop plant or part thereof of embodiment 16, wherein about 5 to about 25 nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second approved transgenic locus is excised.

[00165] 18. The elite crop plant or part thereof of embodiment 16, wherein the crop plant is a maize plant and wherein first approved transgenic locus comprises a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-

Ø1981-5, 98140, TC1507 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.

[00166] 19. The elite crop plant or part thereof of embodiment 16, wherein the second approved transgenic locus comprising a DAS-59122-7, DP-4114, MON87411, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, is excised.

[00167] 20. The elite crop plant or part thereof of embodiment 16, wherein the plant further comprises a third transgenic locus comprising a selectable marker gene which confers a selectable marker trait of a DAS-59122-7, DP-4114, MON87411, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus.

[00168] 21. The elite crop plant or part thereof of embodiment 16, wherein the crop plant is a soybean plant and wherein the first approved transgenic locus comprises a A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, SYHT0H2 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic soybean plant genome.

[00169] 22. The elite crop plant or part thereof of embodiment 16, wherein the crop plant is a soybean plant and wherein the second approved transgenic locus comprises a A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, SYHT0H2 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic soybean plant genome.

[00170] 23. The elite crop plant or part thereof of embodiment 16, wherein the crop plant is a cotton plant and wherein the first approved transgenic locus comprises a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, MON88913 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic cotton plant genome.

[00171] 24. The elite crop plant or part thereof of embodiment 16, wherein the crop plant is a cotton plant and wherein the second approved transgenic locus comprises a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, MON88913 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic cotton plant genome.

[00172] 25. The elite crop plant or part thereof of embodiment 16, wherein the crop plant is a canola plant and wherein the first approved transgenic locus comprises a GT73, HCN28, MON88302, MS8 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic canola plant genome.

[00173] 26. The elite crop plant or part thereof of embodiment 16, wherein the crop plant is a canola plant and wherein the second approved transgenic locus comprises a GT73, HCN28, MON88302, MS8 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic canola plant genome.

[00174] 27. The elite crop plant or part thereof of any one of embodiments 16 to 26, wherein the plant further comprises a targeted genetic change.

[00175] 28. A method for obtaining the elite crop plant of any one of embodiments 16 to 27, comprising the steps of: (a) obtaining a crop plant comprising at least the approved first transgenic locus or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, and a second transgenic locus; (b) introgressing the first approved transgenic locus or modification thereof and the second approved transgenic locus into the germplasm of the elite crop plant; (c) excising a DNA segment comprising the second approved transgenic locus from the elite crop plant of step (b) with genome editing molecules and optionally inducing at least one targeted genetic change in the genome of the crop plant of step (b) with one or more genome editing molecules; and (d) selecting an elite crop plant comprising: (i) the approved first transgenic locus or modification thereof and a transgenic locus excision site wherein all of the second approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second approved transgenic locus is excised; and optionally (ii) the targeted genetic change.

[00176] 29. The method of embodiment 28, wherein the introgression comprises: (i) crossing the crop plant of (a) to a plant comprising the elite crop germplasm but lacking both the first and the second transgenic locus; (ii) selecting a progeny plant comprising the first and the

second transgenic locus; (iii) backcrossing the progeny plant to a recurrent parent crop plant comprising the elite crop germplasm but lacking the first and second transgenic locus; and (iv) selecting a progeny plant comprising the first and the second transgenic locus.

[00177] 30. A method for obtaining a bulked population of inbred seed for commercial seed production comprising selfing the elite crop plant of any one of embodiments 16 to 27 and harvesting seed from the selfed elite crop plants.

[00178] 31. A method of obtaining hybrid crop seed comprising crossing a first crop plant comprising the elite crop plant of any one of embodiments 16 to 27, to a second crop plant and harvesting seed from the cross.

[00179] 32. The method of embodiment 31, wherein the first crop plant and the second crop plant are in distinct heterotic groups.

[00180] 33. The method of embodiment 31, wherein either the first or second crop plant are pollen recipients which have been rendered male sterile.

[00181] 34. The method of embodiment 33, wherein the crop plant is rendered male sterile by emasculation, cytoplasmic male sterility, a chemical hybridizing agent or system, a transgene, and/or a mutation in an endogenous plant gene.

[00182] 35. The method of any one of embodiments 31 to 34, further comprising the step of sowing the hybrid crop seed.

[00183] 36. DNA comprising a transgenic locus excision site wherein all of an approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the approved transgenic locus is excised.

[00184] 37. The DNA of embodiment 36, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus.

[00185] 38. The DNA of embodiment 36, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

[00186] 39. The DNA of embodiment 36, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.

[00187] 40. The DNA of embodiment 36, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

[00188] The DNA of any one of embodiments 36 to 40, wherein the DNA is purified or isolated.

[00189] 41. A nucleic acid marker adapted for detection of genomic DNA or fragments comprising a transgenic locus excision site wherein all of an approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the approved transgenic locus is excised and wherein the marker does not detect an approved transgenic locus which has not been excised.

[00190] 42. The nucleic acid marker of embodiment 41, comprising a polynucleotide of at least 18 nucleotides in length which spans the selectable marker gene excision site.

[00191] 43. The nucleic acid marker of embodiment 41, wherein the marker further comprises a detectable label.

[00192] 44. The nucleic acid marker of embodiment 41, wherein the approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 transgenic locus.

[00193] 45. The nucleic acid marker of embodiment 41, wherein the approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

[00194] 46. The nucleic acid marker of embodiment 41, wherein the approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.

[00195] 47. The nucleic acid marker of embodiment 41, wherein the approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

[00196] 48. A biological sample comprising plant genomic DNA or fragments thereof, said genomic DNA or fragments comprising a transgenic locus excision site wherein all of an approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the

plant genome in the 5' and a 3' DNA junction polynucleotide of the approved transgenic locus is excised.

[00197] 49. The biological sample of embodiment 48, wherein the approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 transgenic locus.

[00198] 50. The biological sample of embodiment 48, wherein the approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

[00199] 51. The biological sample of embodiment 48, wherein the approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLCotton25, MON15985, MON88701, and/or MON88913 transgenic locus.

[00200] 52. The biological sample of embodiment 48, wherein the approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

[00201] 53. A method of identifying the plant, DNA, or biological sample of any one of embodiments 36 to 52, comprising detecting a polynucleotide comprising a transgenic locus excision site wherein all of an approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the approved transgenic locus is excised with a nucleic acid detection assay.

[00202] 54. The method of embodiment 53, wherein the detection assay does not detect the approved transgenic locus which was excised.

[00203] 55. The method of embodiment 53, wherein the detection assay comprises contacting the biological sample with the nucleic acid marker of any one of embodiments 41 to 47.

[00204] 56. An elite crop plant or part thereof comprising at least one approved first transgenic locus or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, and a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of a second approved transgenic locus is excised.

[00205] 57. The elite crop plant or part thereof of embodiment 56, wherein the crop plant is a maize plant and wherein the first approved transgenic locus comprises a Bt11, DAS-59122-7,

DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.

[00206] 58. The elite crop plant or part thereof of embodiment 56, wherein the crop plant is a maize plant and wherein the second approved transgenic locus comprising a DAS-59122-7, DP-4114, MON87411, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, transgenic locus is excised.

[00207] 59. The elite maize plant or part thereof of embodiment 58, wherein the crop plant is a maize plant and wherein the plant further comprises a third transgenic locus comprising a selectable marker gene which confers a selectable marker trait of a DAS-59122-7, DP-4114, MON87411, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, or TC1507 transgenic locus.

[00208] 60. The elite crop plant or part thereof of embodiment 56, wherein the crop plant is a soybean plant and wherein the first approved transgenic locus comprises a A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, SYHT0H2 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic soybean plant genome.

[00209] 61. The elite crop plant or part thereof of embodiment 56, wherein the crop plant is a soybean plant and wherein the second approved transgenic locus comprises a A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, SYHT0H2 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic soybean plant genome.

[00210] 62. The elite crop plant or part thereof of embodiment 56, wherein the crop plant is a cotton plant and wherein the first approved transgenic locus comprises a DAS-21023-5,

DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, MON88913 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic cotton plant genome.

[00211] 63. The elite crop plant or part thereof of embodiment 56, wherein the crop plant is a cotton plant and wherein the second approved transgenic locus comprises a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, MON88913 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic cotton plant genome.

[00212] 64. The elite crop plant or part thereof of embodiment 56, wherein the crop plant is a canola plant and wherein the first approved transgenic locus comprises a GT73, HCN28, MON88302, MS8 transgenic locus, or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic canola plant genome.

[00213] 65. The elite crop plant or part thereof of embodiment 56, wherein the crop plant is a canola plant and wherein the second approved transgenic locus comprises a GT73, HCN28, MON88302, or MS8 transgenic locus in a transgenic canola plant genome

[00214] 66. The elite crop plant or part thereof of any one of embodiments 56 to 65, wherein the plant further comprises a targeted genetic change.

[00215] 67. A method for obtaining the elite crop plant of any one of embodiments 56 to 66, comprising the steps of: (a) obtaining a crop plant comprising at least the approved first transgenic locus or modification thereof and a second transgenic locus or modification thereof; (b) introgressing the first approved transgenic locus or modification thereof and a second approved transgenic locus or modification thereof into the germplasm of the elite crop plant; (c) excising a DNA segment comprising the second approved transgenic locus from the elite crop plant of step (b) with genome editing molecules and optionally inducing at least one targeted genetic change in the genome of the crop plant of step (b) with one or more genome editing molecules; and (d) selecting an elite crop plant comprising: (i) the approved first transgenic locus or modification thereof and a transgenic locus excision site wherein all of the second approved transgenic locus or modification thereof and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second approved transgenic locus or modification thereof is excised; and optionally (ii) the targeted genetic change.

[00216] 68. The method of embodiment 67, wherein the introgression comprises: (i) crossing the crop plant of (a) to a plant comprising the elite crop germplasm but lacking both the first and the second transgenic locus; (ii) selecting a progeny plant comprising the first and the second transgenic locus; (iii) backcrossing the progeny plant to a recurrent parent crop plant comprising the elite crop germplasm but lacking the first and second transgenic locus; and (iv) selecting a progeny plant comprising the first and the second transgenic locus.

[00217] 69. A method for obtaining a bulked population of inbred seed for commercial seed production comprising selfing the elite crop plant of any one of embodiments 56 to 66 and harvesting seed from the selfed elite crop plants.

[00218] 70. A method of obtaining hybrid crop seed comprising crossing a first crop plant comprising the elite crop plant or part thereof of any one of embodiments 56 to 66, to a second crop plant and harvesting seed from the cross.

[00219] 71. The method of embodiment 70, wherein the first crop plant and the second crop plant are in distinct heterotic groups.

[00220] 72. The method of embodiment 71, wherein either the first or second crop plant are pollen recipients which have been rendered male sterile.

[00221] 73. The method of embodiment 72, wherein the crop plant is rendered male sterile by emasculation, cytoplasmic male sterility, a chemical hybridizing agent or system, a transgene, and/or a mutation in an endogenous plant gene.

[00222] 74. The method of any one of embodiments 70 to 73, further comprising the step of sowing the hybrid crop seed.

[00223] 75. DNA comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of an approved transgenic locus is excised.

[00224] 76. The DNA of embodiment 75, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, or TC1507 transgenic locus.

[00225] 77. The DNA of embodiment 75, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

[00226] 78. The DNA of embodiment 75, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.

[00227] 79. The DNA of embodiment 75, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

[00228] 80. The DNA of any one of embodiments 75 to 79, wherein the DNA is purified or isolated.

[00229] 81. A nucleic acid marker adapted for detection of genomic DNA or fragments comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of an approved transgenic locus is excised and wherein the marker does not detect an approved transgenic locus which has not been excised.

[00230] 82. The nucleic acid marker of embodiment 81, comprising a polynucleotide of at least 18 nucleotides in length which spans the transgenic locus excision site.

[00231] 83. The nucleic acid marker of embodiment 81, wherein the marker further comprises a detectable label.

[00232] 84. The nucleic acid marker of embodiment 81, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 transgenic locus.

[00233] 85. The nucleic acid marker of embodiment 81, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

[00234] 86. The nucleic acid marker of embodiment 81, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.

[00235] 87. The nucleic acid marker of embodiment 81, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

[00236] 88. A biological sample comprising the plant genomic DNA or fragments thereof, said genomic DNA or fragments comprising a transgenic locus excision site wherein all but at

least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of an approved transgenic locus is excised.

[00237] 89. The biological sample of embodiment 81, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus.

[00238] 90. The biological sample of embodiment 81, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

[00239] 91. The biological sample of embodiment 81, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.

[00240] 92. The biological sample of embodiment 81, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

[00241] 93. A method of identifying the plant, DNA, or biological sample of any one of embodiments 56 to 66, 75 to 80, or 81 to 92, comprising detecting a polynucleotide comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of an approved transgenic locus is excised with a nucleic acid detection assay.

[00242] 94. The method of embodiment 93, wherein the detection assay does not detect the approved transgenic locus which was excised.

[00243] 95. The method of embodiment 93, wherein the detection assay comprises contacting the biological sample with the nucleic acid marker of any one of embodiments 81 to 87.

Examples

[00244] The following Examples are provided for purposes of illustration only, and are not intended to be limiting.

[00245] Example 1. Excision of Transgenic Loci

[00246] Transgenic plant genomes containing one or more of the following transgenic loci (events) are contacted with a class 2 type II RdDe (*e.g.*, Cas9) or class 2 type V RdDe (*e.g.*, Cas12) and guide RNAs which recognize the indicated target DNA sites (guide RNA coding plus PAM site for class 2 type II or PAM site plus guide RNA coding for class 2 type V) in the 5' and 3' junction polynucleotides of the event. Plant cells, callus, parts, or whole plants comprising a deletion of the transgenic loci from the transgenic plant genome are selected.

[00247] Table 5. Use of pre-existing Genomic DNA target and PAM sites in Event (transgenic loci) 5' Junction and 3' Junction polynucleotides to excise transgenic loci with Class 2 type II RdDe

CORN EVENT NAME	Selectable Marker Gene	Selectable Marker Gene Flanking DNA 1 polynucleotide target DNA (Guide RNA coding sequence+ PAM for Class 2 type II)	Selectable Marker Gene Flanking DNA 2 polynucleotide target DNA (Guide RNA coding sequence+ PAM for Class 2 type II)
DAS-59122-7	PAT	GAAGAAAATCTTCGTCAAC ATGG (SEQ ID NO:35)	TCCAGGGCGAGCTCGGTACC CGG (SEQ ID NO:36)
DP-4114	PAT	GGCCGCGGACCGAATTCCC ATGG (SEQ ID NO:37)	ATCGTGGCCTCTTGCTCTTC AGG (SEQ ID NO:38)
MON87411	CP4 EPSPS	CGAGGCAAGCTTGTGCGAAA ATGG (SEQ ID NO: 39)	AAACACTGATAGTTTAAACG CGG (SEQ ID NO: 40)
MIR162	PMI	TGCACTGCAGGCATGCAAG CTGG (SEQ ID NO: 41)	TGTACTGAATTGTCTAGACC CGG (SEQ ID NO: 42)
NK603	pOS-ACT- CP4 EPSPS	CGCGTTAACAAGCTTACTCG AGG (SEQ ID NO: 43)	AGATCGGGGATAGCTTCTGC AGG (SEQ ID NO: 44)
SYN-E3272-5	PMI	TGCACTGCAGGCATGCAAG CTGG (SEQ ID NO: 45)	GGCACCGGTAAATTTCCTGC AGG (SEQ ID NO: 46)
5307	PMI	TGCACTGCAGGCATGCAAG CTGG (SEQ ID NO: 47)	ACTAGATCTGCTAGCCCTGC AGG (SEQ ID NO: 48)
SOYBEAN EVENT NAME			
DAS68416-4		CGCGGCCGCTTAATTAAGGC CGG (SEQ ID NO: 49)	CGGGTTTCTAGTCACCGGTT AGG (SEQ ID NO: 50)
MON89788	EPSPS	TTTGGA CTGAGAATTAGCTT CCTCTCG ((SEQ ID NO: 51; CLASS 2 TYPE V PAM+GRNA CODING)	TTTCTCATCTAAGCCCCCAT TTGGACG (SEQ ID NO: 52) ;CLASS 2 TYPE V PAM+GRNA CODING)
MON89788	EPSPS	TTCTGCAGGTCCTGCTCGAG TGG (SEQ ID NO: 53 ;CLASS 2 TYPE 2 GRNA CODING +PAM)	CGGCCGCTTCGAGTGGCTGC AGG (SEQ ID NO: 54;CLASS 2 TYPE II GRNA CODING +PAM)
COTTON EVENT NAME			
COT102	aph4 (hpt)	GTACGCCATGCTGGCCGCC	CTTGGCTCCAAATCCGGTAC

		GGG (SEQ ID NO: 55)	CGG (SEQ ID NO: 56)
CANOLA EVENT NAME			
MON88302	EPSPS	TTCTGCAGGTCCTGCTCGAG TGG (SEQ ID NO: 57)	ATCGATGCGGCCGCTTCGAG TGG (SEQ ID NO: 58)

[00248] Table 6. Use of pre-existing Genomic DNA target and PAM sites in Event (transgenic loci) 5' Junction and 3' Junction polynucleotides to excise transgenic loci with Class 2 type V RdDe

MAIZE EVENT NAME	5' Junction polynucleotide target DNA (PAM+ Guide RNA coding sequence)	3' Junction polynucleotide target DNA (PAM + Guide RNA coding sequence)
DAS- 59122-7	TTTCCCGCCTTCAGTTTAAACTATCAG (SEQ ID NO: 59)	TTTAATGTACTGAATTGCGT ACGATTG (SEQ ID NO: 60)
DP-4114	TTTAAACGCTCTTCAACTGGAAGAGCG (SEQ ID NO: 61)	TTTAATGTACTGAATTGTCT AGTAGCG (SEQ ID NO: 62)
MON87411	TTTATGACTTGCCAATTGATTGACAAC (SEQ ID NO: 63)	TTTAATCATATTGTAAAGGA TATAATT (SEQ ID NO: 64)
MON89034	TTTGCGCGCCAAATCGTGAAGTTTCT (SEQ ID NO: 65)	TTTGCGCGCCAAATCGTG AAGTTTCT (SEQ ID NO: 66)
MIR162	TTTCCCGCCTTCAGTTTAAACTATCAG (SEQ ID NO: 67)	TTTAATGTACTGAATTGTCT AGACCC (SEQ ID NO: 68)
NK603	TTTGACTATCCCGACTCTCTTCTCAA (SEQ ID NO: 69)	TTTGAGTGGATCCTGTTATC TCTTCTC (SEQ ID NO: 70)
SYN- E3272-5	TTTCCCGCCTTCAGTTTAAACTATCAG (SEQ ID NO: 71)	TTTGTTTACACCACAATATA TTTCAAG (SEQ ID NO: 72)
TC1507	TTTGTGGGACAGTATGTCTGCCACTTT (SEQ ID NO: 73)	TTTGCCAGTGGGCCCAGCCT GGCCCAG (SEQ ID NO: 74)
5307	TTTGTGGGACAGTATGTCTGCCACTTT (SEQ ID NO: 75)	TTTGCCAGTGGGCCCAGCCT GGCCCAG (SEQ ID NO: 76)
SOYBEAN EVENT NAME	5' Junction polynucleotide target DNA (PAM + Guide RNA coding sequence)	3' Junction polynucleotide target DNA (PAM + Guide RNA coding sequence)
MON87701	TTTGACACACACTAAGCGTGCCTGG (SEQ ID NO: 77)	TTTCCTAAATTAGTCCTACT TTTTGAT (SEQ ID NO: 78)
MON89788	TTTAAACTATCAGTGTTTGGAGCTTGA (SEQ ID NO: 79)	TTTATAATAACGCTCAGACT CTAGTGA (SEQ ID NO: 80)
COTTON EVENT NAME	5' Junction polynucleotide target DNA (PAM + Guide RNA coding sequence)	3' Junction polynucleotide target DNA (PAM + Guide RNA coding sequence)

COT102	TTTGTTTACCTGAATATTTGCCTTTT (SEQ ID NO: 81)	TTTAATAAATATGGGCAAT CTTCCCT (SEQ ID NO: 82)
CANOLA EVENT NAME	5' Junction polynucleotide target DNA (PAM + Guide RNA coding sequence)	3' Junction polynucleotide target DNA (PAM + Guide RNA coding sequence)
MON88302	TTTCCCGCCTTCAGTTTAACTATCAG (SEQ ID NO: 83)	TTTACAATTGACCATCATAC TCAACTT (SEQ ID NO: 84)

[00249] The breadth and scope of the present disclosure should not be limited by any of the above-described embodiments.

WHAT IS CLAIMED IS:

1. A method of producing an elite maize plant comprising a targeted genetic change and at least one approved transgenic locus comprising steps of:
 - (i) inducing at least one targeted genetic change in the genome of the maize plant with one or more genome editing molecules in an elite maize plant comprising a first approved transgenic loci or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA and a second approved transgenic loci or modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA;
 - (ii) excising a DNA segment comprising all or most of the first approved transgenic locus with genome editing molecules by (a) contacting genomic DNA of said plant with: (i) at least a first and at least a second guide RNA directed to genomic DNA adjacent to two PAM sites, wherein the PAM sites are operably linked to a 5' and a 3' DNA junction polynucleotide of the first approved transgenic locus; and (ii) one or more RNA dependent DNA endonucleases (RdDe) which recognize the PAM sites; and
 - (iii) selecting an elite maize plant wherein the first approved transgenic locus or modification thereof is excised, the second approved transgenic locus or modification thereof is present, and the targeted genetic change is present.
2. The method of claim 1, wherein steps (i) and (ii) are performed sequentially.
3. The method of claim 1, wherein steps (i) and (ii) are performed simultaneously.
4. The method of claim 1, wherein the targeted genetic change confers a desirable agronomic or quality trait.
5. The method of claim 1, wherein all of the first approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide is excised.
6. The method of claim 1 wherein all but at least one nucleotide of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of the first approved transgenic locus is excised
7. The method of claim 1, further comprising the step of obtaining the elite crop plant comprising a first approved transgenic loci and a second approved transgenic loci of step (i).
8. The method of any one of claims 1 to 7, wherein the first approved transgenic locus is selected from the group consisting of Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus and a modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.
9. The method of any one of claims 1 to 8, wherein the second approved transgenic locus is selected from the group consisting of Bt11, DAS-59122-7, DP-4114, GA21, MON810,

MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507, and a modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.

10. An elite maize plant or part thereof comprising at least one approved first transgenic locus and a transgenic locus excision site wherein all of a second approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second approved transgenic locus is excised.

11. The elite maize plant or part thereof of claim 10, wherein about 5 to about 25 nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second approved transgenic locus is excised.

12. The elite maize plant or part thereof of claim 10, wherein the first approved transgenic locus is selected from the group consisting of Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 and modifications thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.

13. The elite maize plant or part thereof of claim 10, wherein the second approved transgenic locus comprising a DAS-59122-7, DP-4114, MON87411, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, or TC1507 transgenic locus is excised.

14. The elite maize plant or part thereof of claim 13, wherein the plant further comprises a third transgenic locus comprising a selectable marker gene which confers a selectable marker trait of a DAS-59122-7, DP-4114, MON87411, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, or TC1507 transgenic locus.

15. The elite maize plant or part thereof of any one of claims 1 to 14, wherein the plant further comprises a targeted genetic change.

16. A method for obtaining the elite maize plant of any one of claims 1 to 15, comprising the steps of: (a) obtaining a maize plant comprising at least the approved first transgenic locus and a second transgenic locus; (b) introgressing the first approved transgenic locus and a second approved transgenic locus into the germplasm of the elite maize plant; (c) excising a DNA segment comprising the second approved transgenic locus from the elite maize plant of step (b) with genome editing molecules and optionally inducing at least one targeted genetic change in the genome of the maize plant of step (b) with one or more genome editing molecules; and (d) selecting an elite maize plant comprising: (i) the approved first transgenic locus and a transgenic

locus excision site wherein all of the second approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second approved transgenic locus is excised; and optionally (ii) the targeted genetic change.

17. The method of claim 16, wherein the introgression comprises: (i) crossing the maize plant of (a) to a plant comprising the elite maize germplasm but lacking both the first and the second transgenic locus; (ii) selecting a progeny plant comprising the first and the second transgenic locus; (iii) backcrossing the progeny plant to a recurrent parent maize plant comprising the elite maize germplasm but lacking the first and second transgenic locus; and (iv) selecting a progeny plant comprising the first and the second transgenic locus.

18. A method for obtaining a bulked population of inbred seed for commercial seed production comprising selfing the elite maize plant of any one of claims 1 to 15 and harvesting seed from the selfed elite maize plants.

19. A method of obtaining hybrid maize seed comprising crossing a first maize plant comprising the elite maize plant or part thereof of any one of claims 1 to 15, to a second maize plant and harvesting seed from the cross.

20. The method of claim 19, wherein the first maize plant and the second maize plant are in distinct heterotic groups.

21. The method of claim 19, wherein either the first or second maize plant are pollen recipients which have been rendered male sterile.

22. The method of claim 19, wherein the maize plant is rendered male sterile by emasculation, cytoplasmic male sterility, a chemical hybridizing agent or system, a transgene, and/or a mutation in an endogenous plant gene.

23. The method of any one of claims 19 to 22, further comprising the step of sowing the hybrid maize seed.

24. DNA comprising a transgenic locus excision site wherein all of an approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the approved transgenic locus is excised.

25. The DNA of claim 24, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, or TC1507 transgenic locus.

26. The DNA of claim 24, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

27. The DNA of claim 24, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.
28. The DNA of claim 24, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.
29. A nucleic acid marker adapted for detection of genomic DNA or fragments comprising a transgenic locus excision site wherein all of an approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the approved transgenic locus is excised and wherein the marker does not detect an approved transgenic locus which has not been excised.
30. The nucleic acid marker of claim 29, comprising a polynucleotide of at least 18 nucleotides in length which spans the selectable marker gene excision site.
31. The nucleic acid marker of claim 29, wherein the marker further comprises a detectable label.
32. The nucleic acid marker of claim 29, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 transgenic locus.
33. The nucleic acid marker of claim 29, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.
34. The nucleic acid marker of claim 29, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.
35. The nucleic acid marker of claim 29, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.
36. A biological sample comprising plant genomic DNA or fragments thereof, said genomic DNA or fragments comprising a transgenic locus excision site wherein all of an approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the approved transgenic locus is excised.
37. The biological sample of claim 36, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 transgenic locus.

38. The biological sample of claim 36, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.
39. The biological sample of claim 36, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.
40. The biological sample of claim 36, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.
41. A method of identifying the plant, DNA, or biological sample of any one of claims 1 to 15, 24 to 28, or 36 to 40, comprising detecting a polynucleotide comprising a transgenic locus excision site wherein all of an approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the approved transgenic locus is excised with a nucleic acid detection assay.
42. The method of claim 41, wherein the detection assay does not detect the approved transgenic locus which was excised.
43. The method of claim 41, wherein the detection assay comprises contacting the biological sample with the nucleic acid marker of any one of claims 29-35.
44. An elite maize plant or part thereof comprising at least one approved first transgenic locus and a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of a second approved transgenic locus is excised.
45. The elite maize plant or part thereof of claim 44, wherein the first approved transgenic locus comprises a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus, or a modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, in a transgenic maize plant genome.
46. The elite maize plant or part thereof of claim 44, wherein the second approved transgenic locus comprising a DAS-59122-7, DP-4114, MON87411, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, TC1507 transgenic locus, or a modification thereof comprising a deletion of at least one selectable marker gene and/or non-essential DNA, is excised.
47. The elite maize plant or part thereof of claim 46, wherein the plant further comprises a third transgenic locus comprising a selectable marker gene which confers a selectable marker trait of a DAS-59122-7, DP-4114, MON87411, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403,

MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, or TC1507 transgenic locus.

48. The elite maize plant or part thereof of any one of claims 44 to 47, wherein the plant further comprises a targeted genetic change.

49. A method for obtaining the elite maize plant of any one of claims 44 to 48, comprising the steps of: (a) obtaining a maize plant comprising at least the approved first transgenic locus and a second transgenic locus; (b) introgressing the first approved transgenic locus and a second approved transgenic locus into the germplasm of the elite maize plant; (c) excising a DNA segment comprising the second approved transgenic locus from the elite maize plant of step (b) with genome editing molecules and optionally inducing at least one targeted genetic change in the genome of the maize plant of step (b) with one or more genome editing molecules; and (d) selecting an elite maize plant comprising: (i) the approved first transgenic locus and a transgenic locus excision site wherein all of the second approved transgenic locus and one or more nucleotides of endogenous chromosomal DNA of the plant genome in the 5' and a 3' DNA junction polynucleotide of the second approved transgenic locus is excised; and optionally (ii) the targeted genetic change.

50. The method of claim 49, wherein the introgression comprises: (i) crossing the maize plant of (a) to a plant comprising the elite maize germplasm but lacking both the first and the second transgenic locus; (ii) selecting a progeny plant comprising the first and the second transgenic locus; (iii) backcrossing the progeny plant to a recurrent parent maize plant comprising the elite maize germplasm but lacking the first and second transgenic locus; and (iv) selecting a progeny plant comprising the first and the second transgenic locus.

51. A method for obtaining a bulked population of inbred seed for commercial seed production comprising selfing the elite maize plant of any one of claims 44 to 48 and harvesting seed from the selfed elite maize plants.

52. A method of obtaining hybrid maize seed comprising crossing a first maize plant comprising the elite maize plant or part thereof of any one of claims 44 to 48, to a second maize plant and harvesting seed from the cross.

53. The method of claim 52, wherein the first maize plant and the second maize plant are in distinct heterotic groups.

54. The method of claim 52, wherein either the first or second maize plant are pollen recipients which have been rendered male sterile.

55. The method of claim 54, wherein the maize plant is rendered male sterile by emasculation, cytoplasmic male sterility, a chemical hybridizing agent or system, a transgene, and/or a mutation in an endogenous plant gene.

56. The method of any one of claims 52 to 55, further comprising the step of sowing the hybrid maize seed.

57. DNA comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of an approved transgenic locus is excised.

58. The DNA of claim 57, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, or TC1507 transgenic locus.

59. The DNA of claim 57, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, or SYHT0H2 transgenic locus.

60. The DNA of claim 57, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, or MON88913 transgenic locus.

61. The DNA of claim 57, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

62. The DNA of any one of claims 57 to 61, wherein the DNA is purified or isolated.

63. A nucleic acid marker adapted for detection of genomic DNA or fragments comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of an approved transgenic locus is excised and wherein the marker does not detect an approved transgenic locus which has not been excised.

64. The nucleic acid marker of claim 63, comprising a polynucleotide of at least 18 nucleotides in length which spans the transgenic locus excision site.

65. The nucleic acid marker of claim 63, wherein the marker further comprises a detectable label.

66. The nucleic acid marker of claim 63, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 transgenic locus.

67. The nucleic acid marker of claim 63, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

68. The nucleic acid marker of claim 63, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.

69. The nucleic acid marker of claim 63, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

70. A biological sample comprising the plant genomic DNA or fragments thereof, said genomic DNA or fragments comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of an approved transgenic locus is excised.

71. The biological sample of claim 70, wherein the original approved transgenic locus is a Bt11, DAS-59122-7, DP-4114, GA21, MON810, MON87411, MON87427, MON88017, MON89034, MIR162, MIR604, NK603, SYN-E3272-5, 5307, DAS-40278, DP-32138, DP-33121, HCEM485, LY038, MON863, MON87403, MON87403, MON87419, MON87460, MZHG0JG, MZIR098, VCO-Ø1981-5, 98140, and/or TC1507 transgenic locus.

72. The biological sample of claim 70, wherein the original approved transgenic locus is an A5547-127, DAS44406-6, DAS68416-4, DAS81419-2, GTS 40-3-2, MON87701, MON87708, MON89788, MST-FGØ72-3, and/or SYHT0H2 transgenic locus.

73. The biological sample of claim 70, wherein the original approved transgenic locus is a DAS-21023-5, DAS-24236-5, COT102, LLcotton25, MON15985, MON88701, and/or MON88913 transgenic locus.

74. The biological sample of claim 70, wherein the original approved transgenic locus is a GT73, HCN28, MON88302, or MS8 transgenic locus.

75. A method of identifying the elite plant, DNA, or biological sample of any one of claims 44 to 48, 57 to 62, or 70 to 74, comprising detecting a polynucleotide comprising a transgenic locus excision site wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of an approved transgenic locus is excised with a nucleic acid detection assay.

76. The method of claim 75, wherein the detection assay does not detect the approved transgenic locus which was excised.

77. The method of claim 75, wherein the detection assay comprises contacting the biological sample with the nucleic acid marker of any one of claims 63 to 69.

DAS59122-7 (11922 bp)

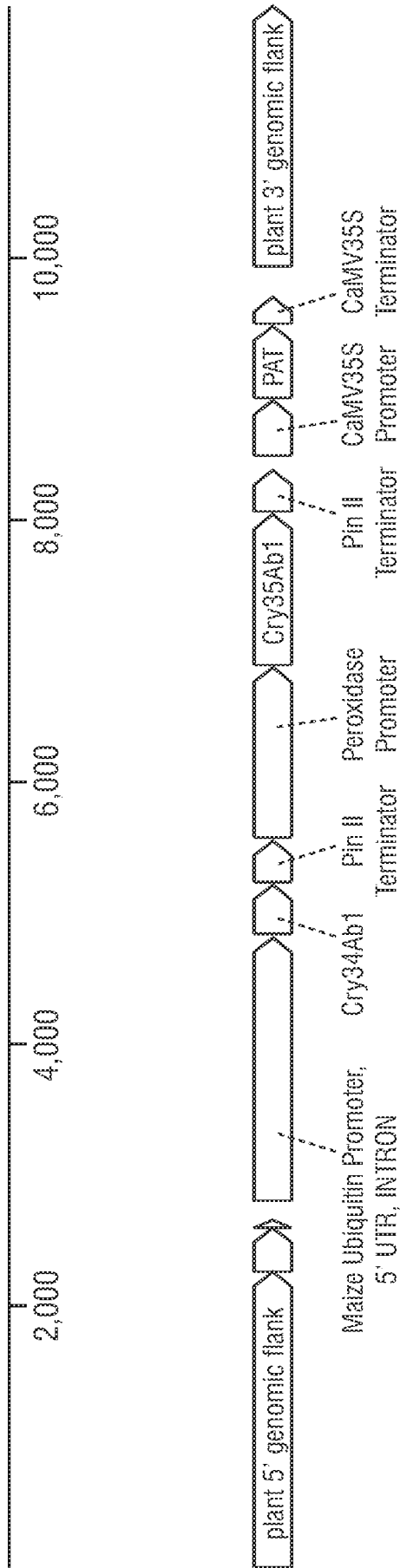


FIG. 1

DP-4114

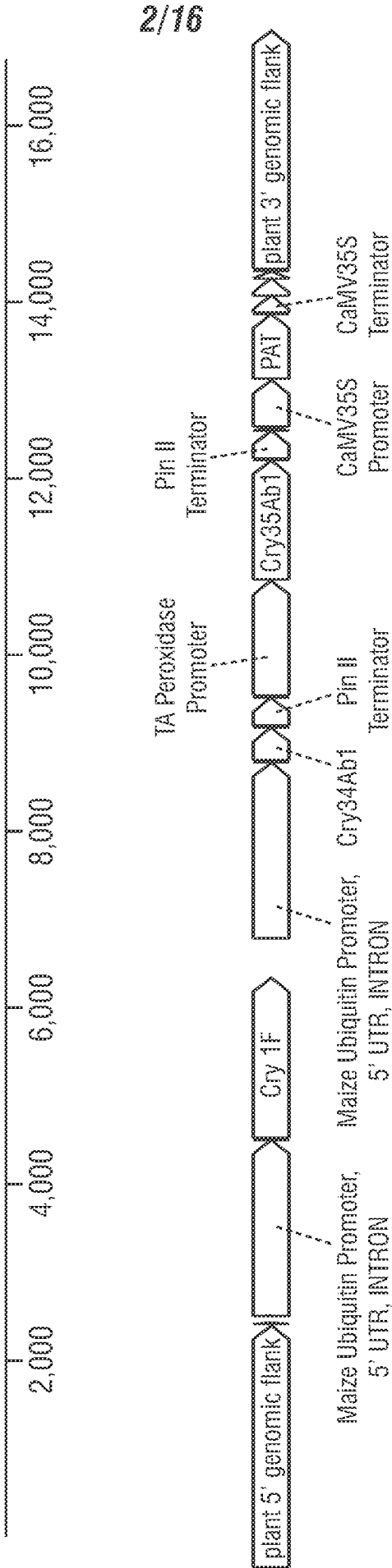


FIG. 2

MON87411

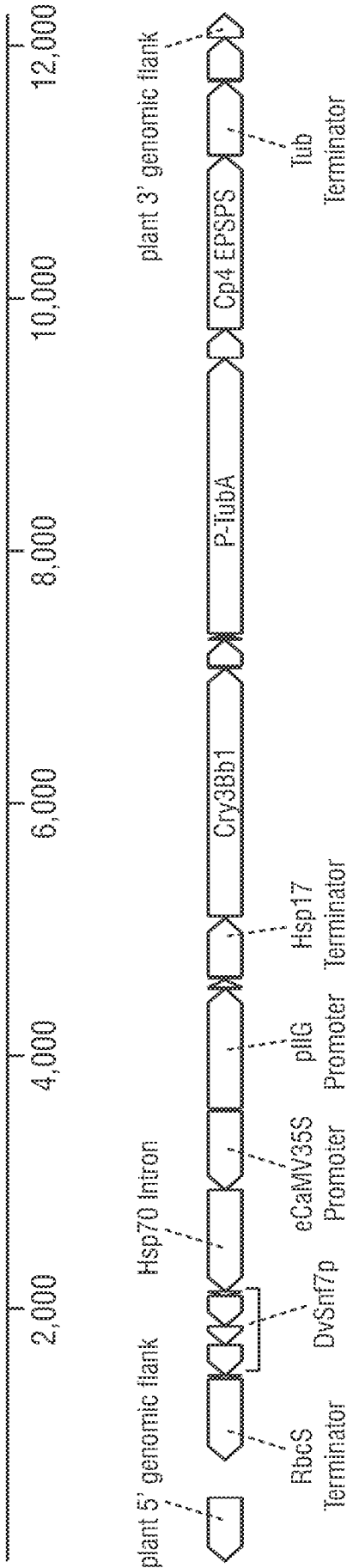


FIG. 3

MON89034 (12282)

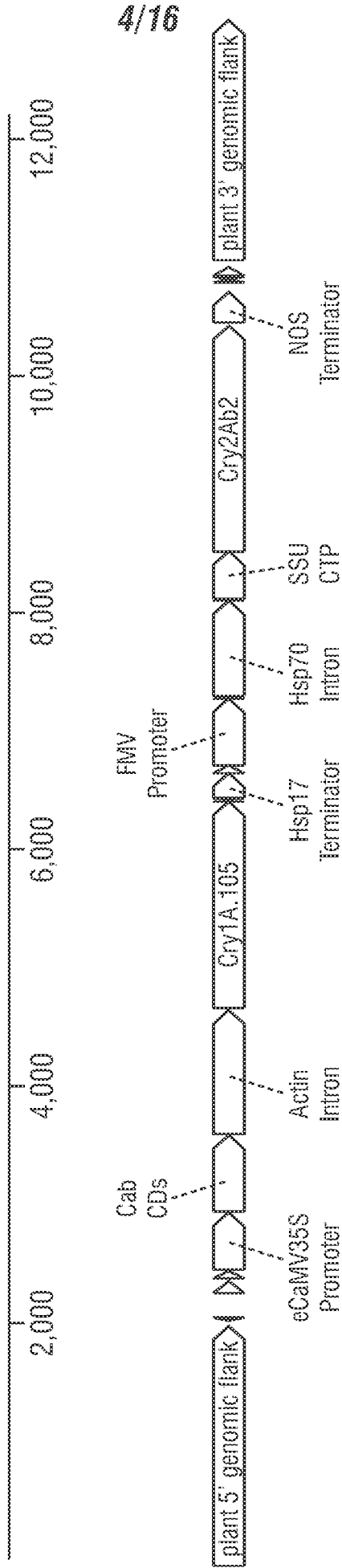


FIG. 4

MIR162 (10579 bp)

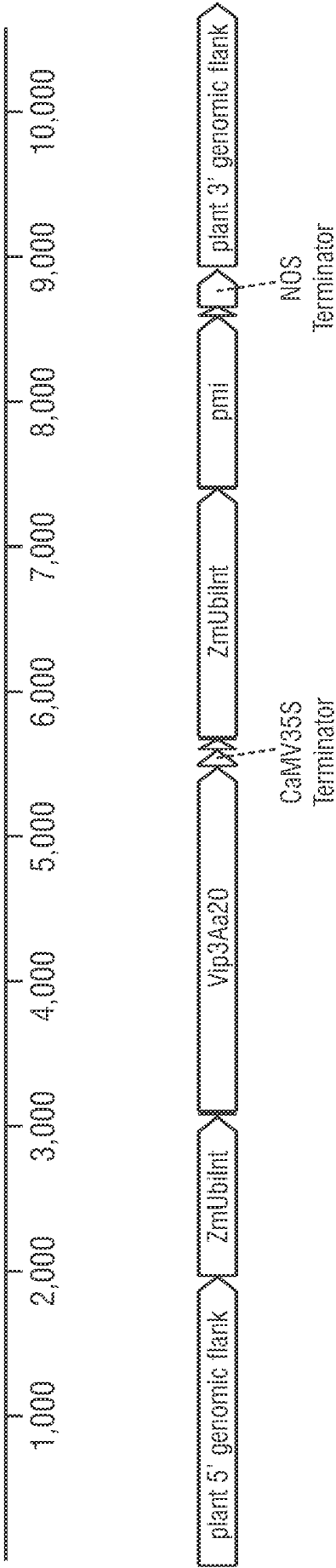


FIG. 5

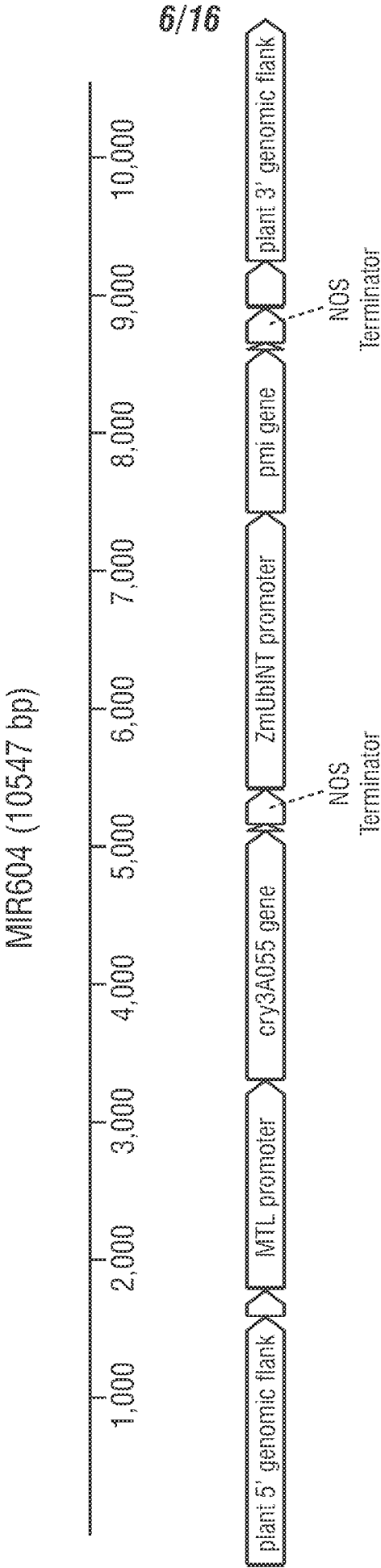


FIG. 6

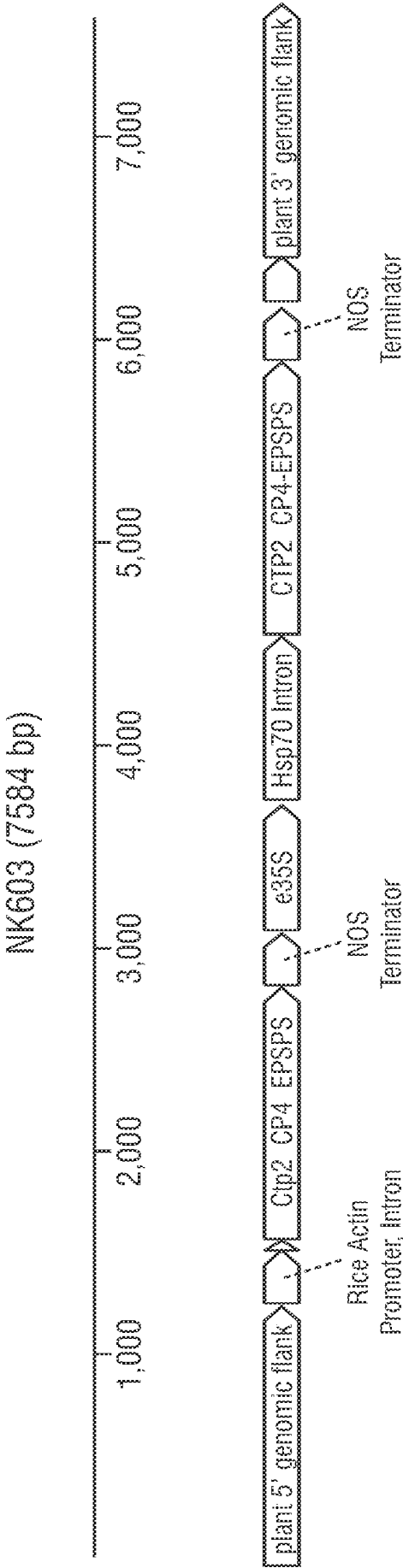


FIG. 7

SYN-E3272-5 (9067 bp)

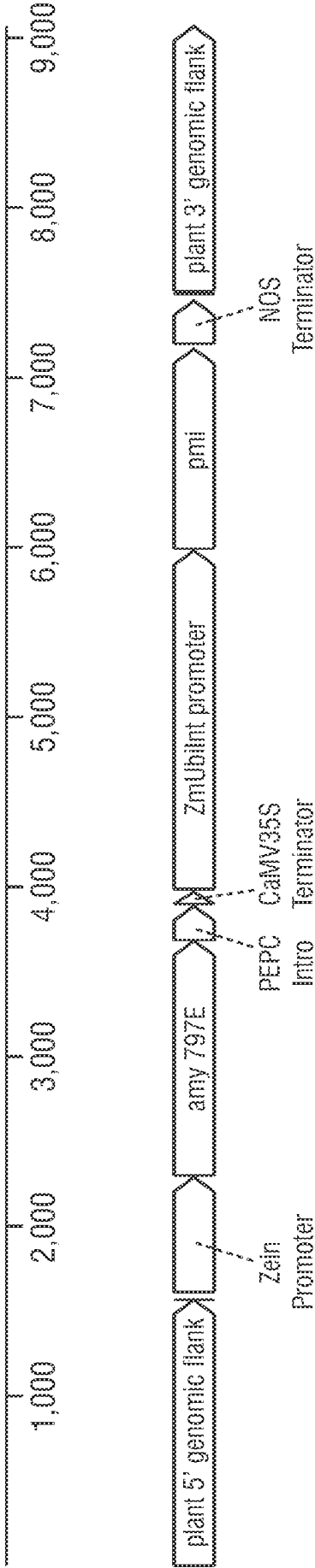


FIG. 8

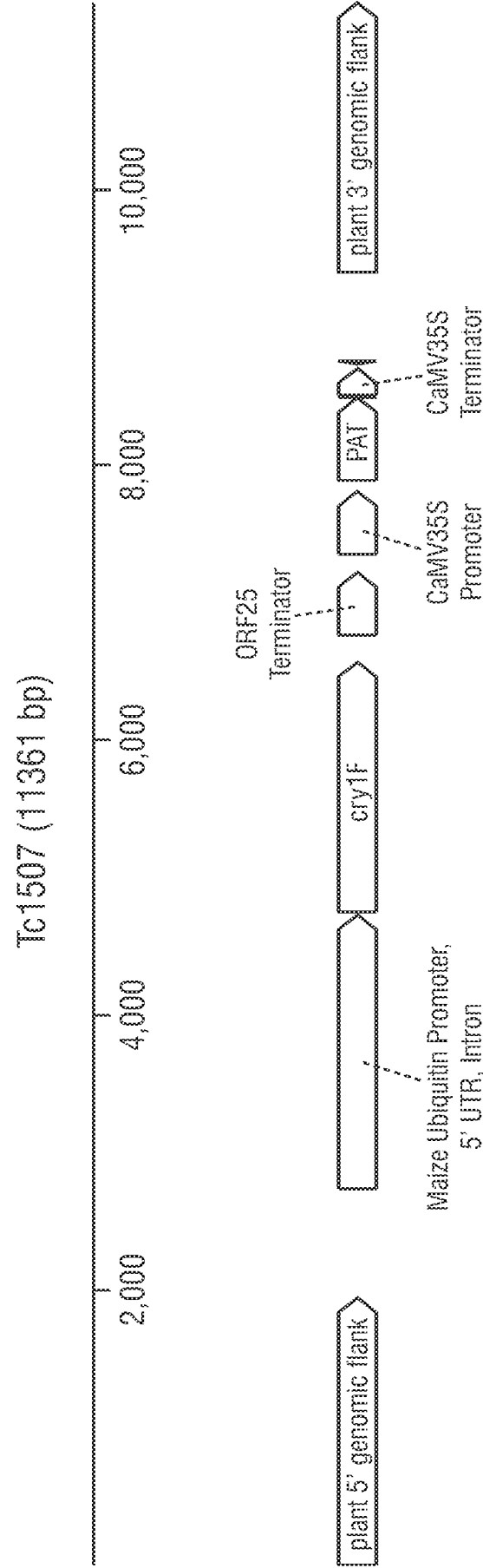


FIG. 9

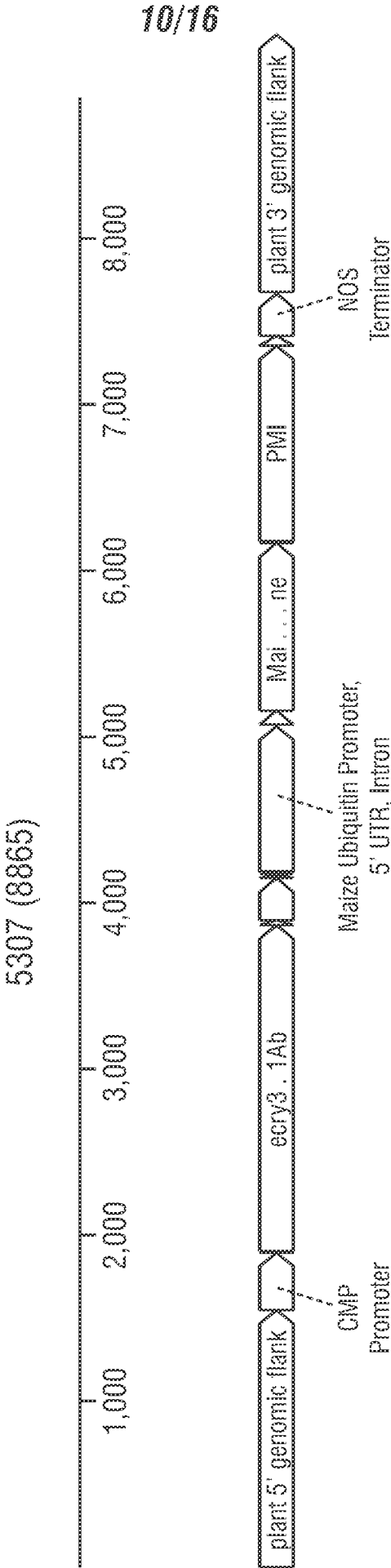


FIG. 10

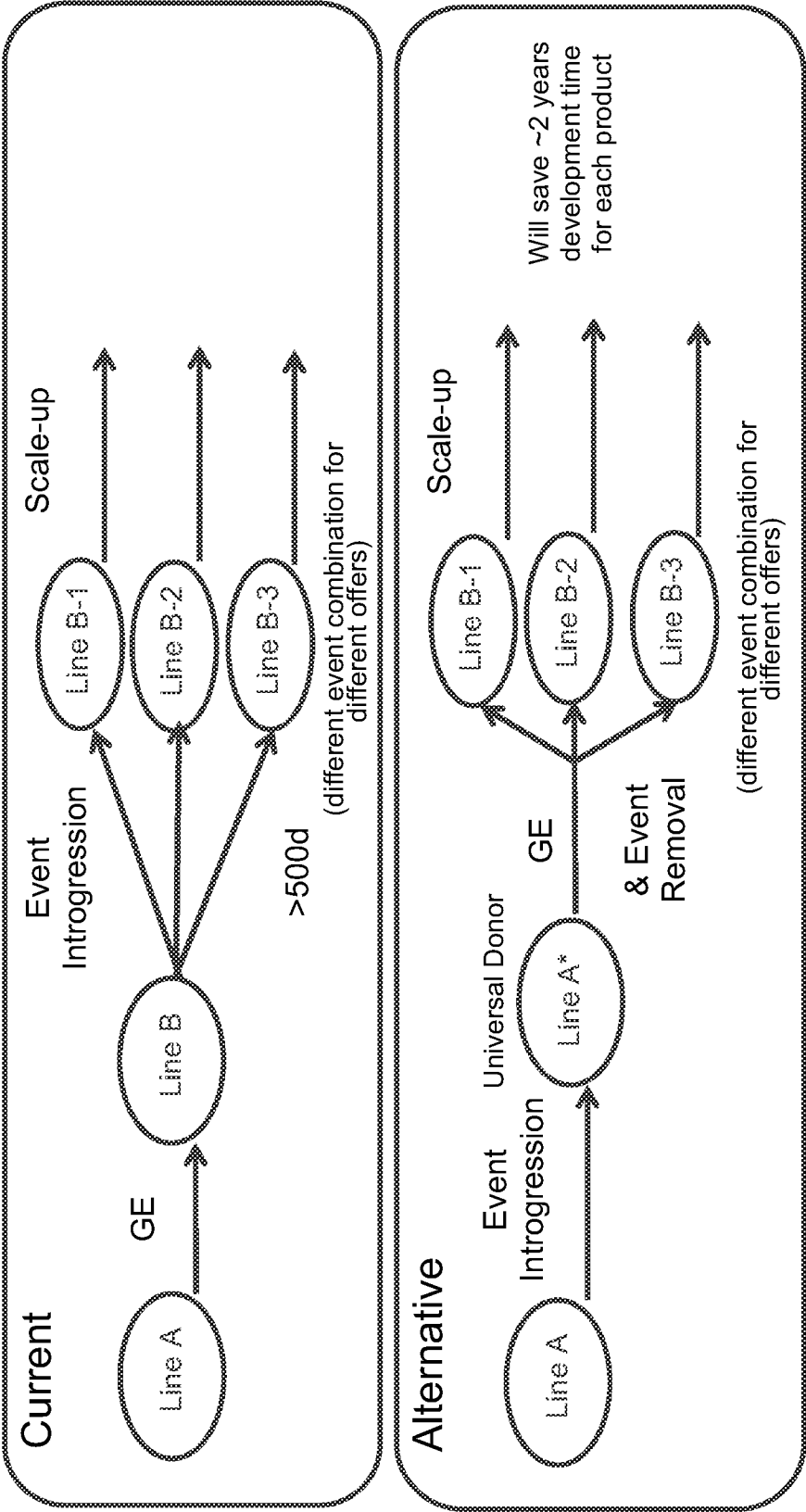


FIG. 11

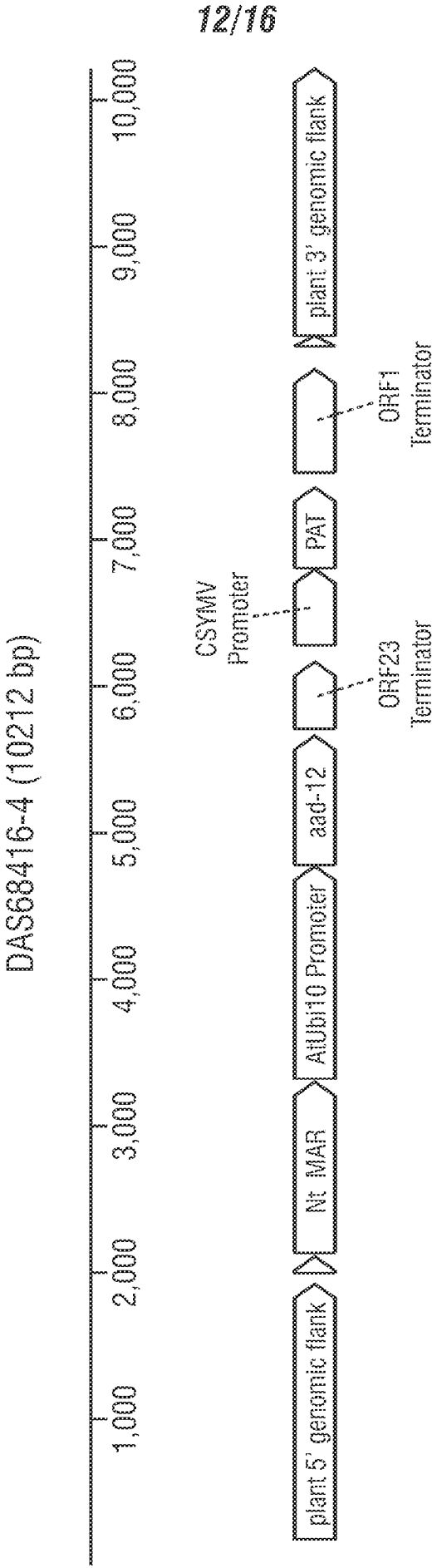


FIG. 12

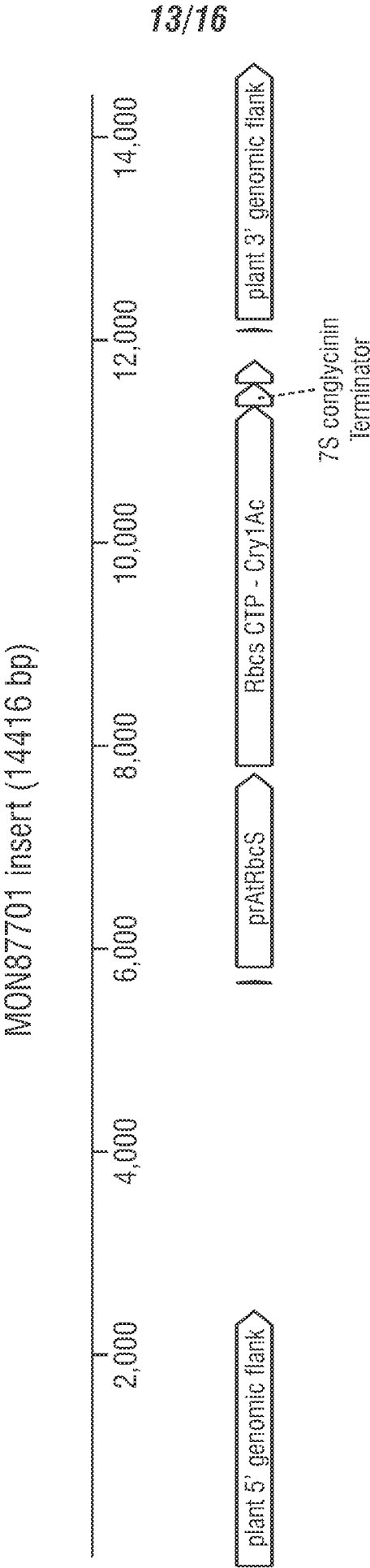


FIG. 13

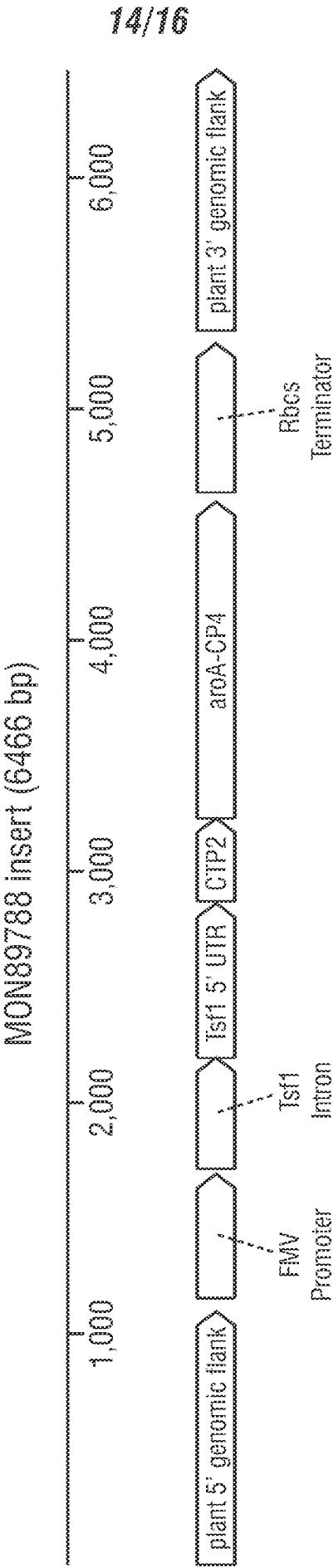


FIG. 14

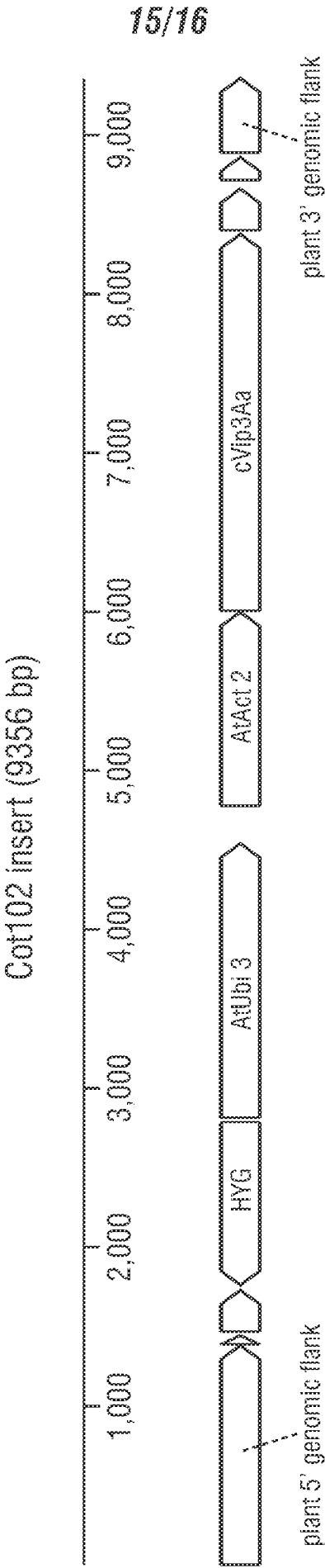


FIG. 15

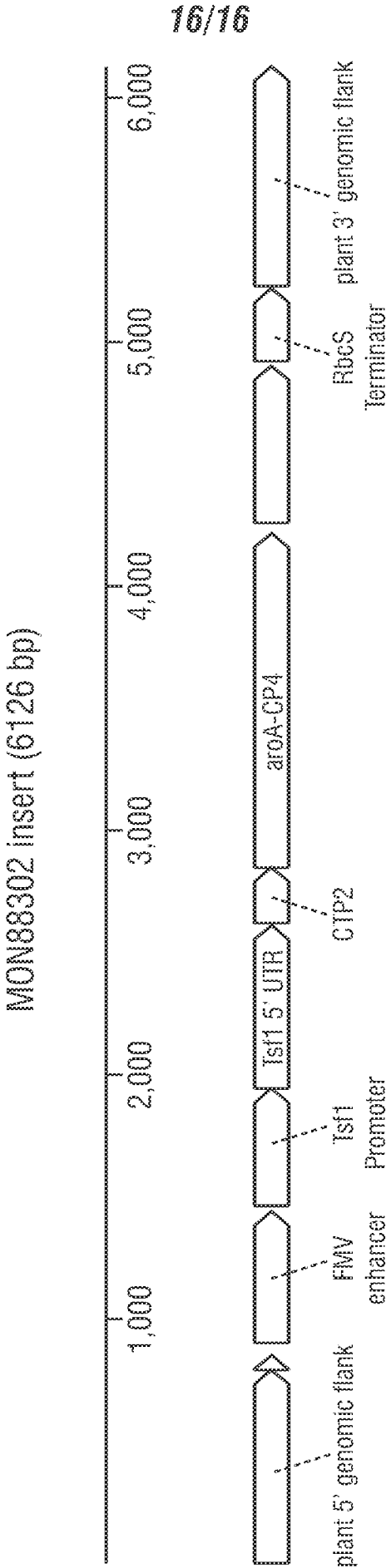


FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/43170

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/82; A01H 1/04; A01H 1/06; C12N 15/10 (2021.01)

CPC - C12N 15/8201; C12N 15/8205; C12N 15/8209; A01H 1/12; A01H 1/04; A01H 1/06; C12N 15/102; C12N 2310/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2019/0352655 A1 (INARI AGRICULTURE, INC.) 21 November 2019; paragraphs [0009], [0025], [0056], [0059], [0063], [0065], [0067], [0116], [0153], [0156], [0160], [0236], [0237], [0316], [0317], [0318], [0319], [0320]	24-25, 36-37 --- 26-35, 38-40 --- 1-8, 10-14
Y --- A	(STINE, HH) Request for Extension of Determination of Nonregulated Status to the Additional Regulated Article: Maize Line HCEM485. USDA Extension Petition (online). Stine. 14 February 2011 [retrieved on 8 December 2021]. Retrieved from the Internet: [URL: https://www.aphis.usda.gov/brs/aphisdocs/09_06301p_a3.pdf]; pages 1-2; page 1, 1st and 2nd paragraphs.	24-40 --- 1-8, 10-14
Y ---	(THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE, USDA) Approval of AgrEvo Request (98-014-01p) Seeking Extension of Determination of Non-regulated Status For Glufosinate Resistant Soybean Transformation Event A5547-127. USDA report (online). USDA. April 1998 [retrieved on 8 December 2021]. Retrieved from the Internet: [URL: https://www.aphis.usda.gov/brs/aphisdocs2/98_01401p_com.pdf]; pages 1-4; page 1, 3rd and 4th paragraphs.	26, 33, 38
Y ---	(THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE, USDA) Syngenta Petition 03-155-01p for Determination of Nonregulated Status for Lepidopteran Resistant Cotton Event COT102. USDA report (online). USDA. May 2005 [retrieved on 8 December 2021]. Retrieved from the Internet: [URL: https://www.aphis.usda.gov/brs/aphisdocs2/03_15501p_com.pdf]; pages 1-3; page 2, 1st paragraph; page 3, 2nd paragraph.	27, 34, 39

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 December 2021 (11.12.2021)

Date of mailing of the international search report

JAN 05 2022

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/43170

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(THE ANIMAL AND PLANT HEALTH INSPECTION SERVICE, USDA) Response to AgrEvo Petition 98-278-01 p for Determination of Nonregulated Status for Canola Transformation Events MS8 and RF3 Genetically Engineered for Pollination Control and Tolerance to Glufosinate Herbicide. USDA report (online). USDA. March 1999 [retrieved on 8 December 2021]. Retrieved from the Internet: [URL: https://www.aphis.usda.gov/brs/aphisdocs2/98_27801p_com.pdf]; pages 1-4; page 3, 2nd paragraph; page 4, 2nd paragraph.	28, 35, 40
Y	(DU, D et al.) Construction of Marker-Free Genetically Modified Maize Using a Heat-Inducible Auto-Excision Vector. Genes. 17 May 2019, Vol. 10, No. 374; pages 1-17; abstract; page 7, 2nd paragraph; figs. 3c and 4b; DOI: 10.3390/genes10050374	30
A	(US DEPARTMENT OF AGRICULTURE) BE Corn Crop Summary. USDA report (online). USDA. December 20, 2018 [retrieved on 10 December 2021]. Retrieved from the Internet: [URL: https://www.ams.usda.gov/sites/default/files/media/BECornCropSummary.pdf]; pages 1-5; page 5, 1st paragraph.	1-8, 10-14
A	(MOOKKAN, M et al.) Selectable marker independent transformation of recalcitrant maize inbred B73 and sorghum P898012 mediated by morphogenic regulators BABY BOOM and WUSCHEL2. Plant Cell Reports. September 2017, Epub 5 July 2017, Vol. 36, No. 9; pages 1477-1491; abstract; DOI: 10.1007/s00299-017-2169-1	1-8, 10-14
A	US 2010/0162428 A1 (BROWN, JA et al.) 24 June 2010; entire document	1-8, 10-14, 24-40

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/43170

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed:
 - ☐ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
 - ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/43170

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 9, 15-23, 41-43, 49-56, 75-77
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group I, Claims 1-8, 10-14, 24-40

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

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-Continued From Box No. III: Observations where unity of invention is lacking-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-8, 10-14, 24-40 are directed toward a method of producing an elite maize plant, the maize plant produced, DNA comprising an excision site in the plant, a biological sample from the plant, and a nucleic acid marker for detection therefor.

Group II, claims 44-48, 57-74 are directed toward an elite maize plant, the maize plant produced, DNA comprising an excision site in the plant, a biological sample from the plant, and a nucleic acid marker for detection therefor

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include PAM sites, not present in Group II; the special technical features of Group II include wherein all but at least one to 50 nucleotides of the heterologous DNA of the 5' and/or 3' DNA junction polynucleotide of a second approved transgenic locus is excised, not present in Group I.

Groups I and II share the technical features including: an elite maize plant or part thereof comprising at least one approved first transgenic locus and a transgenic locus excision site wherein a second approved transgenic locus is excised; DNA comprising a transgenic locus excision site wherein an approved transgenic locus is excised; a nucleic acid marker adapted for detection of genomic DNA or fragments comprising a transgenic locus excision site wherein an approved transgenic locus is excised and wherein the marker does not detect an approved transgenic locus which has not been excised; a biological sample comprising the plant genomic DNA or fragments thereof, said genomic DNA or fragments comprising a transgenic locus excision site wherein an approved transgenic locus is excised.

However, these shared technical features are previously disclosed by US 2003/0088081 A1 to Maliga, et al. (hereinafter 'Maliga').

Maliga discloses an elite maize plant or part thereof (an elite maize plant or part thereof; paragraph [0092], wherein the deletion of the marker is applied to the maize plant) comprising at least one approved first transgenic locus and a transgenic locus excision site wherein a second approved transgenic locus is excised (comprising at least one transgenic plastid locus (approved first transgenic locus) and a transgenic locus comprising a marker gene to be excised (excision site wherein a second approved transgenic locus is excised; paragraphs [0004], [0006]); DNA comprising a transgenic locus excision site wherein an approved transgenic locus is excised (plastid DNA comprising a heterologous marker to be excised (transgenic locus excision site wherein an approved transgenic locus is excised); paragraphs [0004], [0006]); a biological sample (a biological sample; paragraph [0018]) comprising the plant genomic DNA or fragments thereof (comprising amplified plant genomic DNA or fragments thereof; paragraph [0018]), said genomic DNA or fragments comprising a transgenic locus excision site wherein an approved transgenic locus is excised (said genomic DNA or fragments comprising a transgenic locus excision site wherein an approved transgenic locus is excised; paragraphs [0004], [0006], [0018]); and a nucleic acid marker adapted for detection of genomic DNA or fragments comprising a transgenic locus excision site wherein an approved transgenic locus is excised (a probe (nucleic acid marker adapted for detection of genomic DNA or fragments) complementary to (comprising) a transgenic locus excision site wherein an approved transgenic locus is excised paragraph [0088]). Maliga does not disclose a nucleic acid marker adapted for detection of genomic DNA or fragments comprising a transgenic locus excision site wherein an approved transgenic locus is excised and wherein the marker does not detect an approved transgenic locus which has not been excised. However, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to have modified the disclosure of Maliga to have specifically detected a desired excision product using a nucleic acid marker, such as a probe, rather than the absence of binding of a probe to the starting material, as a more accurate means of detecting a desired product.

Since none of the special technical features of the Groups I and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Maliga reference, unity of invention is lacking.