1. Information about Requestor

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2. Does the request contain Confidential Business Information (CBI)?*

Yes.

CBI Justification Statement:

This document contains Confidential Business Information (CBI) which is divided up into five categories justified below.

1. Genetic information including associated phenotypes

Genes and sequences are generally recognized as CBI according to the USDA CBI guidelines. These harbor information that is the result of detailed analysis and experimentation that could reveal internal strategies or mechanisms that give our company a competitive advantage. These are generally held as trade secrets and may also be subject to pending patent submissions prior to publication.

2. Methods

We are fundamentally a plant breeding company and creation of new plant varieties through our methods is what differentiates us from our competitors. For this reason our methods are CBI as they are held as trade secrets and may also be subject to pending patent submissions prior to publication. This includes all elements of breeding from pollination to production of seed, trialing design, and disposal of plant material.

3. Location

Locations may reveal commercial partnerships or investor relationships which could reveal financial or other commercial partnership information. In some cases this could also violate the terms of a nondisclosure agreement. The locations of our operations are also carefully selected based on latitude, elevation, and other criteria which gives us an advantage in certain crops – these criteria are held as a trade secret. **4. Varieties and numbers of plants/seeds** Disclosing varieties used would reveal commercial partnerships and suppliers which could also reveal financial or commercial information and strategies. In some cases revealing this could also violate the terms of a nondisclosure agreement. The number of plants/seeds may also reveal the stage of the plant or invention which may reveal trial and financial information which is held as a trade secret. **5. Company information and personnel** Any additional company information such as financials, contractual information or agreements is declared as CBI. Other CBI may include personally identifiable information such as staff names, locations and expertise as this information could reveal staffing expertise. **3. Description of the comparator plants:** **Scientific name (genus, species, subspecies)*:** *Solanum tuberosum* **Ploidy** Tetraploid **Common Name:** Potato **Cultivars:** [ ]	CBI-Deleted
4. Genotype of the modified plant. A. No foreign DNA was inserted into the plant B. If genetic material is not inserted into the genome: **Nature of modification(s)*:**	

DNA-free and marker free [ ] nonfunctional, weak, or missense mutations. **Overview of modifications:**	CBI-Deleted CBI-Deleted CBI-Deleted
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Figure 1: Alignment of edited haplotypes to the wild type gene. A) IGV genome browser screenshot depicting alignments of three edited haplotype sequences to the wild type gene. The wild type gene is depicted in blue with the largest rectangles representing protein coding sequences. The three gray alignments represent the haplotype sequences for, from top to bottom, [ ] **B**) The same as (**A**) but zoomed in to the interval containing the mutations. Black lines in the gray bars represent deletions relative to the wild type sequence.	CBI-Deleted CBI-Deleted
Sequence of the Modification*	

Below is the first sequence of the modification and flanking sequence: [ ]	CBI-Deleted CBI-Deleted

Below is the second sequence of the modification and flanking sequence: [ ]	CBI-Deleted CBI-Deleted

Below is the third sequence of the modification and flanking sequence: [ ]	CBI-Deleted CBI-Deleted

Sequence comparison* Below is the comparison sequence: [ ]	 CBI-Deleted

5. Description of new trait

Intended trait:

Increased pro-vitamin A and alteration of tuber color.

Intended phenotype:*

Potato lines and cultivars with a higher concentration of beta-carotene in tubers which may also result in a yellow or orange color.

Description of the MOA*

MOA Summary:

[ ] in tubers and other plant parts.

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MOA Background:

Plant carotenoids, including Xanthophylls and carotenes, are important components of accessory light harvesting complexes involved in photosynthesis and photoprotection (Niyogi et al. 1997). Carotenoids are also involved in other metabolic processes that influence plant growth, development, and flavor (Nisar et al. 2015). Carotenoids are also prized for their contributions to food color and nutrition with the concentrations of carotenoids varying tremendously within and between species (Khoo et al. 2011).

Potato tubers often contain the following Xanthophylls: antheraxanthin, lutein, neoxanthin, violaxanthin, and rarely zeaxanthin in orange-fleshed diploids (Nesterenko et al. 2005, Haynes et al. 2011). While beta-carotene concentration is typically low or not detected in commercial tetraploid potatoes, it is occasionally abundant in the sexually comaptible wild relatives *Solanum stenotomum* and *Solanum phureja* (Brown et al. 1993, Haynes et al 2011). These relatives can be used in breeding programs to develop orange-fleshed tetraploid potatoes using various traditional breeding methods.

The carotenoid biosynthetic pathway is highly conserved in plants and the relative abundance of carotenoids are governed by the relative expression and efficiencies of conserved lycopene cyclases (LCY-e, LCY-b), beta-hydroxylases (CHY1, CHY2), and zeaxanthin epoxidase (ZEP) and other factors such as degradation of specific carotenoids by carotenoid cleavage dioxygenases (CCD) which remove specific carotenes or xanthophylls and generate volatile compounds and phytohormones (Nissar et al. 2015).

Most critical steps in the carotenoid biosynthetic pathway have been investigated in potato by utilizing natural variation and biotechnological approaches such as RNA interference. Using antisense transgenic lines, Dirreto et al 2006 suppressed epsilon cyclase (LCY-e), an enzyme that drives the beta-epsilon branch of carotenoid biosynthesis. These lines resulted in as much as a 2.5-fold increase in beta-carotene. Later studies found that the [ ] **MOA detail:** In the present case we deployed learnings from various studies to generate various potato plants with novel [ ] which results in an accumulation of beta-carotene that is in the range of beta carotene observed in potato germplasm. Because beta-carotene and other xanthophylls are substrate to produce various phytohormones and volatile molecules, we anticipate that alterations in tuber sprouting, tuber shape or other plant morphology could be possible due to differences in substrate availability for carotenoid cleavage dioxygenase CCD. However, given that non-functional [ ] alleles already exist in natural germplasm, these changes would fall in the natural range for the species.	CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted
MOA References: Brown, C.R., C.G. Edwards, C.-P. Yang, and B.B. Dean. "Orange Flesh Trait in Potato: Inheritance and Carotenoid Content." Journal of the American Society for Horticultural Science 118, no. 1 (January 1993): 145–50. https://doi.org/10.21273/JASHS.118.1.145. [ ] [	CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted CBI-Deleted

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Khoo, Hock-Eng, K. Nagendra Prasad, Kin-Weng Kong, Yueming Jiang, and Amin Ismail. "Carotenoids and Their Isomers: Color Pigments in Fruits and Vegetables." *Molecules* 16, no. 2 (February 18, 2011): 1710–38. https://doi.org/10.3390/molecules16021710 Nesterenko, Sergey, and Kenneth C. Sink. "Carotenoid Profiles of Potato Breeding Lines and Selected Cultivars." *HortScience* 38, no. 6 (October 2003): 1173–77. https://doi.org/10.21273/HORTSCI.38.6.1173. Nisar, Nazia, Li Li, Shan Lu, Nay Chi Khin, and Barry J. Pogson. "Carotenoid Metabolism in Plants." *Molecular Plant* 8, no. 1 (January 2015): 68–82. https://doi.org/10.1016/j.molp.2014.12.007. Niyogi, Krishna K., Olle Björkman, and Arthur R. Grossman. "The Roles of Specific Xanthophylls in Photoprotection." *Proceedings of the National Academy of Sciences* 94, no. 25 (December 9, 1997): 14162–67. https://doi.org/10.1073/pnas.94.25.14162.	
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