

## Plant Breeding Innovation (PBI) Product Information Sheet (PIS)

<b>Part I. Background Information</b>	
1. Name of Product Developer	Tropic Biosciences (CEO: Gilad Gershon)
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<b>Part II. Description of the PBI Product</b>	
1. Name of the PBI Product	Reduced Browning Banana (TRB011002)
2. Identification of the PBI Product (organism)	Scientific Name: <i>Musa acuminata</i>
	Common Name: Cavendish subgroup Grande Naine cultivar
3. Phenotypic feature before and after genetic change (Explain in detail.)	<p>Polyphenol oxidase (PPO) enzymes are released from plastids upon mechanical damage of banana fruits, including peeling, bruising and slicing. The released PPO enzymes oxidize phenolic compounds in fruit tissues, resulting in discoloration known as enzymatic browning. This process ultimately lowers the quality of banana fruits.</p> <p>Reducing <i>PPO</i> expression has proven an effective strategy for reducing enzymatic browning in fruits and vegetables. Knockout of <i>PPO</i> genes in potato and apple resulted in reduction in PPO activity and reduction in tuber and fruit browning. Therefore, reducing <i>PPO</i> expression in banana fruit tissue could result in reduced browning fruit.</p> <p>To produce a reduced browning banana, Tropic Biosciences first performed genomic and transcriptomic analyses on <i>M. acuminata</i>. Genomic analyses identified nine functional <i>PPO</i> genes encoded in the triploid Cavendish <i>M. acuminata</i> genome (AAA).</p>

	Gene editing of a highly expressed <i>PPO</i> gene in the fruit flesh introduced mutations that are expected to prevent functional PPO protein production and significantly reduce PPO levels in the banana fruit resulting in reduced enzymatic browning and a reduced browning banana variety.
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<b>Part III. Description of the Plant Breeding Innovation (PBI) Procedure Used (To Be Used)</b>	
1. Purpose of the PBI	Develop banana ( <i>Musa acuminata</i> ) with reduced browning
2. PBI procedure	SDN1
3. Genetic change in the organism	
a. Name of the molecular tools used	CRISPR / Cas9
b. Delivery system	Agrobacterium-mediated
c. Nature of DNA changes	Insertion and deletions
4. Experimental evidence showing the final PBI product has no new combination of genetic material in the form of foreign DNA insert or sequences from gene editing tool construct using appropriate molecular techniques.	<p><b>Molecular characterization for the absence of plasmid DNA integration</b></p> <p>In the initial screen, genomic DNA was extracted from a single leaf of the banana plants regenerated from the <i>Agrobacterium</i>-treated embryogenic cells which transiently expressed genes from the T-DNA. Absence of the T-DNA in these banana plants was assessed using quantitative PCR (qPCR) with primers designed to amplify from the two elements in the T-DNA.</p> <p>To further confirm the absence of plasmid DNA in the genome of the edited banana plants, genomic DNA was extracted from at least two distinct leaves of the plants for additional qPCR analyses. These qPCR investigations were performed using primer pairs targeting regions of the T-DNA and plasmid backbone, including the elements encoding Cas9, the sgRNA cassettes, the bacterial and plant resistance markers, and the left and right T-DNA borders. The plasmid-specific primers failed to specifically amplify from genomic DNA extracted from the reduced browning banana plants. This was also the case for DNA from the negative control wild-type banana plant. In contrast, these primers successfully amplified from a positive control DNA sample of a plasmid carrying a single copy of each genetic element being probed for. The control qPCR reactions using a primer pair targeted to the endogenous banana gene, successfully amplified from all banana genomic DNA samples. These analyses therefore confirmed that plasmid sequences are absent from the genome of the reduced browning banana plants.</p>

	Finally, the WGS data for the reduced browning banana plants was assessed to further test for the integration of plasmid DNA. Aligning the sequencing reads to the plasmid map revealed that plasmid DNA is not present in the genome of the reduced browning banana plants.
5. Any existing regulatory precedence on the PBI Product in the issuing country and purpose of the decision (if applicable).	Columbia Instituto Colombiano Agropecuario (ICA) determined that the reduced browning banana plants were equivalent to conventional cultivars (notification received 27 Feb 2024).