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BENSON HILL

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September 8, 2022
Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services

Re: **22-207-01cr:** Request for Confirmation of Exemption for Benson Hill Improved Productivity Soy (IPS) GM200003

Confidential Business Information Deleted

Dear Ms. Juarez,

Benson Hill, Inc (BH) respectfully requests confirmation from the USDA-APHIS's Biotechnology Regulatory Services (BRS) regarding the regulatory status of a soybean we intend to develop using CRISPR gene editing technology. BH is developing technology that will enable food crops to be more efficient, flavorful, and productive as food and feed. One of the products that BH is focused on is Improved Productivity Soy (IPS). Improved Productivity Soy is designed to have improved seed morphology and composition. The proposed soybean product contains a deletion in the soybean [ ] **CBI-deleted** gene [ ]. The request is described as follows: **CBI-deleted**

A. Requestor's name and contact information
Susan Martino-Catt
Benson Hill, Inc
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B. Description of the plant's genus, species
Order: *Fabales*
Family: *Fabaceae*
Genus: *Glycine*
Species: *Glycine max* (L.) Merr

C. Regulatory exemption we are claiming
We believe our product concept is eligible for exemption from regulation under the BRS SECURE rule published in 7 CFR part § 340.1(b)(1). The single genetic modification described in this proposal is a deletion in a single gene resulting from the cellular repair of a targeted DNA break in the absence of an externally provided repair template. The final plant line does not contain any DNA from a known plant pest.

D. Description of the trait
GM200003 soy contains a single gene edit resulting in the knockout of the soybean [**CBI-deleted**] gene involved in the regulation of organ size. GM200003 has **CBI-deleted** changes in both seed morphology and composition.

E. Description of reagents used for genome editing
Single cotyledon whole embryonic axes explants from soybean variety [ ] were **CBI-deleted** transformed with construct F135486 (see Table 1) containing a selectable marker, [ ] **CBI-deleted** CRISPR nuclease, and BH303-4 guide targeting the [ ] gene using Agrobacterium **CBI-deleted** transformation strain AGL1 using standard protocols (Karmakar et al., 2019; Li et al., 2017; Paz et al., 2006).

Transformed plants were identified utilizing the selectable marker and screened for T-DNA copy number. Edited T0 plants were identified by droplet digital PCR (BioRad Droplet Digital PCR, BioRad Rare Mutation Detection), selfed, and the resulting T1 plants were analyzed by amplicon sequencing to confirm the edit (Clement et al., 2019; Illumina MiSeq System Guide, 2018; Illumina MiSeq System Guide, 2019). Additional PCR-based assays were performed to confirm absence of the T-DNA insert (Applied Biosystems User Bulletin #2). The homozygous T1 plant, P181358.1:38, containing the desired edit with no T-DNA was selected to carry forward and is designated with the name GM200003. This plant was analyzed and confirmed to contain wild-type sequence at the potential off-target site, [ ] gene (Figure 2). The T1 plant was free of foreign DNA and contains a homozygous deletion [ ] of the [ ] gene. **CBI-deleted** **CBI-deleted**

Table 1. Genetic elements of transformation vector F135486 used to create GM200003 soy

Plasmid Genetic Elements – Construct F135486			
Element	**Function**	**Source**	
Left Border	Enables T-DNA transfer and integration	*Agrobacterium tumefaciens*	
NOSt	Terminator for Plant Selectable Marker	Terminator sequence of the Nopaline synthase gene from *Agrobacterium tumefaciens*	
BH268	Plant Selectable Marker	[ ]	**CBI-deleted** **CBI-deleted** **CBI-deleted** **CBI-deleted**
AtUbi10p	Promoter for Plant Selectable Marker	Polyubiquitin 10 promoter from *Arabidopsis thaliana*	
CAP binding site	Catabolite activator protein increases binding ability of RNA polymerase in lac operon	*Escherichia coli*	
Lac promoter	Lac operon binding site for RNA polymerase	*Escherichia coli*	
Lac operator	Lac operon binding site for repressor protein	*Escherichia coli*	
35S-ENp	Promoter for Visual Marker	Promoter sequence from the 35S transcript from Cauliflower mosaic virus	
BH193	GFP: Plant Visual Marker	Green fluorescent protein gene from *Aequorea victoria*	
SEKDEL	Localizes BH193 to the Endoplasmic Reticulum	Amino Acid Sequence Ser-Glu-Lys-Asp-Glu-Leu (SEKDEL) occurring in plants	
CaMVt	Terminator for Visual Marker	Terminator sequence of the 35S transcript from Cauliflower mosaic virus	
PsUBI3p	Promoter for [ ] Nuclease	Polyubiquitin 3 promoter from *Pisum sativum*	**CBI-deleted**
5' UTR	Aids in Ribosome Recruitment	Polyubiquitin 3 5'untranslated region from *Pisum sativum*	
5'UTR Intron	Non-coding Sequence Involved in Splicing	Polyubiquitin 3 intron from *Pisum sativum*	
N0033	[ ] CRISPR Nuclease	CRISPR nuclease from [ ]	**CBI-deleted**

AtHSP18.2t	Terminator for [ ] Nuclease	Heat shock protein 18.2 terminator from *Arabidopsis thaliana*	CBI-deleted
AtUbi11p + Intron	Promoter for crRNA	Polyubiquitin 11 promoter from *Arabidopsis thaliana*	
FnHP and HH ribozyme	Mediates cleavage of RNA, RNA stability	Hammer head ribozyme structure from avocado sunblotch viroid	
Fn Mature	Direct Repeat sequence (crRNA Hairpin Leader)	Direct repeat sequence from CRISPR locus of [ ]	CBI-deleted
BH303-4	crRNA	crRNA sequence for [ ] from *Glycine max*	CBI-deleted
HDV Ribozyme	Mediates cleavage of RNA, RNA stability	Hepatitis delta virus ribozyme from hepatitis delta virus	
NOSt	Terminator for crRNA	Terminator sequence of the Nopaline synthase gene from *Agrobacterium tumefaciens*	
Right Border	Enables T-DNA transfer and integration	*Agrobacterium tumefaciens*	

F. Description of intended deletion in [ ] gene in GM200003 CBI-deleted

Below is the description of the change obtained in the target sequence after applying the technique. GM200003 contains a homozygous deletion [ ] of the target gene, [ ]. CBI-deleted

[

] CBI-deleted

G. No plant pest sequences remain in GM2000003

Absence of foreign DNA in the genome was confirmed through real-time PCR-based assays utilizing fluorescent-labelled hybridization probes. The hybridization probes were specific to regions within the T-DNA borders as well as regions spaced across the transformation backbone. Each reaction included a separately labelled internal reference gene native to *Glycine max*. A sample from the wild-type soybean variety that was used for the transformation was included as the negative control. Synthetic DNA matching the Agrobacterium vector F135486 was included as the positive control. Data was analyzed utilizing the comparative Ct method as detailed in Applied Biosystems User Bulletin #2. The negative control did not produce an amplification signal with any of the hybridization probes, indicating no exogenous DNA was present. The positive control produced an amplification signal with each hybridization probe, indicating exogenous DNA was present. No amplification signal was detected with any of the hybridization probes for GM200003, confirming no exogenous DNA remained in the edited line. Based on this analysis, we conclude there is no exogenous DNA present in GM200003.

H. Off-target Analysis

In our guide design process, we select guides that are specific to the target gene with no matches to other parts of the genome. When this is not possible, any guide with the potential to generate an off-target edit that contains 3 or less mismatches is monitored by PCR amplification of the off-target region and sequencing (Bae et al., 2014). For guide BH303-4, bioinformatic analysis demonstrated potential for a single off-target edit in the [ ] gene CBI-deleted (Figure 1). Sequencing of GM200003 confirmed the edit to be present only in the target gene, [ ], and wild-type sequence at the potential off-target gene, [ ] (Figure 2). CBI-deleted

Figure 1. BH303-4 Guide Design and Potential Off Target

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Figure 2. Sequence of Target and Potential Off-Target Genes

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I. References

Applied Biosystems (2001), User Bulletin #2 ABI Prism 7700 Sequence Detection System https://assets.thermofisher.com/TFS-Assets/LSG/manuals/cms_040980.pdf

Bae, S., Park, J., & Kim, J. S. (2014). Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. *Bioinformatics,* 30:1473-1475. DOI: 10.1093/bioinformatics/btu048

BioRad, Droplet Digital PCR Applications Guide, Bulletin#6407 https://www.bio-rad.com/sites/default/files/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf

BioRad, Rare Mutation Detection Best Practices Guidelines, Bulletin# 6628 https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6628.pdf

Clement, K., Rees, H., Canver, M. C., Gehrke, J. M., Farouni, R., Hsu, J. Y., Cole, M.A., Liu, D. R., Joung, J. K., Bauer, D. E., & Pinello, L. (2019). CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nat Biotechnol.*, 37(3):224-226. DOI: 10.1038/s41587-019-0032-3. PubMed PMID: 30809026

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Illumina (2018), MiSeq System Guide for MiSeq Reporter, Document# 1000000061014 https://support.illumina.com/downloads/miseq-system-user-guide-for-miseq-reporter-1000000061014.html

Illumina (2019), MiSeq System Denature and Dilute Libraries Guide, Document# 15039740-10 https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/miseq-denature-dilute-libraries-guide-15039740-10.pdf

Karmakar, S., Molla, K. A., Gayen, D., Karmakar, A., Das, K., Sarkar, S. N., Datta, K. & Datta, S. K. (2019). Development of a rapid and highly efficient *Agrobacterium*-mediated transformation system for pigeon pea [Cajanus cajan (L.) Millsp]. *GM Crops & Food*, 10(2):115-138. DOI:10.1080/21645698.2019.1625653

Li, S., Cong, Y., Liu, Y., Wang, T., Shuai, Q., Chen, N., Gai, J., & Li, Y. (2017). Optimization of *Agrobacterium*-Mediated Transformation in Soybean, *Front. Plant Sci.*, 8:246. DOI:10.3389/fpls.2017.00246

Paz, M. M., Martinez, J. C., Kalvig, A. B., Fonger, T. M., & Wang, K. (2006). Improved cotyledonary node method using an alternative explant derived from mature seed for efficient *Agrobacterium*-mediated soybean transformation. *Plant Cell Rep*, 25(3):206-213. DOI 10.1007/s00299-005-0048-7

Please contact me at (314) 605-7071 or smartino-catt@bensonhill.com if you have questions or need additional information. Thank you for your time in reviewing this request.

Sincerely,

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