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March 27, 2024

Bernadette Juarez
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Contains Confidential Business Information

Subject: Request for Regulatory Status Review of Gene Edited Blackberry

Dear Ms. Juarez,

Pairwise Plants Services, Inc., a food technology company based in Durham, North Carolina, USA, respectfully requests a Regulatory Status Review under 7 CFR § 340.4(a)(1) of a Pairwise product produced using CRISPR/Cas gene editing technology and described in detail below.

The following description has been provided to assist in your review. Please do not hesitate to contact us if you have questions or need additional information.

Thank you in advance for your attention to this request.

Sincerely,

A handwritten signature in black ink that reads "Daniel Jenkins".

Dan Jenkins, M.S., J.D.
Vice President, Regulatory and Government Affairs
Pairwise

**Part I: Requestor**

Pairwise Plants Services, Inc
Attn: Dan Jenkins, M.S., J.D.
Vice President, Regulatory and Government Affairs
807 East Main Street, Suite 4-100
Durham, NC 27701
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+1 (919) 321-0820

Part II: Confidential Business Information (CBI) Statement

Pairwise Plants Services, Inc. (hereafter the "Company") has marked certain information in this Regulatory Status Review as confidential business information (CBI). Information marked by the Company as CBI is exempt from release and/or public disclosure under Freedom of Information Act (FOIA) Exemption 4, 5 U.S.C. Section 552(b)(4), and is prohibited from release pursuant to the Trade Secrets Act, 18 U.S.C. Section 1905. Release of information marked by the Company as CBI would provide competitive information about the nature of the research, development, and commercialization plans of the Company and could jeopardize protection of its intellectual property rights. Access to this information would cause competitive and/or financial harm to the Company.

Information claimed as CBI is consistent with the Guidance for Claiming Confidential Business Information (CBI) in Submission to USDA APHIS BRS (Document ID BRS-GD-2020-0004) v. December 20, 2021, found at https://www.aphis.usda.gov/brs/pdf/CBI_Submission_Guidance.pdf and include, but are not necessarily limited to the following:

Genotype, phenotype, mechanism of action, gene or genetic sequence names, gene descriptions, including the function of the native sequence in blackberry or other plant species, and supporting citations which disclose or allude to such information.

Sequence information, including DNA and amino acid sequences, whole or partial, of the target sequence, including the wild type and the modified sequences.

Information in each of these categories constitutes trade secrets related to the design, production, and benefit of edited blackberry which was developed at the expense of the Company. This information is commercially valuable, is used in the Company business, and is held by the Company as strictly secret. The release of this information would cause harm to the Company and provide a competitive advantage to others.

Part III: Description of Domesticated Blackberry, the Comparator Plant

Family: Rosaceae

Genus: *Rubus* L.

Subgenus: *Rubus*

Common name: Domesticated varieties of blackberry¹

The domesticated varieties of blackberry do not include *R. fruticosus*, wild blackberry, or other federally listed noxious weed *Rubus* species.

The focus of this Regulatory Status Review is on domesticated varieties of blackberry (*Rubus* subgenus *Rubus*) intentionally developed as part of academic, government, and/or industry led breeding efforts. Examples of such varieties can be found in plant patents for new varieties of domesticated blackberry, which typically describe the new variety using the nomenclature *Rubus* subgenus *Rubus* (Clark and Finn, 2011; Hall and Funt, 2017), although the list of domesticated blackberry varieties found in plant patents is not exhaustive. Domesticated varieties of blackberry are biologically distinct from domesticated varieties of red raspberry (*Rubus idaeus*) and black raspberry (*Rubus occidentalis*). Taxonomic delineation between blackberry and raspberry varieties is defined by the fruit structure, specifically that in *Rubus* the fruit, called a drupe, consist of a number of fleshy drupelets, each containing a single seed, structured around a central torus, or receptacle. In blackberries the torus is picked with the fruit, maintaining fruit integrity, whereas in raspberries (*Rubus* subgenus *Idaeobatus*), the torus remains on the plants upon fruit removal (Clark et al., 2007).

The genus *Rubus* includes blackberry, dewberry, and raspberry species as well as their hybrids. There are more than 700 *Rubus* species/varieties distributed across North and South America, Europe, Africa, and Asia (Lu and Boufford, 2003) which can be found in nature as wild species or as varieties which have been developed as part of breeding programs for commercial production purposes. Of the several hundred *Rubus* species, only a few have been utilized in breeding programs which have resulted in the purposeful development of domesticated blackberry varieties (Thompson, 1995). The genus *Rubus* is divided into 15 subgenera. *Rubus* subgenus *Rubus* encompasses all blackberry species/varieties, wild and domesticated, and is further divided into twelve sections (Hall and Funt, 2017; Watson, 1958), often classified by growth habit and geographical range, with ten of the twelve sections having ranges in North America. Taxonomic classification of *Rubus* species is complicated due to interspecific hybridizations, polyploidy, union of nonreduced gametes, and various forms of apomixis (Meng and Finn, 2002; Thompson, 1995). Slight variations tend to persist and be recognized as different species or hybrids, and not simply different varieties. This coupled with inconsistent nomenclature and records describing new varieties, makes species level classification of some domesticated blackberry varieties nearly impossible.

Efforts to breed domesticated blackberry varieties from wild species began in the 1800's (Hedrick, 1925; Stafne and Clark, 2005). Blackberry breeding programs have predominately utilized blackberry species/varieties from sections *Allegheniensis*, *Arguti*, *Rubus*, and *Ursini* (Clark and Finn, 2011) with the four species *Rubus allegheniensis*, *Rubus argutus*, *Rubus ulmifolius*, and *Rubus ursinus* being the primary contributors to the development of modern domesticated blackberry varieties grown in North America (Clark and Finn, 2011; Stafne and Clark, 2004). None of these four species are listed as noxious weed

¹Domesticated blackberry varieties are distinguishable from *Rubus fruticosus*, wild blackberry, and *Rubus moluccanus*, wild raspberry, which are wild relatives native to Eurasia (Hall and Funt, 2017).

species of *Rubus* (USDA, 2010). Cross-pollination between varieties is possible, however, crosses between diploid and tetraploid varieties, or crosses between species/varieties of different subgenera, often lead to sterility (Clark and Finn, 2011). This could be due to the fact that the offspring resulting from these crosses are likely to have odd number ploidy (e.g., triploid) which often have poor fertility (Meng and Finn, 2002). *Rubus* subgenus *Rubus* species/varieties range in ploidy from $2n = 2x = 14$, in the diploid state, up to $12x$, or possibly higher (Meng and Finn, 2002). *R. allegheniensis*, *R. argutus*, and *R. ulmifolius* are parental contributors to domesticated varieties of eastern tetraploid blackberry ($2n = 4x = 28$), with *R. allegheniensis* being the major parental contributor. Western cultivated blackberry varieties are predominately derived from *R. ursinus* as the major parental contributor with genetic contributions from other *Rubus* species and these domesticated varieties can vary in ploidy. In a survey of wild blackberry populations on the west coast of the United States, examples of hybridization between wild species and domesticated blackberry varieties were rarely detected. When hybrids were identified the wild species was the pollen donor suggesting gene flow from domesticated varieties of blackberry to the wild species has a low likelihood (Clark and Jasieniuk, 2012; Clark and Jasieniuk, 2017). This would also suggest that the wild species predominantly reproduce asexually either by apomixis or vegetative spread (Clark and Jasieniuk, 2012). Asexual reproduction by apomixis is a trait commonly found in invasive species and is not found in domesticated varieties, based on lack of spread (Hall and Funt, 2017).

Domesticated varieties of blackberry are distinguishable from the federally listed noxious weed *Rubus* species *R. fruticosus*, wild blackberry, and *R. moluccanus*, wild raspberry, (USDA, 2010). The main points of differentiation between domesticated blackberry varieties and the *Rubus* weed species is that the domesticated varieties exhibit physical characteristics favorable to commercial fruit production, such as having a more upright and open plant architecture, having fruit that is easily picked, and some domesticated varieties are thornless, while the wild species are characterized as prickly, woody shrubs with biennial semi-erect canes that form a dense thicket that can outcompete native species (CABI, 2022; EPPO, 2022; Hall and Funt, 2017). *R. fruticosus* is not listed as being native to the United States (USDA, 2022a) and can range in ploidy from $2x$, $3x$, and $4x$ (Clark and Finn, 2011). Cross-pollination between diploid or triploid *R. fruticosus* and domesticated varieties of blackberry is possible, however, crosses between diploid and tetraploids varieties, or crosses between species/varieties of different subgenera, often lead to sterility (Clark and Finn, 2011). This could be due to the fact that the offspring resulting from these crosses are likely to have odd number ploidy (e.g., triploid) which often have poor fertility (Meng and Finn, 2002). Additionally, the wild species demonstrate more pervasive and invasive spreading than domesticated varieties. This was exemplified at the HortResearch facility in Riwaka, New Zealand, where observations of a collection of wild and domesticated *Rubus* found that the apomictic wild species spread into the local environment while no spread was observed for the domesticated varieties which were not apomictic (Hall and Funt, 2017).

While domesticated blackberry varieties are self-fertile, with flowers comprised of both male (stamens) and female (pistils) parts, modern fruit production practices have implemented the use of introduced pollinators, particularly honeybees, about 3-4 hives per 100,000 feet² (1 hectare) (Cane, 2005), to increase pollination events and ensure a productive and profitable fruit harvest. Domesticated blackberry breeders and commercial propagators rely on asexual (vegetative) propagation methods to produce clones of desirable varieties. Various methods can be employed such as tip layering, cane cuttings (soft and hard), root cuttings, and tissue culture (Caldwell, 1984; Takeda et al., 2011). Desirable traits being introduced into domesticated blackberry varieties include modifying fruit shape and size, removing thorns, reducing seed size, altering growth habit, expanding production timing, and increasing

yield. Blackberry breeding programs also focused on expanding growing environments as well as disease resistance and overall plant quality (Clark and Finn, 2011; Threlfall et al., 2016).

The focus of this RSR is a domesticated blackberry with product quality and agronomic traits resulting in a plant that is seedless, thornless, and has an []. These phenotypes have already been developed through traditional breeding methods in domesticated varieties of blackberry or in related species. The seedless trait has been developed through traditional breeding methods and introduced in crop species in the family Rosaceae, of which blackberry is a member. The traits for thornless and [] have been developed using traditional breeding methods to introduce these naturally occurring traits into domesticated blackberry varieties. Example varieties from the University of Arkansas program that have both the thornless and [] traits include []

[]. When considering the genetic compatibility between wild *Rubus* and domesticated varieties factors such as ploidy and the presence of the apomictic trait in wild *Rubus* should be considered as challenges to outcrossing. However, even if such outcrossing were to occur, the product quality and agronomic traits presented in this RSR for a domesticated blackberry variety have already been produced, or could be produced, in varieties of domesticated blackberry using traditional breeding methods.

Part IV: Genotype of the Modified Plant

The blackberry described in this RSR contains modifications that have been made to multiple native sequences within the plant.

1. Nature of the Modifications

Multiple modifications (Table 1) have been made to domesticated blackberry. The modifications for the product quality trait are intended to produce a blackberry that is seedless. Botanically speaking, blackberries contain a seed that is surrounded by a lignified endocarp. The term “seedless” is being used in the context of this RSR to refer to the entire structure of the seed and endocarp and is not expected to result in a complete physical loss of this structure but rather a shrinking or softening of this structure as is found in other seedless varieties, like table grape (*Vitis vinifera* L.) []

[]. There are two agronomic quality traits, the modifications for which are intended to produce a blackberry that is thornless and [] trait both of which are found in domesticated varieties of blackberry produced using traditional breeding.

Botanically speaking, blackberries do not have thorns, they have prickles, also called spines, which are similar to trichomes. The term “thorn” or “thornless” is being used in the context of this RSR to refer to the outgrowths on blackberries. Botanically speaking, [] refers to the ability of the []

[] trait has been developed using traditional breeding to [] of blackberries and this naturally occurring trait is being used to model the [] trait in modified blackberry. Modification providing in vitro selection traits, for which there would be no selection pressure applied in a commercial production setting, are also considered in this RSR.

The phenotypes associated with the proposed modifications are reduced [], reduced thorn development on stems, [], as well as a selection trait based on insensitivity to an antibiotic, which is intended to be used to identify plants during the development process but is not intended to be used in commercial blackberry cultivation. The genetic differences between the modified

domesticated blackberry relative to a comparator plant are alterations to existing endogenous genetic sequences that could introduce non-templated deletions, insertions, inversions, and/or base pair substitution(s) resulting in reduced or complete loss of function of the native protein. See Part V for a mechanism of action discussion.

Table 1. Summary of modified domesticated blackberry.

Trait	Phenotype	Function of Native Sequence	Mechanism of Action
Product Quality	Soft Seeds	NAC transcription factor associated with [] biosynthesis pathway	Loss or reduction of DNA binding capacity of transcription factor [] ¹
		NAC transcription factor associated with [] biosynthesis pathway	Loss or reduction of DNA binding capacity of transcription factor []
		NAC transcription factor associated with [] biosynthesis pathway	Loss or reduction of DNA binding capacity of transcription factor [] ¹
Agronomic Quality	Reduced thorn development on stems	bHLH transcription factor associated with [] cell development	Loss of DNA binding capacity of transcription factor [] ¹
		Homeodomain transcription factor associated with [] cell development	Loss or reduction of DNA binding capacity of transcription factor [] ¹
	Compact Growth Habit	Transcription co-factor impacting [] in angiosperms	Loss or reduction of function of transcriptional co-factor [] ¹
Selection	Resistance to certain antibiotics []	Plastid transporter associated with chloroplast [] transport but can opportunistically transport certain antibiotics	Loss or reduction of function of a plastid [] transporter [] ¹

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¹Single loss of function modifications to these sequences in blackberry were confirmed to meet the exemption described in 7 CFR Part 340.1(b)(1) (USDA, 2024a).

One of the domesticated blackberry varieties being used for targeted genetic modification is a proprietary eastern tetraploid variety ($2n = 4x = 28$) (Clark and Finn, 2011; Foster et al., 2019). A de novo genome contig assembly was constructed for domesticated blackberry using PacBio Hifi sequencing. Genome assembly was performed with HiCanu software, which produced an assembly with contiguity N50 score of []

[], consistent with the tetraploid genome composition.

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Foundational information on the blackberry genome sequence was established using the Basic Local Alignment Search Tool (BLAST) to query the proprietary blackberry genome for sequences similar to those of known target sequences in other species, followed by ortholog inference using phylogenetic gene tree construction (Table 2). For some target genes, the genome assembly contained collapsed haplotype sequences, which was determined by coverage analysis where a collapse of two haplotypes results in 2X read coverage. The coverage analysis was performed by mapping the PacBio Hifi sequencing reads to the assembly with minimap2 alignment software (Li, 2018) and coverage calculation per gene with sambamba-depth software (Tarasov et al., 2015). Based on this analysis the number and



locations of each sequence being used for targeted modification was identified in the known tetraploid blackberry (Figure 1) [10].

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Table 2. Query sequence used to identify targets in domesticated blackberry.

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Figure 1. Genomic location and distribution target sequences in a domesticated variety of tetraploid blackberry. Shown are chromosomes [] as indicated by the corresponding number. Blue bars represent the relative position of the target sequences.

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2. Sequence of the Modifications

At least [] for each target sequence will be modified. Multiple target modifications may exist together in the same plant or in various sub-combinations. Nucleotide sequence for a representative allele for each target sequence in FASTA format can be found in Appendix A.

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3. Sequence Comparison of the Modifications

The products have been confirmed to have at least [] modified for one or more of the target sequences. Figure 2 shows the modified sequence compared with the unmodified sequence for a representative allele for each target. A summary of the modifications is described in Table 3.

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Table 3. Summary of modified sequences in Pairwise domesticated blackberry.

Target	Modification	Effect
[		
		]

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[

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Figure 2. Edits in domesticated blackberry trait targets of interest. One edited allele per target sequence is aligned against the reference sequence.

Part V: Description of the New Traits

1. Intended Phenotype: Soft Seeds

Blackberries contain a seed that is surrounded by a lignified endocarp. The term “seedless” is being used in the context of this RSR to refer to the entire structure of the seed and endocarp and is not expected to result in a complete physical loss of this structure but rather a shrinking or softening of this structure as is found in other seedless varieties, like table grape (*Vitis vinifera* L.) [CBI-deleted]. Development of seedless traits in plants is predominately the result of breeding efforts [CBI-deleted] as seedless fruit is more desirable and easier to process. Therefore, CBI-deleted seed size and overall feel is part of the evaluation of new domesticated blackberry varieties as blackberry seeds tend to be larger than those of other *Rubus* species, like raspberry, and are more off-

putting to consumers (Daubeney, 1996). Certain trailing varieties of blackberries, primarily developed using *Rubus ursinus* as a parental species, are perceived as being seedless or having reduced seeds due to reduced []. The seedless trait in plants can result from []

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A review by Picarella and Mazzucato (2019) of seedless traits in flowering plants identified 96 taxa, with nearly half (47 taxa) belonging to the phylogenetic group Rosidae, to which blackberries also belong. This includes grapes (*Vitis vinifera* L.), pistachio (*Pistacia vera* L.), fig (*Ficus carica* L.), mulberry (*Morus alba* L.), lemon (*Citrus limon* (L.) Burm.), and orange (*Citrus sinensis* (L.) Osbeck). Among the 96 taxa, six species identified were from the family Rosaceae, the family of which blackberry belongs. The species of Rosaceae observed to have the occurrence of the seedless trait include domesticated apple (*Malus x domestica* Borkh), medlar, a tree with apple-like fruit (*Mespilus germanica* L.), cherry plum (*Prunus cerasifera* Ehrh.), peaches (*Prunus persica* (L.) Batsch), pear (*Pyrus communis* L.), and dog rose, a wild rose species (*Rosa canina* L.). The review by Picarella and Mazzucato (2019) demonstrates that the seedless trait is achievable in nature and by traditional breeding methods.

Description of the Mechanism of Action:

Using a gene editing approach, we achieved a seedless trait in domesticated blackberry varieties. This was accomplished by editing target sequences involved in []. Products include modifications [] for one or more of these gene targets.

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Target sequences for creation of the seedless trait are members of the NAC domain transcription family, specifically [] which control the activation of genes involved in []. []

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were selected as targets because they are expressed in blackberry fruit beginning a few weeks after pollination and impact the deposition of [], acting as a transcription factor associated with []. Loss of function mutations of [] family members in other species leads to reduced accumulation of []. A variety of apricot described as having a [] was also described to have lower expression of an [] family member than an apricot variety with a typical []. Single loss of function modifications to the [] trait were confirmed to meet the exemption described in 7 CFR Part 340.1(b)(1) as “plants with modifications that are exempt pursuant to §340.1(b)(1) are achievable by conventional breeding and unlikely to pose an increased plant pest risk relative to their conventionally bred counterparts” (USDA, 2024a).

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2. Intended Phenotype: Reduced thorn development on stems

The outgrowth on blackberries is commonly referred to as a thorn, however, developmentally this structure is not actually a thorn because it is not derived from an axillary bud that could also have given rise to a leaf or branch. Blackberries actually have prickles, also called spines, which are similar to trichomes as they form from the vascular cortex. Domesticated blackberry varieties show a range in size, density, and firmness of outgrowths. Multiple breeding programs have prioritized creation of thornless blackberry varieties and as a result there are several thornless blackberry varieties that have been the

genetic contribution to the thornless trait in dozens of commercially grown blackberry varieties including ‘Merton Thornless’, ‘Burbank Thornless’, and ‘Austin Thornless’ (Coyner et al., 2005; []).

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The thornless trait has been developed using traditional breeding methods and has been released in varieties of domesticated blackberry from public and private breeding programs, including USDA ARS and the University of Arkansas. Blackberry breeding programs have released domesticated varieties of blackberry that have both the naturally occurring thornless trait as well as a second agronomically important trait for [], which was also developed using traditional breeding methods. Example varieties from the University of Arkansas program that contain both the thornless and [] traits include []. The thornless trait has not been observed in wild species of *Rubus*.

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Description of the Mechanism of Action:

The target sequences for creation of the thornless trait are transcription factors shown to play a role in [] cell differentiation in one or more plant species. One transcription factor is [], a homeodomain transcription factor which has been shown to be expressed in [] tissues and coordinate [] in *Arabidopsis* []. Blackberry linkage mapping studies identified a locus on chromosome [] associated with a prickleless phenotype []. Internal analysis at Pairwise identified a [] gene in this region, with a polymorphism that appears to be associated with a recessive allele causing the thornless phenotype. Using gene editing to modify [] by creating a deletion, insertion, inversion, and/or substitution of one or more base pairs in the target sequence resulting in loss or reduced function of the native protein is expected to prevent development of []. Single loss of function modifications to the [] trait was confirmed to meet the exemption described in 7 CFR Part 340.1(b)(1) as “plants with modifications that are exempt pursuant to §340.1(b)(1) are achievable by conventional breeding and unlikely to pose an increased plant pest risk relative to their conventionally bred counterparts” (USDA, 2024a).

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Another thornless target is the bHLH transcription factor []. The [] protein has been shown to act in the same epidermal differentiation pathway as [], and the two proteins physically interact to activate initiation of []. In roots, [] was shown to interact in another protein complex which controlled epidermal differentiation and [] in specific cells within the tissue []. Given their central role in epidermal development and initiation of outgrowths, using gene editing to modify [] by creating a [] in these sequences can result in reduction or loss of protein function, yielding a thornless trait in blackberry. Single loss of function modifications to the [] trait was confirmed to meet the exemption described in 7 CFR Part 340.1(b)(1) as “plants with modifications that are exempt pursuant to §340.1(b)(1) are achievable by conventional breeding and unlikely to pose an increased plant pest risk relative to their conventionally bred counterparts” (USDA, 2024a).

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3. Intended Phenotype: Compact Growth Habit

Many varieties of domesticated blackberry produce [] which do not []. This means that in the [] the cultivated blackberry plants produce a []

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needs to undergo a dormancy period after which these same [CBI-deleted
CBI-deleted
] will also be produced on the same plant. CBI-deleted
Blackberry breeding programs have been able to develop a [CBI-deleted
allows the plant to produce [CBI-deleted
plants with the [CBI-deleted
] trait produce [CBI-deleted
]. This transition of [CBI-deleted
] could CBI-deleted
be further optimized through gene editing approaches by reducing the amount of [CBI-deleted
] growth CBI-deleted
required prior to the transition, reducing the photoperiod-dependent nature of [CBI-deleted
, CBI-deleted
and/or eliminating dormancy requirement which would allow for [CBI-deleted
]. CBI-deleted

The development of the [CBI-deleted
] trait in domesticated blackberry varieties played a critical CBI-deleted
role in advancing blackberry breeding programs allowing for increased [CBI-deleted
] CBI-deleted
expansion which allowed for near year-round production and availability in grocery stores. [CBI-deleted
, a CBI-deleted
Rubus argutus selection found in North America, is the primary source of the [CBI-deleted
] trait CBI-deleted
in domesticated blackberry [CBI-deleted
]. The [CBI-deleted
] trait has CBI-deleted
been developed using traditional breeding methods and has been released in varieties of domesticated
blackberry from public and private breeding programs, including USDA ARS and the University of
Arkansas. Blackberry breeding programs have released domesticated varieties of eastern tetraploid
blackberry that have both the naturally occurring [CBI-deleted
] trait as well as a second CBI-deleted
agronomically important thornless trait, which was also developed using traditional breeding methods.
Example varieties from the University of Arkansas program that contain both the thornless and
[CBI-deleted
] traits include [CBI-deleted
]. The [CBI-deleted
] trait has not been observed in wild species of *Rubus*. CBI-deleted

Description of the Mechanism of Action:

The target sequence for creation of the [CBI-deleted
] trait is a transcriptional co-factor CBI-deleted
shown to play a role in [CBI-deleted
] in one or more plant species. [CBI-deleted
] binding protein and a major co-transcription factor associated with CBI-deleted
[CBI-deleted
]. Using gene editing to modify [CBI-deleted
] by creating a deletion, insertion, inversion, and/or CBI-deleted
substitution of one or more base pairs in the target sequence resulting in loss or reduced function of the
native protein is a strategy to control [CBI-deleted
] and replicate the naturally occurring [CBI-deleted
]. Genetic modifications in [CBI-deleted
], which lead to [CBI-deleted
], have been identified in many domesticated crop species [CBI-deleted
]. In CBI-deleted
these examples the [CBI-deleted
] is believed to be achieved by disrupting the native function of the CBI-deleted
transcriptional co-factor resulting in loss or reduced function leading to the [CBI-deleted
] transcriptional co-factor of [CBI-deleted
]. Single loss of function CBI-deleted
modifications to the [CBI-deleted
] trait was confirmed to meet the exemption described in 7 CFR Part CBI-deleted
340.1(b)(1) as “plants with modifications that are exempt pursuant to §340.1(b)(1) are achievable by
conventional breeding and unlikely to pose an increased plant pest risk relative to their conventionally
bred counterparts” (USDA, 2024a). CBI-deleted

4. *Intended Phenotype:* Resistance to certain antibiotics [CBI-deleted

Insensitivity to certain antibiotics, such as [CBI-deleted
] will be used to identify plants during the CBI-deleted
development process but is not intended to be used in commercial blackberry production. CBI-deleted

Description of the Mechanism of Action:

The target sequence for creation of the antibiotic resistance trait is a plasmid transporter shown to play a role in [], is a CBI-deleted
 plastid transporter whose primary function is as a chloroplast envelope protein that is primarily associated with [] but can opportunistically transport certain antibiotics. Genetic CBI-deleted
 modifications in [], which lead to resistance to certain [], has CBI-deleted
 been demonstrated in *Arabidopsis* using ethyl methanesulfonate (EMS) mutagenesis [CBI-deleted
]. Using gene editing to modify [] by creating a deletion, insertion, CBI-deleted
 inversion, and/or substitution of one or more base pairs in the target sequence resulting in loss or
 reduced function of the native protein is a strategy to create a selection system by which to recover
 plants in tissue culture [] loss of function CBI-deleted
 mutants but this can be overcome with sufficient [] in the growing environment []. CBI-deleted
 Single loss of function modifications to the [] trait was confirmed to meet the exemption CBI-deleted
 described in 7 CFR Part 340.1(b)(1) as “plants with modifications that are exempt pursuant to
 §340.1(b)(1) are achievable by conventional breeding and unlikely to pose an increased plant pest risk
 relative to their conventionally bred counterparts” (USDA, 2024a).

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