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1. Requestor

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2. Confidential Business Information (CBI)

This document contains Confidential Business Information (CBI)

CBI Justification Statement

This document contains Confidential Business Information (CBI) with plant phenotypes, the genetic constructs used and the methodology the subject of current patent preparations and are therefore not publicly available. The information marked with square brackets ([ ]) is commercially valuable, currently being used at Insignum AgTech, LLC and is maintained in secrecy. The document is marked as containing Confidential Business Information and a separate version of this document has been prepared with this information deleted and marked as CBI-Deleted

The details of the genetic construct used to transform each plant line is a valuable trade secret. The genomic site of modification, described in Figure 1, is integral to the function of our technology and has not been disclosed. The genetic pieces used in creating our technology have not been and will not be disclosed. These constructs were created in-house and are the sole property of Insignum AgTech.

The phenotypes of the plants under review are a critical component of the product that Insignum AgTech is creating. The photographs provided are for explanatory purposes and is proprietary. The DNA sequence of the final product (supplied in Appendix 1) further details proprietary information.

The method of selection of modified plants, and the suggested NGS depth could provide enough information to competitors about the nature of the modification used to create our products.

3. Introduction and background

Insignum AgTech is developing corn (*Zea mays*) lines that will signal attack by fungal diseases by utilizing the existing anthocyanin pathway in these plants. To achieve this, Insignum AgTech utilizes gene-editing techniques to [

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Although gene-editing involves the use of foreign DNA, no foreign DNA will be present in the final product and [

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], only sequences known to occur in the maize gene pool CBI del
are present in the final product.

4. Comparator Plant

Zea mays L. Common name corn or maize. This crop plant is already very widely utilized and Insignum assumes that a Plant Reference Document already exists for this plant.

5. Genotype of the modified plant

Description of genetic modification

Figure 1 below illustrates the genetic modification in Insignum AgTech's modified corn plants. **[** CBI del
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The expected DNA sequence of the modification compared to the non-modified line is attached in Appendix 1. Confirmation of this sequence is part of the selection process described below.

Selection of modified plants

After initial selection of transgenic plants with bialaphos, plants with the correct integration of [ ] in the intended location will be identified via DNA sequence analysis. The inserted DNA elements identified above as 1, 2, 3 and 4 will be removed by selective breeding to produce plants with [ ], but with no foreign DNA.

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Selected plants will be confirmed as having the specific intended insertion through sequence analysis, via whole-genome next-generation sequencing (NGS) carried out with Illumina, PacBio or Oxford Nanopore platforms [ ]. This selection will result in plants with a single insertion in the desired gene and will also confirm the lack of any inserted foreign DNA fragments from the transformation construct remaining in the final product. The use of existing platforms for DNA sequencing will allow the use of established algorithms for the detection of foreign DNA. [

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6. Intended Phenotype

Phenotype of modified plant

Corn plants developed by Insignum AgTech will signal infection by pathogen through the production of the anthocyanin pigment. This will allow for rapid identification of infection before any symptom of the infection. Figure 2 below shows an example of a modified plant with purple anthocyanin pigmentation around the site of infection.

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Figure 2. Modified plant showing [ ] CBI del

Mode of Action

The mode of action for this trait is to use the existing anthocyanin pathway to signal pathogen infection by placing [ ]. In this way, CBI del
infection by a pathogen that would trigger expression of [ ] gene would result in the production CBI del
of anthocyanin and thereby indicate infection prior to the development of symptoms the disease itself.

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] Therefore, a CBI del
change in phenotypes other than the color change described above is not expected.

Anthocyanin pigmentation in corn plants is a common occurrence and has not been linked to any deleterious effect on human or animal health or in the environment. Cell-specific anthocyanin pigmentation was noted in early studies on the transfer of genes within the corn genome through the action of transposable elements. Barbara McClintock used anthocyanin production as a visual indicator to track what was later termed transposition (McClintock, 1953). Since her seminal work, others have identified and described not only the structural genes involved in anthocyanin biosynthesis, but the transcription factors that regulate them (Chatham, *et al.*, 2019). In fact, although this particular integration of the anthocyanin-producing gene has not been identified, it is quite within the expected natural genetic variation of corn that such changes as proposed by Insignum AgTech could occur without human intervention and thus be considered to be within the range of natural variation.

Anthocyanin pigments are Generally Regarded As Safe (GRAS, 21CFR73.169) by the Food and Drug Administration. Anthocyanins as visible markers have been used by maize breeders in the production of doubled-haploid inbred lines for over 50 years (Nanda and Chase, 1966). This mode of action is not considered to produce any potential hazard as expression of the anthocyanin pigment in maize plants is very common and not associated with any negative impacts. Blue or purple corn chips (with high levels of anthocyanin) are found in supermarkets (https://www.kroger.com/p/simple-truth-organic-blue-corn-tortilla-chips/0001111086062), and supplements of concentrated purple corn extract claim to have high antioxidant activity and immune system benefits (https://cellfend.com/pages/pce)

References

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Appendix 1

The sequences herein were obtained from two sources.

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