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Certified Reference Materials

AOCS 0711-C3

Report of the certification process for

Rf2

Canola Certified Reference Materials

Third Batch

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ISO 17034:2016
A2LA Certificate 3438.01

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Abstract

This report describes the preparation and certification of the canola CRM AOCS 0711-C3 produced by AOCS Technical Services in 2018. The CRMs have been prepared according to ISO Guide 17034:2016 and are intended to serve as control material for third party testing of canola for transformation events. The presence of Rf2 in the canola was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory). AOCS 0711-C3 is available in 0.5 ml skirted screw-cap self-sealing tubes. The canola Rf2 DNA was provided by BASF Agricultural Solutions Seed US LLC and was extracted from clean leaves. The leaf DNA extract sample shall be stored dry in a sealed container at +4 °C in the dark.

Acknowledgements

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Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	A related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food
DNA	Deoxyribonucleic Acid is the linear, double-helix macromolecule that makes up the genetic material of most organisms
Detection Limit	Lowest level at which target DNA can be detected in a sample.
EC	European Commission
Genome	The full set of genes and associated DNA characteristic of an organism
GMO	Genetically modified/engineered organism: an organism in which the genetic material has been changed through modern biotechnology in a way that does not occur naturally by multiplication and/or natural recombination.
ISO	International Organisation for Standardisation
PCR	Polymerase Chain Reaction: technique used to determine whether a sample of plant tissue contains a particular DNA sequence. PCR relies on primer sets that bind to a particular target DNA sequence and a special DNA-copying enzyme

(DNA polymerase) that exponentially amplifies the target sequence for identification and measurement

Qualitative PCR	PCR methods that determine the presence or absence of a specific target DNA sequence at a particular level of detection
Quantitation Limit	Lowest level at which the amount of target DNA sequence in a sample can be reliably quantitated
Quantitative PCR	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules
Trait: Rf2	Glufosinate ammonium herbicide tolerance and male sterility

Introduction

Plant genetic modification is an extension of traditional plant breeding. It allows plant breeders to develop crops with specific traits including insect, disease, and herbicide resistance; processing advantages; and nutritional enhancement. An important component for identifying these new traits is a Certified Reference Material created from leaf, seed, or grain containing the new trait as well as a CRM created from the conventionally bred matrix. The European Commission has mandated that from 18 April 2004, a method for detecting a new event derived from transgenic technology and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new crop derived from transgenic technology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above regulatory requirements for GMO determination, AOCS 0711-C3 was manufactured from canola according to ISO Guide 17034:2016 and in accordance with EC No 1829/2003. The CRM is available from AOCS.

Materials and Methods

BASF Agricultural Solutions Seed US LLC prepared the bulk material by taking source leaf material from plants which had been tested individually using several quality standards and was grown from seeds harvested from plants that had themselves passed the same criteria. Plants not meeting the quality standards were removed and destroyed. Leaf material was harvested from the plants which met the quality standards and frozen immediately and stored at -70 °C. The genomic DNA was extracted from leaves of one or more plants according to CTAB-based (Doyle JJ and Doyle JL, 1987) protocol. The integrity and concentration of the genomic DNA was determined by ethidium bromide-stained 1% agarose gel electrophoresis and compared to lambda molecular weight standards by digital imaging quantification. The concentration measurement was done in triplicate, repeated in three different gels. No indications for physical degradation were apparent and the DNA migrated at positions higher than 40 Kb.

BASF Agricultural Solutions Seed US LLC delivered 2 mg of Rf2 canola to AOCS. Five (5) working samples of DNA, 10 µg each, were prepared from the composite and sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for the presence of the intended event, Rf2. This testing was for presence confirmation as well as homogeneity purposes.

The leaf used to manufacture the Rf2 materials was shown to contain the Rf2 event using PCR protocols at BASF Agricultural Solutions Seed US LLC. The Rf2 canola leaf DNA was packaged by SGS-Midwest Seed Services in sterile, 0.5 ml skirted screw-cap self-sealing tubes in aliquots of 10 µg.

AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of gene presence, homogeneity, and to rule out degradation during packaging. Sample numbers AOCS 0711-C3: 55, 139, 163, 171, and 187 were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for Rf2 presence in the samples.

Stability

Stability of these CRMs has been listed as 1 year from the introduction date. The materials were sealed and stored in the dark at 4 °C, therefore not exposed to air and are expected to be stable for longer than the estimated expiration date. The stability of the leaf DNA extract material will be reevaluated annually. If the samples still test positive for the presence of the trait, the certificates will be extended.

Results and Discussion

Sample Homogeneity

The PCR data for the Rf2 homogeneity samples is presented in Table 1.

Table 1. Results of the homogeneity testing performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) on the Rf2 bulk material 0711-C3 provided by BASF Plant Science L.P.	
Sample	Rf2 Presence
Homogeneity Sample 1	Positive
Homogeneity Sample 2	Positive
Homogeneity Sample 3	Positive
Homogeneity Sample 4	Positive
Homogeneity Sample 5	Positive

Prepared Sample Verification

After the bulk material was packaged, five (5) samples were identified by the Microsoft Excel Random Number Generator and sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis. These results are presented in Table 2. This data confirms the presence of the Rf2 gene after the packaging of AOCS 0711-C3. These results are consistent with the homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0711-C3 Rf2 canola 0711-C3 material as tested by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) with Rf2 event-specific, qualitative PCR analysis.	
Sample	Rf2 Presence
AOCS 0711-C3 55	Positive
AOCS 0711-C3 139	Positive
AOCS 0711-C3 163	Positive
AOCS 0711-C3 171	Positive
AOCS 0711-C3 187	Positive

References

Center for Environmental Risk Assessment GM Database

http://www.cera-gmc.org/?action=gm_crop_database

Eurofins-GeneScan; 2219 Lakeshore Drive, Suite 400, New Orleans, LA 70122;

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<https://www.eurofinsus.com/gmotesting/>

International Seed Testing Association, International Rules of Seed Testing: Seed Science and Technology Rules, 2012

ISO Guide 30:2015 (E/F), Reference Materials – Selected Terms and Definitions

ISO Guide 31:2015 (E), Reference Materials – Contents of Certificates, Labels, and Accompanying Documentation

ISO Guide 17034:2016 (E) General Requirements for the Competence of Reference Material Producers

ISO Guide 35:2006 (E) Certification of Reference Materials – General and Statistical Principles

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