

Responses to questions received on 7th May 2026

Assessment of unintended genetic changes (Section 7a)

Section 7a of the marketing notice describes the assessment of unintended genetic changes by reference to in silico prediction of potential off-target sites following guide RNA (gRNA) design. To assist ACRE's consideration, please clarify:

a. Which genome sequence(s) were used to assess potential off-target sites using the Breaking-Cas tool referenced? (e.g. a published reference genome, an in-house reference genome, or other sequence resource).

Response:

The public reference genome published by Martin et al. (2016), which is known as DH-Pahang v2, was used to assess for potential off-target sites using the Breaking-Cas tool. DH-Pahang is a doubled-haploid *Musa acuminata* genotype of the wild subspecies *malaccensis* that contributed to the A genomes of cultivated Cavendish banana (AAA) (D'Hont et al., 2012). DH-Pahang is commonly used as a public reference for genomic analyses in banana (Wang et al., 2015; Kalungi et al., 2025; Van den Broeck et al., 2025). Further information on the DH-Pahang v2 genome can be found on the Ensembl Plants genomic database, which is publicly accessible via the following link:
https://plants.ensembl.org/Musa_acuminata/Info/Annotation/#assembly.

Date links accessed:

11th May 2026

References:

Kalungi, F., Mulindwa, J., Mustafa, A. S., Namanya, P. B., Kubiriba, J., Barekye, A., & Tindamanyire, J. M. (2025). Efficient CRISPR/Cas 9-mediated genome editing of phytoene desaturase in *Musa*-AAA: a critical step for genetic improvement of east African highland bananas. *Frontiers in Plant Science*, 16, 1677409.

Martin, G.; Baurens, F.C.; Droc, G.; Rouard, M.; Cenci, A.; Kilian, A.; Hastie, A.; Dolezel, J.; Aury, J.M.; Alberti, A.; Carreel, F.; D'Hont, A. (2016) Improvement of the banana "*Musa acuminata*" reference sequence using NGS data and semi-automated bioinformatics methods. *BMC Genomics* 17:243. ISSN: 1471-2164.

Van den Broeck, S., Ngapout, Y., Panis, B., & Vanderschuren, H. (2025). An Agrobacterium-mediated base editing approach generates transgene-free edited banana. *New Phytologist*, 247(4), 1790-1804.

Wang, Z., Jia, C., Li, J., Xu, B., & Jin, Z. (2015). Identification of six mitogen-activated protein kinase (MAPK) genes in banana (*Musa acuminata* L. AAA group, cv. Cavendish) under infection of *Fusarium oxysporum* f. sp. cubense Tropical Race 4. *Acta Physiologiae Plantarum*, 37(6), 115.

b. Whether and if so how, the whole-genome sequencing (WGS) data for the edited line TRB011002 described at Section 9 of the marketing notice, were assessed in relation to potential unintended genetic changes (e.g. off-target edits), or alternatively why the WGS data was not used for this purpose (e.g. technical or methodological limitations relevant to the detection of off-target edits).

Response:

For TRB011002, gRNA sequence evaluation was conducted using the Breaking-Cas tool to assess for potential off-target sites. No potential off-target sites with ≤ 2 mismatches with a target sequence were identified, indicating that off-target editing is highly unlikely to occur (Tang et al., 2018). Accordingly, no off-target edits are considered to be present in the genome of the reduced browning banana line TRB011002. If any such potential off-target sites had been identified using the Breaking-Cas tool, the WGS data would have been analysed to assess the sites for novel nucleotide variation in TRB011002 compared to the unedited wild-type control (i.e., the banana line used to generate TRB011002).

References:

Lemke, S., Tao, X., & Kushner, G. J. (2022). Assuring the food safety of crops developed through breeding. *ACS Agricultural Science & Technology*, 2(6):1151-1165.

Technology OGTR (2023) The Biology of *Musa* L. (banana) Version 3: February 2023
Australian Government Office of the Gene Technology Regulator
https://www.ogtr.gov.au/sites/default/files/2023-02/the_biology_of_musa_l_banana.pdf.

Tang, X., Liu, G., Zhou, J., Ren, Q., You, Q., Tian, L., Xin, X., Zhong, Z., Liu, B., Zheng, X., Zhang, D., Malzahn, A., Gong, Z., Qi, Y., Zhang, T., Zhang, Y. (2018) A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. *Genome Biol.* 19:84. <https://doi.org/10.1186/s13059-018-1458-5>.

c. Whether WGS data is available for the non-edited parental line used to generate TRB011002, and if so,

whether they were used in assessing unintended genetic changes. If not, a brief confirmation is sufficient.

Response:

WGS was performed for the reduced browning banana line TRB011002 and the unedited wild-type control (i.e., the banana line used to generate TRB011002). If any potential off-target sites with ≤ 2 mismatches with a gRNA target sequence had been identified in the Breaking-Cas analysis, the WGS data for TRB011002 and the wild type control would have been analysed for those sites to assess for novel nucleotide variation in TRB011002 compared to the unedited wild-type control. As there were no such potential off-target sites identified in silico with Breaking-Cas, no assessment for off-target editing in the WGS data for TRB011002 and the unedited wild type control was performed.