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U.S. DEPARTMENT OF AGRICULTURE

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Guide for Requesting a Confirmation of Exemption from Regulations under 7 CFR part 340

The information contained in this document is intended solely as guidance. Except where noted, persons may choose to follow APHIS guidance or follow different procedures, practices, or protocols that meet applicable statutes and regulations.

Language implying that guidance is mandatory (e.g., "shall," "must," "required," or "requirement") should not be construed as binding unless the terms are used to refer to a statutory or regulatory requirement.

Following the guidance contained in this document should not be construed as a guarantee of compliance with applicable statutes and regulations.

Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
United States Department of Agriculture

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GUIDE INFORMATION

ISSUING AGENCY/OFFICE:	Animal and Plant Health Inspection Service (APHIS)/ Biotechnology Regulatory Services (BRS)
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SUMMARY:	This document provides recommendations on preparing requests for confirmation of exemption from regulations under 7 CFR part 340. APHIS protects and enhances U.S. agricultural and natural resources using a science-based and risk-based regulatory framework to ensure the safe movement – including importation, interstate movement, and confined environmental release – of organisms developed using genetic engineering. APHIS receives its regulatory authority from the Plant Protection Act of 2000, and oversees organisms developed using genetic engineering in accordance with its regulations under 7 CFR part 340 (*Movement of Organisms Modified or Produced Through Genetic Engineering*).
DISCLAIMER:	The contents of this document do not have the force and effect of law and are not meant to bind the public in any way. This document isintended only to provide clarity to the public regarding existing requirements under the law or agency regulations.

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INTRODUCTION TO THE CONFIRMATION OF EXEMPTION PROCESS

APHIS regulations at 7 CFR part 340 govern the movement of organisms that are modified or produced through genetic engineering that could pose a risk to plant health. The regulations specify certain plants that are exempt from the regulations. A person may, but is not required to, request confirmation from APHIS that a plant is exempt from the regulations based on the provisions in § 340.1 of the regulations. APHIS will provide a written response within 120 days of receiving a sufficiently detailed confirmation request, except in circumstances that could not reasonably have been anticipated. Upon completion, APHIS will post confirmation requests and responses on the APHIS-BRS website, typically, within 1-2 business days of providing the response to the requestor, with any information claimed as Confidential Business Information (CBI) or personal identifying information redacted, as appropriate. APHIS is providing the following guide to help with preparing a request for confirmation of exemption from regulations in part 340. We recommend discussing your request for an exemption with APHIS prior to your first submission.

IMPORTANT DEFINITIONS

Gene pool - Germplasm within which sexual recombination is possible as a result of hybridization, including via methods such as embryo culture or bridging crosses (§ 340.3).

Mechanism of Action (MOA) - The biochemical process(es) through which genetic material determines a trait (§ 340.3).

Trait - An observable (able to be seen or otherwise identified) characteristic of an organism (§ 340.3).[1]

EXEMPTIONS

There are two categories of exemptions defined in § 340.1 of the regulations: 340.1(b) and 340.1(c).

EXEMPTION FOR PLANTS WITH MODIFICATIONS ACHIEVABLE THROUGH CONVENTIONAL BREEDING

The first category (**340.1(b)**) covers modified plants that could otherwise have been developed through conventional breeding techniques. This category covers plants with the types of modifications described below:

[1] Phenotype. A set of observable characteristics of an organism resulting from the interaction of its genotype with the environment. A genetic locus controlling a trait within a species can have two or more alleles producing different observable characteristics producing different phenotypes. For example, flower color is a trait, and red and white flower colors are two different phenotypes for the flower color trait. When referring to an organism's observable characteristics, trait and phenotype are sometimes used interchangeably, but because a phenotype is more specific, it is often more helpful.

(b)(1): A change resulting from cellular repair of a targeted DNA break in the absence of an externally provided repair template[2]

(b)(2): A targeted single base pair substitution[3]

(b)(3): The introduction of a gene known to occur in the plant's gene pool; or changes in a targeted sequence to correspond to a known allele of such a gene or to a known structural variation present in the gene pool[4]

(AM1): An indel or contiguous deletion of any size, made at a targeted location, with or without insertion of DNA if generated without using a repair template, or without insertion of DNA if generated using a repair template

(AM2): A plant with up to twelve (12) modifications, made simultaneously or sequentially, if each modification individually qualifies for exemption and occurs in a different gene

How to Apply and Count Modifications (See Appendix 1)

AM2 allows up to 12 modifications of the type described in (b)(1), (b)(2), (b)(3), and (AM1) to be made simultaneously or sequentially to a plant.

A modification made to a single allele counts as one modification. Functionally equivalent modifications made to a pair of alleles on homologous chromosomes also count as one modification (functionally equivalent modifications need not be identical). When all alleles of a given locus are modified, the maximum number of modified loci is:

- 12 in diploid plants
- 6 in tetraploid and triploid plants
- 4 in hexaploid and pentaploid plants
- 3 in octoploid plants

Case Study 1: Exemption of Brassica oleraceae longata (walking stick cabbage) possessing a CRISPR induced mutation of CAULIFLOWER.

An undergraduate researcher has developed a line of walking stick cabbage that possesses homozygous alleles of CAULIFLOWER (CAL, LOC106320120) with an 8-base pair (bp) deletion. The 8-bp deletion was obtained using CRISPR, and involved only one, highly specific guide RNA with a unique 20-bp target that corresponds to the region where the 8-bp deletion occurred. This plant would qualify for an exemption under § 340.1(b)(1) provided that no exogenous DNA remained in the final plant.

[2] 7 CFR § 340.1(b)(1). Examples of such changes are insertions, deletions, changes that result in both insertion and deletion during non-templated break repair, and non-templated base pair substitutions.
[3] 7 CFR § 340.1(b)(2). The single base pair substitution results from a templated repair.
[4] 7 CFR § 340.1(b)(3). Such changes generally involve the use of an externally provided template for repair and may involve the insertion and/or replacement of genetic material provided it aligns with a known gene, allele, or structural variant from the plant's gene pool without reshuffling of genetic material. Transgenes from previously reviewed plant-trait-MOA combinations that have been determined not to be regulated are not considered part of the plant's gene pool.

Depending on the number of alleles that are modified at a given locus, the number of modified loci in polyploid plants could exceed the number stated above provided the modified plant contains no more than 12 new modifications. (See examples in Appendix 1)

Additionally, there are at least three instances where multiple DNA breaks or edits can be made, and the resulting change can be "counted" as a single modification:

1. When two guide RNAs are used to cut out a contiguous portion of a gene or to otherwise make a single deletion of any size.
2. When multiple indels are created with one indel being functional while the other indels have no additional effect.
3. When a gene in the gene pool is inserted into the genome or an existing gene is edited several times to correspond to a gene or structural variant in the gene pool ((b)(3) modifications).

Please note: An external repair template cannot be used to make an AM1 modification across subgenomes in polyploid plants when the desired outcome requires making the exact indel or deletion. In this situation, the modification can only be made to one pair of homologous chromosomes and AM2 does not allow the same modification to be made to other pairs of homologous chromosomes. An external template can be used to make nonspecific indels or deletions across more than one subgenome under AM2, such as for gene inactivation where many different indels could result in the same desired outcome.

Successive Modifications to the Same Genetic Locus

Once a modification has been made to a particular genetic locus and that plant is not subject to the regulations under 7 CFR part 340, plants with successive modifications to the same genetic locus will not qualify for exemption because such modifications are not achievable through conventional breeding.

Hypothetical Modifications

APHIS no longer accepts confirmation requests for plants with hypothetical modifications of the types listed under § 340.1(b). Please use the confirmation request process only for plants you have produced that you believe meet the exemption criteria.

Case Study 2: Exemption of Brassica oleraceae longata (walking stick cabbage) possessing a CRISPR induced mutation of APETALA1.

A graduate researcher has developed a line of walking stick cabbage that possesses homozygous alleles of APETALA1 (BoAP1-a, LOC106298286) with a 40-bp deletion. The 40-bp deletion was obtained using CRISPR, and involved two, highly specific guide RNAs with unique 20-bp targets that correspond to the region where the 40-bp deletion occurred. This plant would not qualify for an exemption under § 340.1(b)(1) because to qualify, the change must result from "a" targeted DNA break. However, this plant would qualify for exemption under (AM1) provided that no exogenous DNA remained in the final plant.

EXEMPTION FOR PREVIOUSLY REVIEWED PTMOA (§ 340.1(C))

The second exemption category (**§ 340.1(c)**) covers plants with:

- a plant-trait-MOA combination (PTMOA) that is the same as that in another plant of the same species APHIS previously reviewed in response to a Regulatory Status Review (RSR) submission in accordance with § 340.4 and determined not to be regulated under 7 CFR part 340, or
- PTMOA combination that is the same as that in a plant of the same species APHIS determined to be not regulated in response to a petition submitted prior to October 1, 2021, pursuant to § 340.6 of the previous regulations found at 7 CFR part 340.

When determining whether an MOA combination is the same as that in another plant previously found by APHIS not to be regulated, it is important to recognize the same trait may be conferred by multiple distinct MOAs, and the same MOA can be conferred by distinct genes.

Case Study 3: Exemption of Brassica oleraceae longata (walking stick cabbage) possessing CRISPR induced double mutation of APETALA1 and CAULIFLOWER.

A post-doctoral researcher has developed a line of walking stick kale that possesses both a 55-bp deletion in CAULIFLOWER and a 62-bp deletion in APETALA1. Each deletion was obtained using CRISPR, and each involved two, highly specific guide RNAs with unique 20-bp targets that correspond to the region where the deletions occurred. This plant would qualify for exemption under (AM2) and would consist of two (AM1) modifications.

In the first case, although the same trait may be conferred by multiple distinct MOAs, these MOAs are not equivalent. For example, one MOA for resistance in plants to the herbicide glyphosate relies on inactivation of glyphosate by the protein glyphosate acetyl transferase (GAT), while a second MOA for resistance relies on an inability of glyphosate molecules to bind and inactivate an enzyme called 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS), which is responsible for an essential step in a biochemical pathway for the synthesis of certain amino acids. GAT and EPSPS catalyze different biochemical reactions and are distinct MOAs. Therefore, a glyphosate resistant plant that uses a gat gene would not provide a basis for exempting a glyphosate resistant plant of the same species that uses an epsps gene. As another example, a coleopteran resistance trait can be conferred to a modified plant by expression of a Cry protein or by expression of a hairpin RNA targeting corresponding ribonucleic acids (RNA) of an essential gene in a coleopteran pest. These are different MOAs. A plant modified to confer one of these MOAs would not be exempt from regulation solely because APHIS had previously determined a plant of the same species modified to confer the other MOA was not regulated.

Conversely, the same MOA can be conferred by discrete genes encoding functionally similar proteins from different species. For example, EPSPS-mediated glyphosate resistance has been developed using epsps genes from both corn (mepsps) and a strain of Agrobacterium (CP4 epsps). In both cases, the added gene encodes an EPSPS protein that does not bind to glyphosate. Both proteins catalyze the same biochemical reaction, and the MOAs are equivalent even though the two genes share a low level of sequence similarity. Therefore, a glyphosate resistant plant that uses an epsps gene encoding a glyphosate-insensitive EPSPS protein from one source would provide a basis for exempting a glyphosate resistant plant that uses an epsps gene encoding a glyphosate-insensitive EPSPS protein from another source.

When evaluating whether two PTMOA combinations are the same, APHIS considers whether the introduced sequences result in the same biochemical process. For example, if a specific biochemical reaction is catalyzed by different enzymes with the same enzymatic activity, the MOA is considered the same as long as the different enzymes do not catalyze any additional biochemical reactions that differ between them.

Developmental Context

A separate consideration when determining whether an MOA is the same as that in another plant previously found by APHIS not to be regulated is the concentration of the gene product across time and tissues. In some cases, previous plant pest risk analyses and Regulatory Status Reviews may have evaluated the MOA in the context of specific cell localization, tissues, developmental state, or protein levels. Please refer to the PTMOA table to determine whether and in what way variation in expression was considered as part of the MOA for purposes of applying the exemption in § 340.1(c).

Subspecies Specificity

There may be rare instances where plant pest risk could differ among subspecies of plant varieties modified with the same PTMOA combination. In those instances, APHIS will specify when the subspecies or variety is considered as part of the PTMOA for purposes of applying the exemption in § 340.1(c). Otherwise, once APHIS has determined a PTMOA is not regulated, that determination will apply to all subspecies and varieties of the plant.

ADDITIONAL CONSIDERATIONS FOR § 340.1(b) AND § 340.1(c)

Scope
Any plant not subject to the regulations in part 340 (because it is not modified, previously met the criteria for a regulatory exemption, or has completed the regulatory status review process or legacy petition process) may be modified in accordance with these exemptions and remain not subject to the regulations in part 340.

Breeding Stack
If different modifications that individually qualify for exemption from regulation are subsequently combined through conventional breeding, the resulting offspring will not be subject to regulation under § 340.1.

Molecular Stack
If a plant contains a PTMOA combination that is the same as, or a subset of, a PTMOA combination previously determined to be not regulated, the plant would not be subject to regulation under 7 CFR part 340.

UNINTENDED VERSUS OFF-TARGET MODIFICATIONS

APHIS considers the unintended retention of exogenous DNA inserted as part of the modification process to be an unintended modification (e.g., DNA encoding genome modification machinery such as the Cas9 protein). To qualify for exemption under § 340.1(b) there cannot be any retention of DNA that was intentionally or unintentionally inserted as part of the modification process unless that DNA is part of the gene pool. This includes vector sequences. Similarly, for § 340.1(b)(3) and AM2 involving § 340.1(b)(3) type modifications, only DNA from within the gene pool may be retained in the plant.

Additionally, APHIS considers modifications to DNA sequences that are highly similar to a target sequence as unintended modifications (e.g., sequences found in multigene families that have the same or highly similar sequences as the intended target, pseudogenes, or other conserved sequences), as those sequences are modified at frequencies exceeding background low-similarity promiscuous binding.

Off-target modifications occur at locations in the genome other than the intended and unintended target sites and are indistinguishable from background mutations. APHIS will not review off-target mutations that occur during development of an exempt plant. APHIS does not believe it is necessary to regulate off-target effects of genome editing in plants because (1) the off-target mutation rate from genome editing is low relative to the background mutation rate that occurs in conventional breeding, and (2) whatever changes do occur are likely to be segregated away from the target mutation during the breeding process. Instead, APHIS' review will focus on the targeted modification.

Case Study 4: Brassica oleraceae longata (walking stick cabbage) possessing CRISPR induced mutation and vector fragments.

A company has developed a line of walking stick kale that possesses CRISPR induced mutations in CAULIFLOWER and APETALA1. These changes cumulatively qualify for exemption under (AM2). Although the company has amply screened for the presence of the CRISPR vector used to make the plant line using PCR, breeding, and a selection screen, the company conducts due diligence and subjects their lines to oversight via a third party. The third party conducts whole genome sequencing and discovers a 100-bp non-coding fragment of Cas9 remaining in chromosome 1. This 100-bp fragment is exogenous DNA, and an unintended modification, and the plant lines therefore do not qualify for exemption.

§ 340.1(b)(2) and incidental silent mutations near the target site

In instances where two base pairs are changed within the same codon and the overall amino acid mutation could have been achieved by modifying only one of the base pairs, the modification is eligible for exemption under § 340.1(b)(2). For example, changing an asparagine residue (AAT or AAC) to either serine residue (AGC, AGT) by changing two base pairs (AAT to AGC or AAC to AGT) is a modification that could be claimed using § 340.1(b)(2) because changing a single nucleotide in AAT to AGT or AAC to AGC produces the same outcome. However, a modification involving a change from asparagine (AAT or AAC) to glycine (GGN) would not be eligible for exemption under § 340.1(b)(2) because this modification could not occur by substituting a single base pair.

SUBMITTING A REQUEST FOR CONFIRMATION OF EXEMPTION

If you are seeking confirmation that your plant is exempt from the regulations in 7 CFR part 340, you must electronically submit (via ConfirmationRequests@usda.gov) your confirmation request as a letter containing the information described below to:

Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services

Your letter must include the following information:

- Requestor's Name, Contact Information, and Email address
- The plant's common name, genus, species, and, if relevant, subspecies or ecotypes.
- A brief description of the plant's ploidy.
- A clear statement of the exemption you are claiming and why the plant qualifies for that exemption. Specifically include references to § 340.1(b)(1), § 340.1(b)(2), § 340.1(b)(3), (AM1), (AM2) or § 340.1(c).
    - When claiming (AM2), provide a list or table that enumerates the type of each individual modification (e.g., (b)(1), (b)(2), (b)(3), (AM1). Please number (e.g.,1-12) and include sufficient detail to demonstrate how each modification meets the criteria of the claimed provision. Chromosome maps or similar figures can be helpful in complex situations
    - In general, because (AM1) allows the modification described in (b)(1) and provides additional flexibilities, developers may wish to claim (AM1) for any indel or deletion.
- A description of the trait. It is helpful to include a description or image of the actual phenotype(s) of the plant.
- A description of the actual genetic modification in the plant. This description must be sufficient to enable APHIS to confirm the plant is eligible for the exemption, including:
    - When claiming exemption for plants with (b)(1), (b)(2), or (AM1) modifications, describe the type of genetic modification (e.g., insertion, deletion, single base pair substitution, as applicable), the targeted gene or genetic element, and the method used to make the modification.
    - When claiming exemption for plants with (b)(3) modifications, describe the type of genetic modification, the gene or genetic or structural element, the donor organism or the organism on which the modification is based, and the method used to make the modification. Your submission must include information that demonstrates the modification exists in the gene pool and that the resulting modification in the recipient organism is consistent with the original genetic context.

  - For exemptions under § 340.1(c), the trait(s) and associated MOA(s), including a molecular description of the inserted genetic material and method used to produce the modification.[5]
- The scientific methodology used to verify the plant qualifies for the specified exemption. The details of the methodology must provide information sufficient to enable APHIS to assess reliability and specificity, including a description of the design or verification steps taken to anticipate, reduce, and monitor unintended modifications of highly similar sequences. Details of the methodology used to confirm the absence of exogenous DNA are also particularly important.

Your letter requesting confirmation of exemption from regulations under 7 CFR part 340, may also include the following optional information and data you deem necessary to substantiate your request:

- The function of the modified gene or genetic element, and consequences of its loss or altered function.
- Molecular characterization data (e.g., PCR, Southern blots)
- DNA sequence data
  - Sequence data should encompass the modification
  - Clearly illustrated sequence alignments are encouraged
  - Any sequencing strategy and methodology should be clearly presented

CONFIDENTIAL BUSINESS INFORMATION

If your confirmation request, as well as any follow-up documentation you provide, does not contain Confidential Business Information (CBI), it must be marked "**No CBI.**" If your confirmation request, as well as any documentation you provide, contains CBI, you must submit a CBI copy, a CBI-deleted copy, and a CBI justification, as detailed in the Guide for Submitting Confidential Business Information and in accordance with 7 CFR 340.7.

For additional questions about CBI and CBI formatting, please contact the BRS Document Control Officer:

Joseph Tangredi
301-851-4061
joseph.tangredi@usda.gov

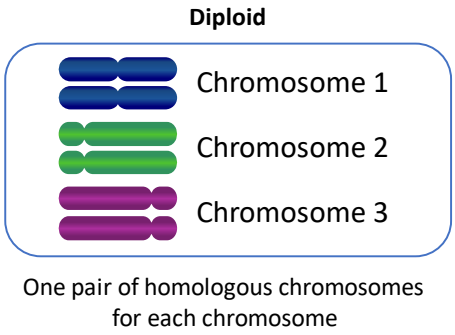
[5] The molecular description could be a list or table identifying the genetic elements introduced into the plant sufficient for APHIS to be able to confirm that the PTMOA combination is the same as, or a subset of, a combination previously determined to be not regulated. The genetic elements need not be identical to those used in the previous combination (e.g., a different promoter or a different gene could be used), as long as the same MOA is conferred.

VERSION HISTORY

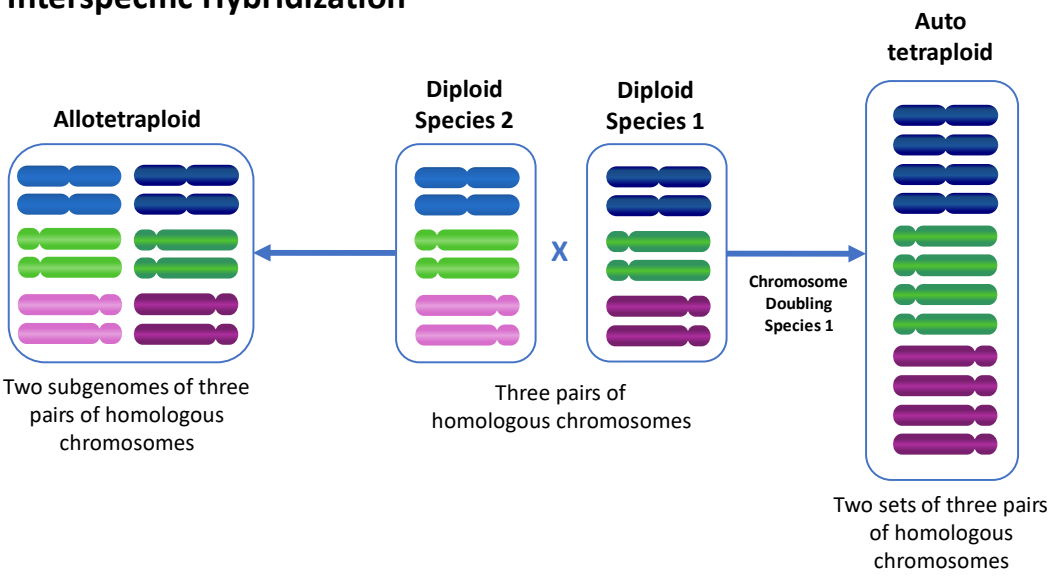
November 7, 2024	Guide for Requesting a Confirmation of Exemption from Regulations under 7 CFR part 340, updates to reflect additional exemption modifications
August 31, 2022	Guide for Requesting a Confirmation of Exemption from Regulations under 7 CFR part 340, minor updates
June 18, 2020	Guide for Requesting a Confirmation of Exemption from Regulations under 7 CFR part 340

APPENDIX I - VISUAL GUIDE TO MODIFICATIONS

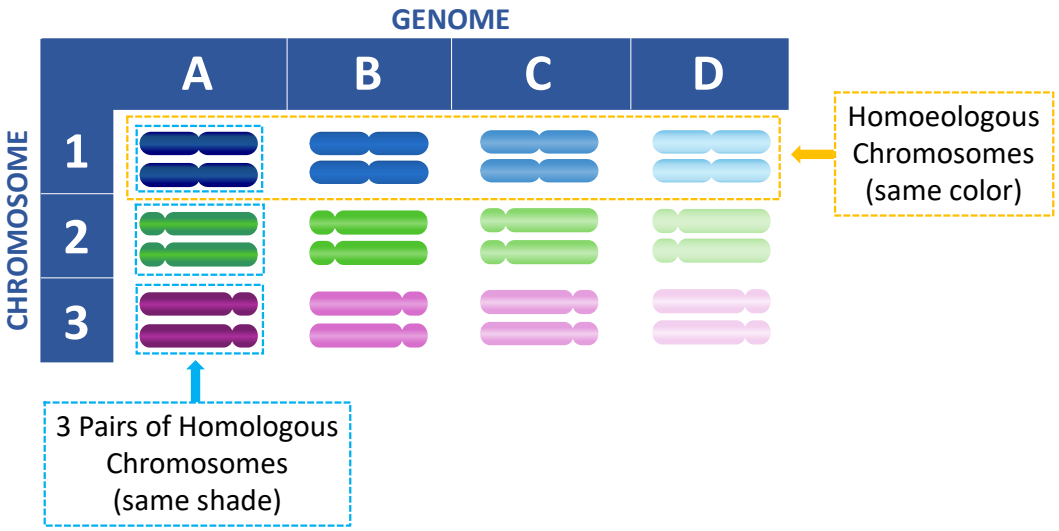
Hypothetical Plant with Three Chromosomes



Interspecific Hybridization

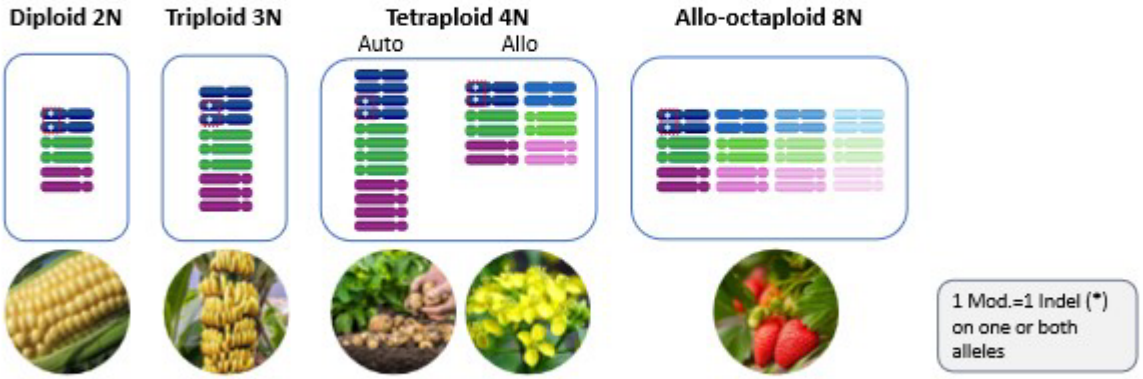


Allopolyploid



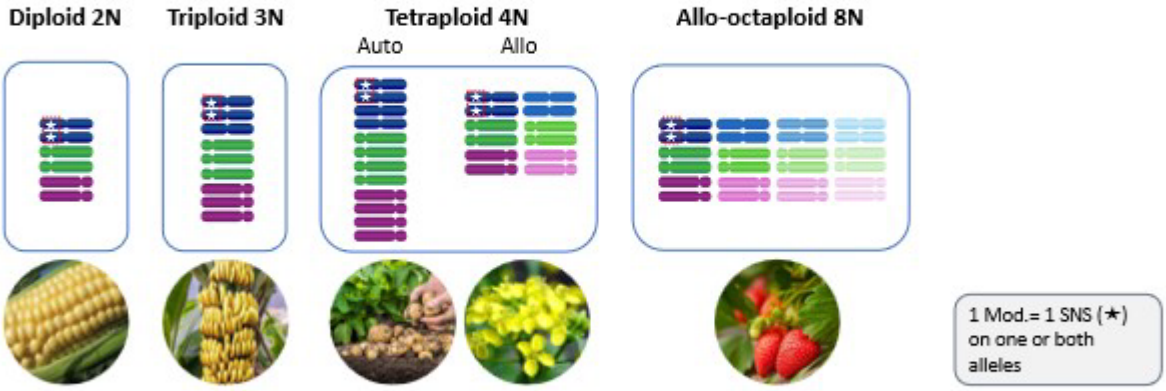
(b)(1)-A targeted DNA break no repair template (i.e., an indel modification)

Only one cut - No external template - One pair of homologous chromosomes



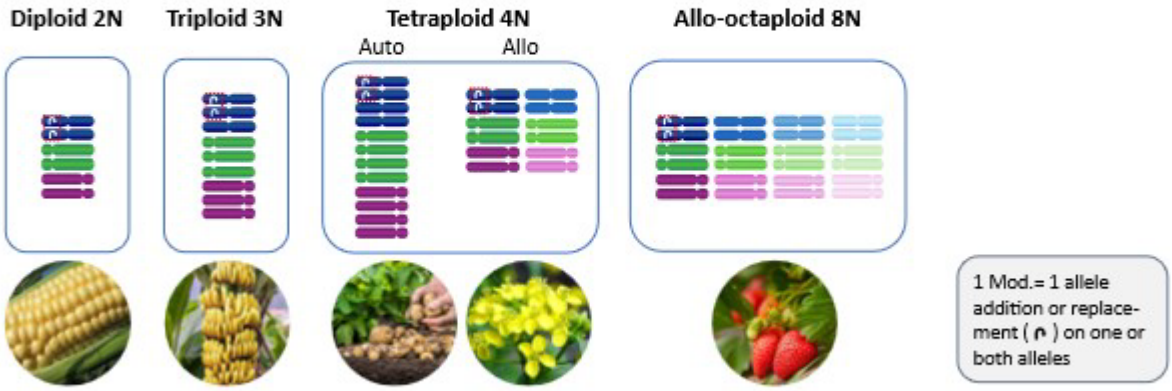
(b)(2)-A Targeted Single Base Pair Substitution

External template allowed - One nucleotide change - One pair of homologous chromosomes



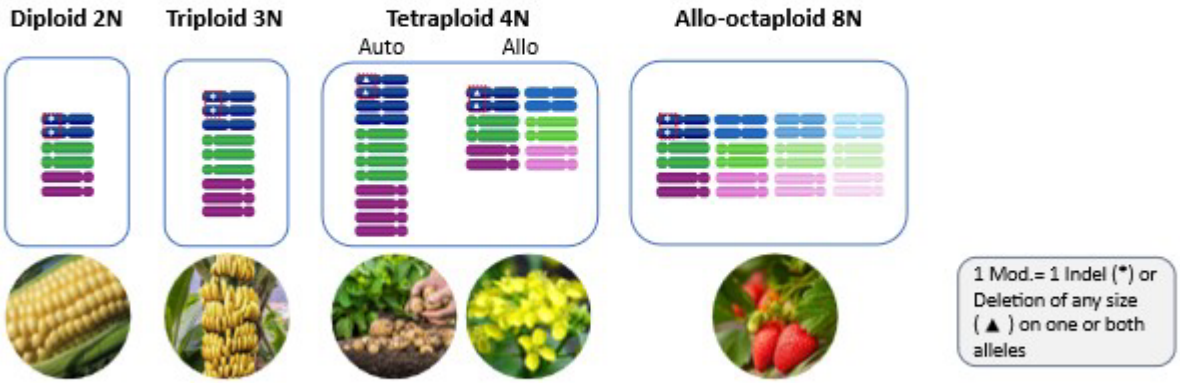
(b)(3)-Introduction of Gene or Structural Variant from the Plant's Gene Pool

External template allowed - No limit on edits to recreate gene - One pair of homologous chromosomes

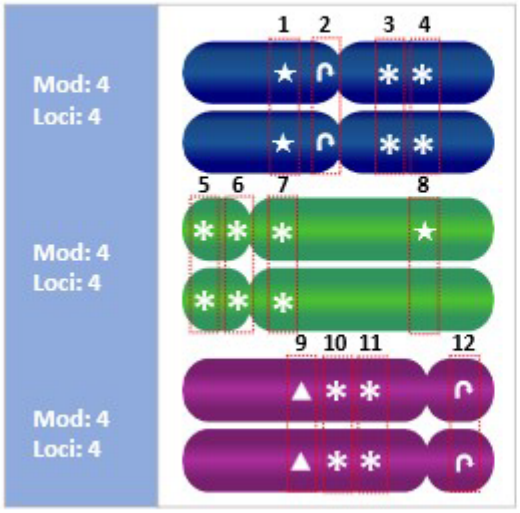


AM1- A Targeted Indel Modification or Deletion of any Size

Single targeted location, Indels on both alleles need not be identical , External repair template for deletions



AM2 Up To Twelve Modifications



AM2: AM1(8), ((b)(2))(2), ((b)(3))(2)

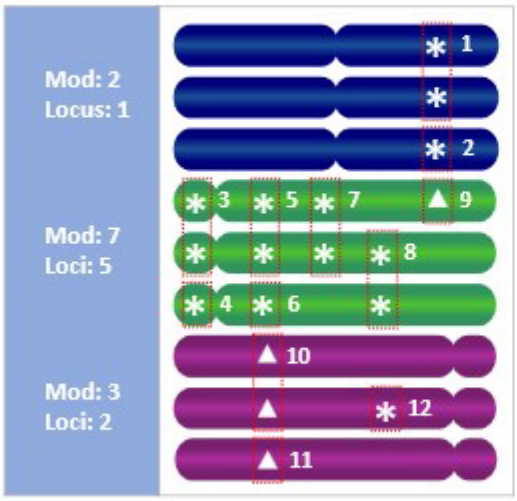
1. AM1 (indel)
2. (b)(3) (allele replacement)
3. AM1 (indel)
4. AM1 (indel)
5. AM1 (indel)
6. AM1 (indel)
7. AM7 (indel)
8. (b)(2) (base pair substitution)
9. AM1 (deletion)
10. AM1 (indel)
11. AM1 (indel)
12. (b)(3) allele replacement

Diploid 2N

One modification on one or both alleles

- Indel (AM1)
- ▲ Deletion of any size (AM1)
- ★ Single base pair substitution(b)(2)
- ∩ Allele replacement(b)(3)

AM2 Up To Twelve Modifications



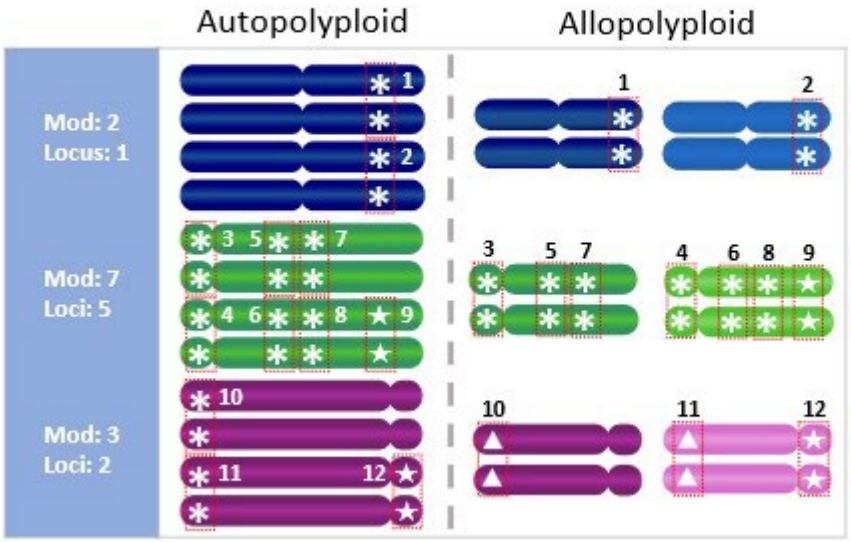
AM2: AM(1)(12)
1. AM1 (indel)
2. AM1 (indel)
3. AM1 (indel)
4. AM1 (indel)
5. AM1 (indel)
6. AM1 (indel)
7. AM1 (indel)
8. AM1 (indel)
9. AM1 (deletion)
10. AM1 (deletion)
11. AM1 (deletion
12. AM1 (indel)

Triploid 3N

One modification on one or both alleles

- Indel (AM1)
- Deletion of any size (AM1)

AM2 Up To Twelve Modifications



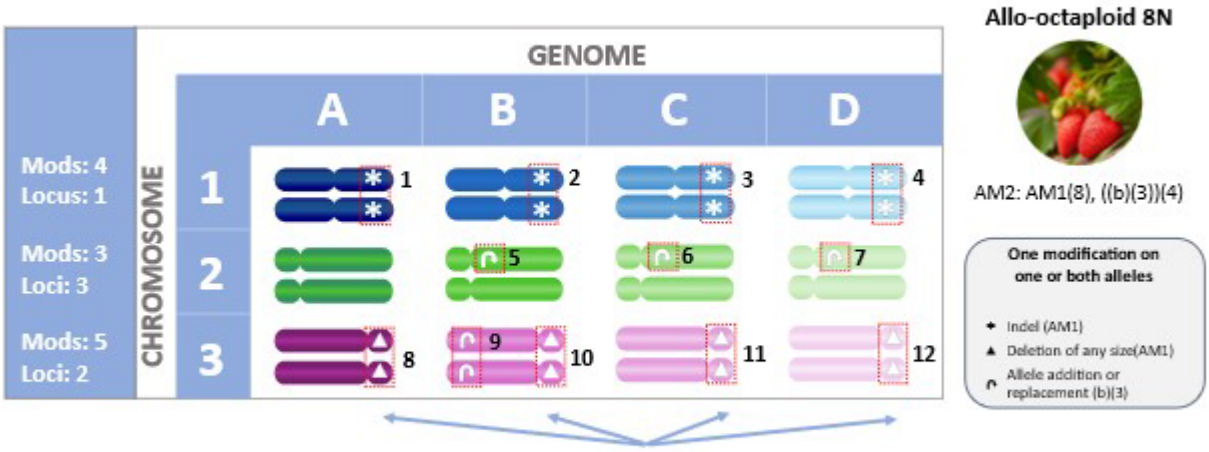
Tetraploid 4N

AM2: AM1(10), ((b)(2))(2)

One modification on one or both alleles

- Indel (AM1)
- Deletion of any size (AM1)
- Single base pair substitution(b)(2)

AM2 Up To Twelve Modifications



Note: An external repair template cannot be used to make an AM1 modification across subgenomes in polyploid plants when the desired outcome requires making the exact indel or deletion