Appendix A

J.R. Simplot Company Request (19-099-02p) for Extension of Determination of Nonregulated Status of Z6 Potatoes with Late Blight Protection, Low Acrylamide Potential, Lowered Reducing Sugars and Reduced Black Spot

OECD Unique Identifier: SPS-000Z6-5

Plant Pest Risk Similarity Assessment

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A. Introduction

The Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) has received an extension request (19-099-02p) from the J.R. Simplot Company (hereafter referred to as JR Simplot). In accordance with §340.6(e)(2), JR Simplot requests that APHIS extend the nonregulated status for the antecedent W8 potatoes with late blight resistance, low acrylamide potential, lowered reducing sugars and reduced black spot potatoes to the genetically engineered (GE) Z6 potato event (late blight tolerance, low acrylamide potential, lowered reducing sugars and reduced black spot potatoes) and any progeny derived from crosses of the Z6 potato event with conventional potatoes or with other GE potato varieties that have received a determination of nonregulated status, or are not considered regulated articles under regulations at 7 CFR Part 340. Previously, USDA announced its determination of nonregulated status for fourteen (14) potato events. These events include:

Ten events with low acrylamide potential and reduced black spot bruise were deregulated on November 10, 2014 (petition 13-022-01p).

- E12 and E24 – Russet Burbank varieties
- F10 and F37 – Ranger Russet varieties
- J3, J55, and J78 – Atlantic varieties
- G11, H37, and H50 - proprietary varieties

One event with late blight resistance, low acrylamide potential, reduced black spot and lowered reducing sugars was deregulated on September 2, 2015 (petition 14-093-01p).

- W8 – Russet Burbank variety

One event with low acrylamide potential and reduced black spot deregulated on January 13, 2016 (extension 15-040-01p).

- V11 – Snowden variety

Two events with late blight resistance, low acrylamide potential, lowered reducing sugars and reduced black spot deregulated on October 28, 2016 (extension 16-064-01p).

- X17 – Ranger Russet variety
- Y9 – Atlantic variety

Presently, JR Simplot intends to pursue commercialization of the Z6 potatoes. Therefore, the antecedent organism identified in the extension request for the Z6 Snowden potato event (hereafter referred to as the Z6 event or Z6 potatoes) is the W8 Russet Burbank potato event (referred to as the W8 event or W8 potatoes).

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under 7 CFR part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any

genera or taxa designated in 7 CFR part 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest. The Z6 event was produced by *Agrobacterium tumefaciens* mediated transformation of potato internode sections (Simplot 2019), and some of the introduced border sequences come from plant pest organisms listed in 7 CFR part 340.2 (Simplot 2019). Therefore, the Z6 event is considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts in this Plant Pest Risk Similarity Assessment are those that pertain to plant pest risk associated with the Z6 potatoes and their progeny and their use in the absence of confinement relative to the antecedent W8 potatoes. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if the Z6 potatoes are any more likely than W8 potatoes to pose a plant pest risk. APHIS specifies in 7 CFR part 340.6(e) that an extension request for nonregulated status shall include information to establish the similarity of the antecedent organism to the regulated article in question.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology'(51 FR 23302 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with USDA-APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on the characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

As a food product, Z6 potatoes are within the scope of the FDA policy statement concerning regulation of food products derived from new plant varieties, including those developed by recombinant DNA techniques. JR Simplot has submitted to the FDA Center for Food Safety and Applied Nutrition for a voluntary safety and nutritional assessment of Z6 potatoes.

Z6 potatoes are also subject to registration by the EPA under the Federal Insecticide, Fungicide and Rodenticide Act because Z6 potatoes contain a plant-incorporated protectant. JR Simplot has submitted to the EPA for a Section 3 registration application for Z6 potatoes. Similar events W8, X17, and Y9 were registered by EPA on January 19, 2017 (8917-1, 8917-2, and 8917-3, respectively). Also, EPA is the U.S. regulatory agency primarily responsible for evaluating safety of the protein against *Phytophthora infestans* from *Solanum venturii* (VNT1) under the Federal Food, Drug, and Cosmetic Act. The EPA has issued a permanent tolerance exemption for the VNT1 protein in potato (40 CFR, §174.534).

B. Development of the Z6 Snowden potato

JR Simplot used genetic engineering to introduce into the background of commercial potato cultivars four traits that are of interest to potato consumers, producers, and

processors: late blight resistance, reduced acrylamide potential, lowered reducing sugars, and reduced black spot (Simplot 2014). The Z6 event is a new potato event produced by introducing the same genes and traits into the Snowden variety, which is the 2nd most popular potato variety used for making potato chips. Multiple traits would be difficult to achieve through conventional breeding. Potato is tetraploid, highly heterozygous and sensitive to inbreeding depression. Each potato parent variety must be independently transformed to achieve the desired phenotype in that variety. The same genetic constructs, pSIM1278 and pSIM1678, used to develop W8 potatoes were used to generate Z6 potatoes. The intended purpose of the antecedent W8 potato event and the Z6 event is to provide the potato processing industry with new varieties with late blight tolerance, low acrylamide potential, lowered reducing sugars and reduced black spot. All of these changes are intended to benefit potato consumers, producers, and processors. Late blight tolerance will lower the need for fungicides for control of late blight disease and lessen the discard of infected potatoes which would lead to increased yield and less chemical residues on potatoes, fields, and waste water. The low acrylamide potential is intended to benefit consumers because of concerns about the health effects of ingesting acrylamide, and to benefit the industry relative to Proposition 65. The proposition requires businesses to provide warnings to Californians about significant exposures to chemicals that cause cancer, birth defects or other reproductive harm. The lowered reducing sugars will allow for storage of potatoes for the fry and chip market at a lower temperature which would lower storage diseases and keep taste/color quality in the desired ranges. The reduced black spot bruise is intended to benefit consumers by providing a higher quality product, to benefit producers by reducing culls at delivery, and to benefit processors by reducing pick-outs.

APHIS completed a detailed plant pest risk assessment (PPRA) and environmental assessment (EA) for the antecedent W8 potatoes (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status). The EA fully addressed all resource areas of potential concern. In the antecedent petition, 14-093-01p, APHIS concluded on the basis of the EA that the impacts would not be significant. The agency issued Findings of No Significant Impacts (FONSI) and made a determination of nonregulated status for the antecedent event.

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the extension request related to the similarity of the Z6 event to the antecedent W8 event and the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop

event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction and the number of loci inserted.

APHIS also assessed data presented in the extension request on whether the genetic modification results in expression of new genes, proteins, or enzymes, suppression of existing genes and their products, or changes in plant metabolism or composition in the Z6 event. The assessment encompasses a consideration of the expressed double stranded RNA (dsRNA) and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, anti-nutrients, or nutrients in harvested potatoes derived from the Z6 event compared to the antecedent W8 event or those in the conventional counterparts and other comparators.

Description of the genetic modification and inheritance of inserted DNA

The same genetic constructs, pSIM1278 and pSIM1678, used to develop the antecedent W8 event, were used to generate the Z6 event (Simplot 2019). The Z6 event was obtained by *Agrobacterium tumefaciens* mediated retransformation of V11 Snowden potato event with genetic construct pSIM1678. The V11 event was generated by transforming Snowden potato variety with genetic construct pSIM1278 (Simplot 2015), and was deregulated in 2016 under extension request 15-040-01p.

The T-DNA from the pSIM1278 construct down-regulates four potato gene transcripts through RNA interference (RNAi):

- The first cassette down-regulates expression of the potato asparagine synthetase-1 gene (*Asn1*) and the potato polyphenol oxidase-5 gene (*Ppo5*). This cassette is comprised of two 404-bp inverted repeat fragments of *Asn*1 and two 143-bp inverted repeat fragments of *Ppo5*. The *Asn1* and *Ppo5* fragments are arranged between the two convergent native potato promoters, the potato *Agp* promoter of the ADP glucose pyrophosphorylase gene (*Agp*) and the potato *Gbss* promoter of the granule-bound starch synthase gene (*Gbss*) that are primarily active in tubers. These promoters drive expression transcription of the inverted repeats to generate double-stranded RNA targeting the native potato genes *Asn1* and *Ppo5* for down-regulation, also known as gene silencing.
- The second cassette lowers reducing sugars by down-regulating the potato phosphorylase-L (*PhL*) gene and the potato starch-associated (*R1*) gene by targeting the down-regulation of their promoters. This cassette is comprised of an inverted repeat fragment of the 508-bp promoter region *PhL* and a 531-bp inverted repeat fragment of *R1*. Similar to the first cassette, the *PhL* and *R1* inverted repeat fragments are transcribed by the convergent potato *Agp* and *Gbss* promoters.

The T-DNA from pSIM1678 was designed to down-regulate the transcription of the potato vacuolar invertase gene (*Vlnv*) through RNAi and to express the *Rpi-vnt1* gene

(resistance against *P. infestans* from *S. venturii*). The pSIM1678 construct consists of the silencing cassette for the *VInv* gene and the expression cassette for *Rpi-vnt1* gene.

- The silencing cassette for the potato *Vlnv* gene consists of aninverted repeat sequence of the potato *Vlnv* gene sense orientation fragment (181bps) and anti-sense orientation fragment (504 bps) flanked by opposing plant promoters, *pGbss* and *pAgp*.
- The *Rpi-vnt1* gene expression cassette consists of the *Rpi-vnt1* gene coding sequence (2676 bps) region regulated by its native promoter and terminator sequences.

Table 1. Summary of Z6 Genes and Intended Traits (Simplot 2019)

Construct	Gene Target	Mechanism	Intended Trait	Intended Benefit
pSIM1278	*Asn1*: asparagine synthetase-1	RNAi down regulation	Reduces free asparagine	Contributes to low acrylamide potential
	R1: water dikinase	RNAi down regulation	Lowers reducing sugars	Contributes to low acrylamide potential
	PhL: phosphorylase L	RNAi down regulation	Lowers reducing sugars	Contributes to low acrylamide potential
	Ppo5: polyphenol oxidase-5	RNAi down regulation	Reduces enzymatic darkening	Reduced black spot
pSIM1678	*VInv*: vacuolar invertase	RNAi down regulation	Lowers reducing sugars	Prevents excess darkening during frying and contributes to low acrylamide potential
	Rpi-vnt1: R-gene	Protein overexpression	Confers protection to *P. infestans*	Late blight protection

Expression of inserted DNA and changes in gene expression, new proteins or metabolism

As with the previously deregulated potato events, the only novel protein expressed is the VNT1 protein for late blight protection. The antecedent W8 event and the Z6 event contain no marker genes. APHIS reviewed the information provided by JR Simplot in the extension request and determined the following:

- The Z6 event contains a single copy of the insert from pSIM1278 (previously deregulated; 15-140-01p) and a single copy of the insert from pSIM1678 as demonstrated by molecular analyses.
- The T-DNA is stably inherited from generation to generation.
- The Z6 event does not contain any back bone sequence of extraneous DNA fragments from the transformation plasmid.
- The Z6 event contains stable, well-characterized inserts similar to the antecedent W8 potato event.
- There were no changes in gene expression, metabolism or additional proteins between the Z6 event and the antecedent W8 event.

D. Potential Plant Pest and Disease Impacts

APHIS assessed data and information presented in the extension request related to the similarity of the Z6 event to the antecedent W8 event to determine whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in the Z6 event that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses. APHIS also assessed whether the Z6 event is more likely to have significantly increased disease and pest susceptibility as compared to antecedent W8 event. Impacts or changes in similarity to the antecedent W8 event to the Z6 event were assessed to determine if they would (1) affect and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States; and supports trade and exports of U.S. agricultural products. PPQ responds to new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be an emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (APHIS 2019), however, none specifically target pests of the Z6 event.

Because the genetic makeup of the GE traits of the Z6 event is identical to the previously deregulated antecedent W8 event, no significant changes in composition are expected from the expression of the inserted genes in the Z6 event. Similarly, the Z6 event is not expected to differ from the antecedent W8 event in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Non-target Organisms Beneficial to Agriculture

APHIS has previously evaluated the potential impacts on non-target organisms beneficial to agriculture that could result from the deregulation of the antecedent W8 event. The W8 event was determined by APHIS to be unlikely to have an adverse effect on non-target organisms in the environment. The genetic constructs, pSIM1278 and pSIM1678, used to develop the deregulated variety contain DNA sequences intended to down-regulate five genes through the mechanism of RNA interference (RNAi) and to express *Rpi-vnt1* (Table 1).

The same genetic constructs, pSIM1278 and pSIM1678, used to develop the previously deregulated W8 event, were used to develop the Z6 event. Therefore, based on the high similarity of the Z6 event to the antecedent W8 event, the compositional similarity of the

Z6 event to its parent variety, the unlikely impacts of non-target effects due to RNAi, and on the finding that the antecedent W8 event was unlikely to harm non-target organisms, APHIS concludes that it is unlikely that the Z6 event will have an adverse effect on non-target organisms, including those beneficial to agriculture.

F. Potential for Enhanced Weediness of the Z6 Snowden Potato

The biology of potato is well studied and understood (OECD 1997, Simplot 2019). As documented in the Plant Pest Risk Assessment (USDA APHIS 2015) of the antecedent W8 event, potatoes are not known to be weedy or persistent; they are incapable of survival outside of cultivation (Holm et al. 1979; Muenscher 1980; Love 1994; OECD 1997).

In addition to considerations of the known biology of potato, APHIS analyzed information submitted in the petition on the antecedent organism on a suite of agronomic characteristics and plant-disease and plant-insect interactions. This agronomic data showed that the antecedent W8 event was not different than its non-transgenic comparator. The assessments concluded that the antecedent was unlikely to become a weed. In addition, APHIS has also assessed the potential weediness of many other GE potato events representing a variety of traits. For both the antecedent and the other GE potato events, it was concluded that the new GE traits would not make the Z6 event more likely to become a weed. Therefore, because of the similarity of the Z6 event to the antecedent W8 event, APHIS has determined that the Z6 event is not likely to be a weed.

G. Potential Impacts on the Weediness of Any Other Plants with which the Z6 Snowden Potato Can Interbreed

APHIS evaluated the potential for gene introgression to occur from the antecedent W8 event to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Those assessments found that among native *Solanum* spp. in the United States, cultivated potato is potentially sexually-compatible only with the two species, *S. jamesii* and *S. stoloniferum* (previously *S. fendleri*) (Spooner et al. 2004). Neither of these species is listed on U.S. or State weed lists (USDA NRCS 2019). Gene flow, hybridization and/or introgression of the introduced genetic material from JR Simplot potatoes to the wild relatives, *S. stoloniferum* and *S. jamesii,* is unlikely to occur. In the case of *S. stoloniferum*, where there is a remote possibility of gene introgression, APHIS concluded that even if such introgression were to occur, this species is not considered a weed.

APHIS concluded that the gene silencing cassettes originating from the antecedent W8 event were unlikely to impact the weediness of this wild species since the antecedent W8event does not exhibit characteristics to be any weedier than other cultivated potatoes. Therefore, the Z6 event is not expected to increase the weed risk potential of other species with which it can interbreed in the United States and its territories based on its similarity to the antecedent W8 event.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from the antecedent W8 event are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

APHIS did not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, rotations, management of volunteers, etc.) from the antecedent potato event and concluded that no impact on plant diseases or pests or their management is likely to occur. Based on the similarity of the Z6 event to the antecedent W8 event with the same GE traits, APHIS concludes that it is unlikely that any significant changes to agriculture or cultivation practices would be associated with the Z6 event and therefore no impact on plant diseases or pests of their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which the Z6 Snowden Potato Cannot Interbreed

APHIS has previously examined the potential for the antecedent W8 event, expressing RNA interference (RNAi) of the *Asn1, R1, PhL*, *Ppo5* and *VInv* genes to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). Potential risks from stable HGT from GE organisms to another organism without reproduction or human intervention has been reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. APHIS has previously reviewed the potential for HGT from GE potato to bacteria, fungi, invertebrates, viruses, and parasitic plants (USDA APHIS 2015).

APHIS previously concluded that HGT of the inserted genetic material from the antecedent W8 event to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. Therefore, APHIS concludes that HGT from the Z6 event to other organisms is also highly unlikely.

J. Conclusion

APHIS has reviewed the information submitted in the extension request, supporting documents, and other relevant information to assess the similarity of plant pest risk of the Z6 event compared to the antecedent W8 event. APHIS concludes that the Z6 event is

not more likely to pose a plant pest risk than the previously deregulated antecedent W8 event.

K. References

51 FR 23302. 1986. *Coordinated Framework for Regulation of Biotechnology*.

57 FR 22984. 1992. *Statement of Policy: Foods Derived from New Plant Varieties*.

APHIS. 2019. *Plant Pest Program Information*. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/planthealth Last accessed 10/8/2019.

Dröge M, Pühler A, and Selbitschka W. 1998. *Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern*. Journal of Biotechnology 64, pp. 75–90. Retrieved from http://www.sciencedirect.com/science/article/pii/S0168165698001059

Holm L, Pancho J, Herberger J, and Plucknett D. 1979. *A geographical atlas of world weeds*. John Wiley and Sons.

Keese P. 2008. *Risks from GMOs due to horizontal gene transfer*. Environ Biosafety Res 7, pp. 123-149. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18801324

Love S. 1994. *Ecological risk of growing transgenic potatoes in the United States and Canada*. American Potato Journal 71, pp. 647-658. Retrieved from https://link.springer.com/article/10.1007/BF02851433

Muenscher W. 1980. *Weeds Secton Edition*. Cornell University Press.

OECD. 1997. *Consensus Document on the Biology of Solanum tuberosum subsp. tuberosum (potato)*. Directorate, Environment. Retrieved from http://www.oecd.org/science/biotrack/46815598.pdf

Simplot. 2014. *Petition (14-093-01p) for Determination of NonRegulated Status for Innate Potatoes with Late Blight Resistance, Low Acrylamide Potential, Reduced Black Spot and Lowered Reducing Sugars: Russet Burbank Event W8.*

Simplot. 2015. *Petition (15-140-01p) for Extension of NonRegulated Status for V11 Potatoes with Low Acrylamide Potential and Reduced Black Spot.*

Simplot. 2019. *Petition (19-099-02p) for Extension of NonRegulated Status for Z6 Potatoes with Late Blight Protection, Low Acrylamide Potential, Lowered Reducing Sugars, and Reduced Black Spot.*

Spooner D, van den Berg R, Rodriguez A, Bamberg J, Hijmans R, and Cabrera S. 2004. *Wild Potatoes (Solanum section Petota; Solanaceae) of North and Central America*. Systematic Botany Monographs 68, pp. 1-209. Retrieved from https://vcru.wisc.edu/spoonerlab/pdf/Syst.%20Bot.%20Monogr.%20vol%2068%202004.pdf

USDA APHIS. 2015. *Plant Pest Risk Assessment on JR Simplot Company Petition (14-093-01p) for Determination of Nonregulated Status for Innate Potatoes with Late Blight Resistance, Low Acrylamide Potential, Reduced Black Spot and Lowered Reducing Sugars: Russet Burbank Event W8*.

USDA NRCS. 2019. *Introduced, invasive, and noxious plants.* United States Department of Agriculture, Natural Resources Conservation Service. Retrieved from https://plants.usda.gov/java/noxiousDriver Last accessed October 8, 2019.

L. Similarity Table

Description		Extension Request SPS-000Z6-5 (Z6) 19-099-02p	Antecedent SPS-000W8-4 (W8) Petition 14-093-01p	Comments
Organism		**Potato**	**Potato**	
Phenotype		Late Blight Protection Low Acrylamide Potential Lowered Reducing Sugars Reduced Black Spot	Late Blight Resistance Low Acrylamide Potential Lowered Reducing Sugars Reduced Black Spot	Same phenotypes Late Blight Resistance was changed to Late Blight Protection
Genotype		**Construct pSIM1278** First cassette ADP glucose pyrophosphorylase promoter from *S. tuberosum*	**Construct pSIM1278** First cassette ADP glucose pyrophosphorylase promoter from *S. tuberosum*	Same genes, promoters, and spacers
	Asn1	*Asn1* from *S. tuberosum* (RNAi)	*Asn1* from *S. tuberosum* (RNAi)	
	Ppo5	*Ppo5* gene from *S. verrucosum* (RNAi)	*Ppo5* gene from *S. verrucosum* (RNAi)	
		10-Kb spacer from *S. tuberosum* to create hairpin	10-Kb spacer from *S. tuberosum* to create hairpin	
		Gbss granule-bound starch synthase promoter from *S. tuberosum*	*Gbss* granule-bound starch synthase promoter from *S. tuberosum*	

		Extension Request SPS-000Z6-5 (Z6) 19-099-02p	Antecedent SPS-000W8-4 (W8) Petition 14-093-01p	Comments
Genotype		Second cassette ADP glucose pyrophosphorylase promoter from *S. tuberosum*	Second cassette ADP glucose pyrophosphorylase promoter from *S. tuberosum*	Same genes, promoters, and spacers.
	R1	fragment of the *R1* promoter sequence from *S. tuberosum* (RNAi)	fragment of the *R1* promoter sequence from *S. tuberosum* (RNAi)	
	PhL	fragment of the *PhL* promoter from *S. tuberosum* (RNAi)	fragment of the *PhL* promoter from *S. tuberosum* (RNAi)	
		Spacer-2, a 257 bp fragment from *S. tuberosum* to create hairpin	Spacer-2, a 257 bp fragment from *S. tuberosum* to create hairpin	
		Construct pSIM1678	**Construct pSIM1678**	Same genes, promoters, and spacers.
	vnt1	*Rpi-vnt1* gene coding sequence (R- Over expression) from *S. venturii.*	*Rpi-vnt1* gene coding sequence (R- Over expression) from *S. venturii.*	

	VInv	**<u>RNAi cassette</u>** *VInv* gene fragment (sense orientation – 498bp + 181) from *S. tuberosum* var. Ranger Russet *VInv* gene fragment (anti-sense orientation – 504bp) from *S. tuberosum* var. Ranger Russet	**<u>RNAi cassette</u>** *VInv* gene fragment (sense orientation – 498bp + 181) from *S. tuberosum* var. Ranger Russet *VInv* gene fragment (anti-sense orientation – 504bp) from *S. tuberosum* var. Ranger Russet	Same genes, promoters, and spacers.
Transformation Method		*Agrobacterium tumefaciens*–mediated	*Agrobacterium tumefaciens*–mediated	Same
Insert and Copy Number		Single intact insertions	Single intact insertions	Same
Description		**Extension Request SPS-000Z6-5 (Z6) 19-099-02p**	**Antecedent SPS-000W8-4 (W8) Petition 14-093-01p**	
Compositional analysis		Compositionally equivalent to conventional potato	Compositionally equivalent to conventional potato	Same
Backbone Absent		Yes	Yes	Same

Mechanism of Action	*Asn1*: reduces free asparagine *R1*: lowers reducing sugars *PhL*: lowers reducing sugars *Ppo5*: reduces enzymatic browning *Rpi-vnt1*: R-gene (over expression) Confers resistance to *P. infestans* and confers late blight resistance *VInv:* vacuolar invertase (RNAi) down-regulation and lowers reducing sugars and prevents excess darkening during frying and contributes to low acrylamide potential.	*Asn1*: reduces free asparagine *R1*: lowers reducing sugars *PhL*: lowers reducing sugars *Ppo5*: reduces enzymatic browning *Rpi-vnt1*: R-gene (over expression) Confers resistance to *P. infestans* and confers late blight resistance *VInv:* vacuolar invertase (RNAi) down-regulation and lowers reducing sugars and prevents excess darkening during frying and contributes to low acrylamide potential.	Same as Antecedent
Date of antecedent EA/ EIS	N/A	July 2014	

Description	Extension Request SPS-000Z6-5 (Z6) 19-099-02p	Antecedent SPS-000W8-4 (W8) Petition 14-093-01p	Comments
Plant Pest Risk			
Disease and pest susceptibilities	Similar to antecedent	Unlikely to change disease and pest susceptibilities	
Impacts on beneficial non-targets	Similar to antecedent	Unlikely to impact beneficial non-target organisms	
Enhanced weediness	Similar to antecedent	Unlikely to enhance weediness	
Enhanced weediness of relatives	Similar to antecedent	Unlikely to enhance weediness of relatives	
Changes to agriculture or cultivation practices	Similar to antecedent	Unlikely to change agriculture or cultivation practices	
Horizontal Gene Transfer	Similar to antecedent	Unlikely to affect the probability of horizontal gene transfer	
Plant Pest Risk	Similar to antecedent	Unlikely to pose a plant pest risk	