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*By mlwanex for BRS Document Control Officer at 7:58 am, Sep 18, 2018*

Dr. Michael J. Firko  
APHIS Deputy Administrator  
Biotechnology Regulatory Services  
4700 River Rd. Unit 98  
Riverdale, MD 20737

September 17, 2018

Dear Dr. Firko,

I am submitting a letter of inquiry to request USDA-APHIS confirmation that our genome edited lettuce lines are not regulated under 7 CFR part 340. Based on our attached evaluation, the resulting lettuce lines are null segregants and do not contain any transgenes or sequences from a plant pest. Moreover, the introduced deletions were ultimately repaired by the plant's naturally occurring repair mechanisms. Lastly, the lettuce lines are themselves are not a plant pest and, therefore, do not meet the definition of a regulated article under 7 CFR Part 340. We believe that all relevant information supporting our case is provided in this letter. This letter contains confidential business information. Please let us know if we can provide any additional information that will aid your review of our request.

Sincerely,



Sekhar Boddupalli, PhD  
President, Ag Bio Division  
Phone: 530-746-6237  
Email: sboddupalli@dna.com

**Product Overview**

Developer Name: Intrexon Corp. 1750 Kraft Drive, Suite 1400, Blacksburg, VA 24060.

Organism: Lettuce [ ]

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Intended activity: Movement and release

Genetic change: Loss of function [ ] due to [ ] deletion

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Vector: [ ]

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Name of construct(s): ID 123

**Introduction**

[ ] gene edited lettuce produced by [ ] and subsequent segregation [ ] is a null segregant, free of plant pest sequences and transgenes [ ]. Lettuce is also not listed as a federal noxious weed pursuant to 7 CFR part 360, and there is no reason to believe that [ ] would increase its weediness in any way.

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Based on our understanding of 7 CFR parts 340 and 360, this gene edited lettuce would not be regulated by USDA.

**Summary of the proposed product concept**

- Lettuce is the lead crop across the globe in the vertical or indoor farming space and the fourth most important vegetable crop grown in greenhouses
- Controlled environment agriculture (CEA), also known as indoor farming, urban farming, and vertical farming, is a rapidly growing market segment in crop agriculture.
- Currently, cut bagged lettuce is subjected to physical and/or chemical treatments to reduce [ ]

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- [ ]

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- [ ]

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- [ ]

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- Gene edited [ ] plants were self-fertilized. Progeny containing [ ] but lacking [ ]

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[ ], were identified by PCR. These plants contain [ ] but no longer contain plant pest derived DNA elements or any transgenes.

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- [

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### **Background**

Lettuce is one of the most commonly consumed “ready to eat” leafy vegetables in the United States. The United States alone produces over 8 billion pounds of lettuce, worth \$1.9B (USDA-ERS: Vegetable and Pulses Data-2017). At least 90% of that total is consumed domestically. Cut lettuce, however, is a highly perishable commodity and significant loss occurs [ ] during storage and distribution. Several practices such as optimized growing conditions (reduced irrigation/nitrogen and early harvesting) and post-harvest practices (application of antioxidant chemicals, low oxygen packing, and low temperature storage) are all utilized [ ]. However, these methods are not completely effective, in addition to adding significant costs to production and storage. [

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] We are hopeful that [

] and may contribute to cost savings and reduced food waste at all levels of commercial production, retail distribution, and by consumers.

### **Methods**

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]

Analysis of [ ]  
[ ]

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**Plant Pest Element Screening**

[ ]

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[ ] screened by PCR to ensure that none had plant pest sequences remaining in the genome. [ ]

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[ ] Absence of the plant pest DNA sequences was confirmed by three additional sets of PCR primers designed to amplify different parts [ ]

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[ ] An additional primer set: (A178/A181) which amplifies the endogenous PDF gene was included in all PCR reactions as a positive control for DNA quality. [ ]

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[ ] All primer sets were first used to amplify genomic DNA [ ] ensuring that the primer design was robust and able to produce a single amplification product. All primer sets did amplify the expected size fragment [ ]

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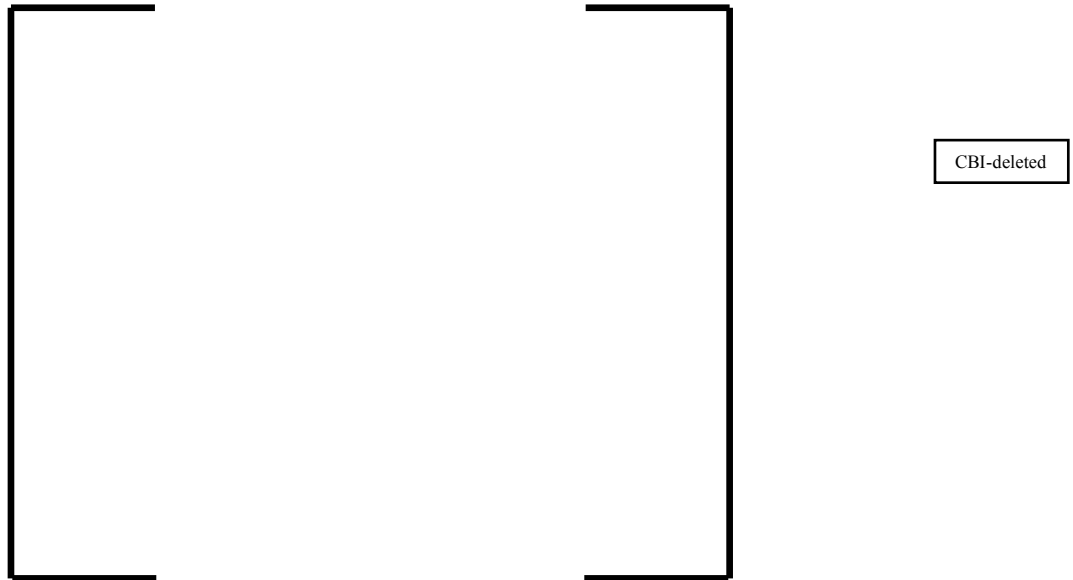
**Conclusion**

[ ]

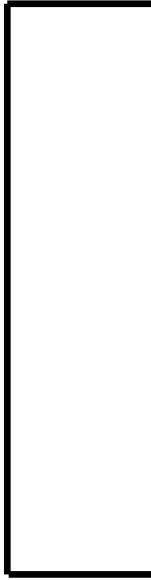
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[ ] We further conclude that the events described herein are free of plant pest sequences and transgenes and, therefore, not subject to regulation under 7 CFR

part 340. Also, lettuce is not currently listed as a noxious weed pursuant to 7 CFR part 360.



**Fig. 1**  
Plasmid map of ID 123



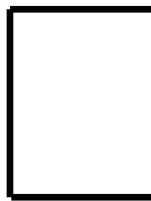
**Fig. 2**  
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**Table 1**  
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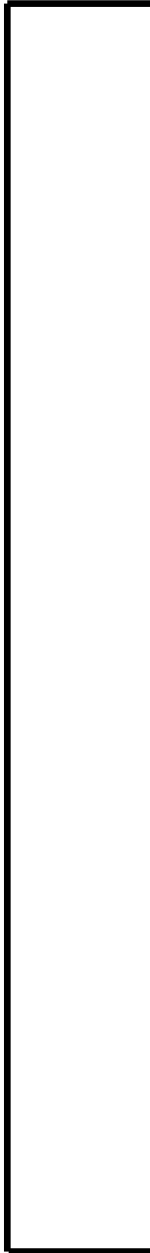
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**Table 2**

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**Table 3**  
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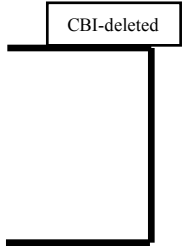
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**Fig. 3**

[



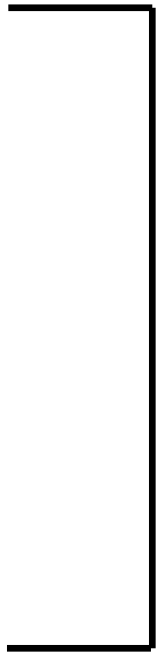
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**Figure 4**

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