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Certified Reference Materials

AOCS 1116-A

Report of the certification process for

Ms11

Canola Certified Reference Materials

First Batch

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ACCREDITED
CERT #3438.01
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Abstract

This report describes the preparation and certification of the canola CRM AOCS 1116-A produced by AOCS Technical Services in 2017. The CRMs have been prepared ac cording to ISO Guides 30-35 and are intended to serve as control material for third party testing of canola for transformation events. The presence of Ms11 in the canola was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory). AOCS 1116-A is available in 0.5ml skirted screw-cap self-sealing tubes. The canola Ms11 N90-740 DNA was provided by BASF Agricultural Solutions Seed US LLC. The canola Ms11 leaf DNA extract was extracted from clean leaves provided by BASF Agricultural Solutions Seed US LLC.
The leaf DNA extract sample shall be stored dry in a sealed container at ambient or be low ambient and in the dark.

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Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Crop variety with no history of transgenic technology and is produced through traditional plant-breeding techniques that rely on selecting and mating parent plants possessing promising traits and repeatedly selecting for superior performance among their offspring
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
ISO	International Organisation for Standardisation
GMO	Organism that has had genetic sequences modified using molecular-level techniques
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 zero in on a particular target DNA sequence and a special DNA-copying enzyme (DNA polymerase) that makes enough copies of 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

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Quantitation Limit	Lowest level at which the amount of targeted DNA sequence in a sample can be reproducibly measured.
Quantitative PCR	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules
Trait: Ms11	Ms11 was produced by genetically engineering plants to be male sterile and tolerant to herbicide glufosinate ammonium (as a selectable marker)

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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 1116-A was manufactured from canola according to ISO Guides 30-35 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 a number of 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^{o}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 electrophoresis in a 1.0% agarose gel and ethidium bromide-staining 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.

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BASF Agricultural Solutions Seed US LLC delivered the Ms11 canola to AOCS. The 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, Ms11. This testing was for presence confirmation as well as homogeneity purposes.

The leaf used to manufacture the Ms11 materials was shown to contain the Ms11 event using PCR protocols at Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory). The Ms11 canola leaf DNA was packaged by SGS-Midwest Seed Services in sterile, 0.5ml 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 1116-A: 44, 201, 38, 126, and 165 were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for event-specific, qualitative PCR analysis to screen for Ms11 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.

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Results and Discussion

Sample Homogeneity

The PCR data for the Ms11 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 Ms11 bulk material 1116-A provided by BASF Agricultural Solutions Seed US LLC.

Sample	Ms11 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 Ms11 gene after the packaging of AOCS 1116-A. These results are consistent with the homogeneity data presented in Table 1.

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Table 2. Results for the verification of AOCS 1116-A Ms11 canola 1116-A material as tested by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) with Ms11 event-specific, qualitative PCR analysis.	
Sample	**Ms11 Presence**
AOCS 1116-A 44	Positive
AOCS 1116-A 201	Positive
AOCS 1116-A 38	Positive
AOCS 1116-A 126	Positive
AOCS 1116-A 165	Positive

The AOCS 1116-A CRM was prepared solely from an identity preserved canola produced by transgenic technology. Sample heterogeneity was not considered because there was no blending of conventional and transgenic technology derived canola into defined mixtures.

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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;Telephone: +1 504 297 4330 Toll Free: +1 866 535 2730 Fax: +1 504 297 4335 http://www.eurofins.com/gmotesting

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

ISO Guide 30:1992 (E/F), Terms and definitions used in connection with reference materials

ISO Guide 31:2000 (E), Reference Materials- Contents of certificates and labels

ISO Guide 34:2009 (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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