Prior Evaluation Form (PEF) for Products of Plant Breeding Innovation (PBI)

"*Commercial-in-Confidence Deleted"*

Part I. Background Information	
1. Name of Product Developer	Tropic Biosciences (CEO: Gilad Gershon)
2. Office Address	Norwich Research Park, Innovation Centre, NR4 7GJ
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5. Website (if any)	https://tropic.bio/
6. Name of Agent	Gabriel Romero
7. Position	North Hill Group Country Head, Philippines
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Part II. Description of the PBI Product	
1. Name of the PBI Product	Reduced Browning Banana
2. Identification of the PBI Product (organism)	Scientific Name: *Musa acuminata*
	Common Name: Cavendish subgroup Grande Naine cultivar
3. Phenotypic feature before and after genetic change (Explain in detail.)	In bananas, PPO enzymes are released from plastids upon mechanical damage of the fruits, including peeling, bruising and slicing. The released PPO enzyme oxidizes phenolic compounds in fruit tissues, resulting in discoloration known as enzymatic browning and ultimately lowering the quality of the bananas. There are four PPO genes in banana with varied expression levels in the fruit. The PPO gene which accounts for the highest mRNA abundance and predominantly expressed was edited to reduce browning in *Musa acuminata*. Gene editing of the PPO gene allele resulted in the loss of function that prevented the functional protein of one of the PPO genes from being produced. As a consequence, the overall PPO enzyme content in the banana fruit is reduced.

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Part III. Description of the Plant Breeding Innovation (PBI) Procedure Used {To Be Used)		Reference/s (If Applicable)
1. Purpose of the PBI	Develop banana *(Musa acuminata)* with reduced browning	
2. PBI procedure	☑ SDN 1	
3. Genetic change in the organism		
a. Name of the molecular tools used	CRISPR / Cas9	
b. Description and nucleotide sequence of the molecular tools used i. Nuclease ii. Vectors	 Cas9 disarmed Ti plasmid	
c. Delivery system	☑ Agrobacterium-mediated	
d. Nature of DNA changes	☑ 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.	**Molecular characterization – PCR amplification and sequencing of targeted PPO gene modifications** In the initial screen, genomic DNA was extracted from a single leaf of banana plants regenerated from *Agrobacterium*-transformed embryogenic cells (transiently expressing the gene from the T-DNA). The two regions of the banana PPO gene targeted by sgRNAs were amplified by PCR and analysed using Sanger sequencing. The relative height of chromatogram peaks was used to assess allelic ratios of the identified modification(s). To confirm genetic modification, analyses were then repeated using genomic DNA extracted from leaves from at least two distinct regions of the plants. The analysis confirmed a deletion in the target PPO gene. The screening and validation of the reduced browning banana is shown in Figure 1. **Molecular characterization – quantitative PCR analyses for absence of plasmid DNA** In the initial screen, genomic DNA was extracted from a single leaf of banana plants regenerated from *Agrobacterium*-transformed embryogenic cells (transiently expressing the gene from the T-DNA). Absence of T-DNA in the banana plants was assessed using quantitative	

	PCR (qPCR) with primers designed to amplify two regions of the T-DNA. To confirm the absence of plasmid DNA in the genome of the plants, genomic DNA extracted from leaves from at least two distinct regions of the plants and qPCR analyses were performed using primers spanning 9 regions of the T-DNA and plasmid backbone, including Cas9, sgRNA cassettes, bacterial and plant resistance markers, and left and right T-DNA borders. As shown in Table 1, plasmid-specific primers failed to amplify target sequences from genomic DNA extracted from reduced browning banana plants. This was also the case for DNA from negative control wild-type plants, whereas these primers did amplify plasmid sequences from genomic DNA extracted from positive control transgenic plants. As an internal control, an endogenous banana genomic region amplified in all samples. These analyses, therefore, confirm that plasmid sequences are absent from the genome of reduced browning banana plants.	
5. Any existing regulatory precedence on the PBI Product in the issuing country and purpose of the decision (if applicable).	a. US confirmation of exemption from regulation b. Honduras confirmation of exemption from regulation	

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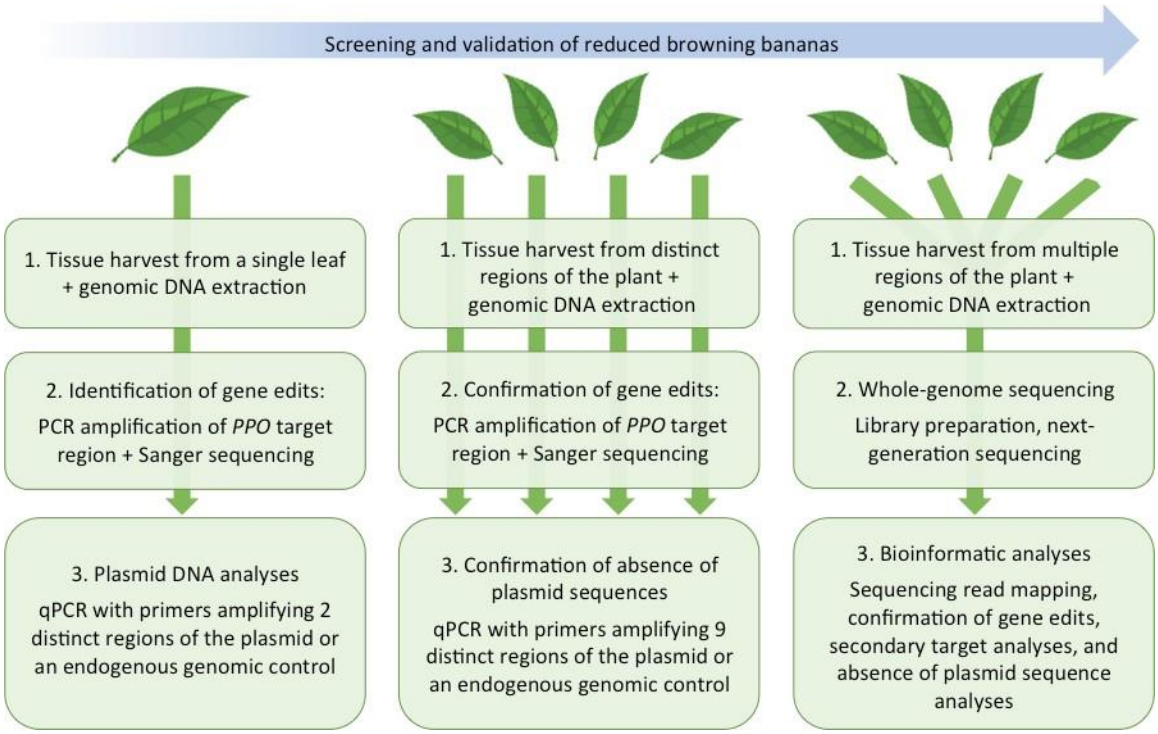

Figure 1: Screening and validation of reduced browning banana using molecular analysis.

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Table 1: Cq values from quantitative PCR analyses

Sample	qPCR amplicons[1]								
	1	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**Endogenous genomic control**
Reduced browning banana plant	NA[2]	NA	NA	NA	NA	NA	NA	NA	27.2
Negative control wild-type banana plant	NA	NA	NA	NA	NA	NA	NA	NA	28.19
Positive control transgenic banana plant	23.1	21.46	21.39	20.84	23.56	22.86	22.28	22.48	30.19

[1]Amplicon IDs from Figure 3

[2]NA = Not Amplified, DNA region not present in the sample

Part IV. Scientific Studies, Experimental Evidences, and Others Submitted with This Form

1. US confirmation of exemption from regulation
2. Honduras confirmation of exemption from regulation