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Certified Reference Materials

AOCS 0306-D5

Report of the certification process for

Nonmodified

Rice Certified Reference Materials

Fifth 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 Rice CRM AOCS 0306-D5 produced by AOCS Technical Services in 2018. The CRMs have been prepared according to ISO 17034:2016 and are intended to serve as control material for third party testing of Rice for transformation events. The absence of LLRice62 was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory). AOCS 0306-D5 is available in 0.5 mL skirted screw-cap self-sealing tubes. The nonmodified rice DNA was provided by Bayer CropScience. Nonmodified rice leaf tissue genomic DNA was extracted from clean leaves at Bayer CropScience. The leaf tissue genomic DNA sample shall be stored dry in a sealed container at +4 ˚C 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 Organization for Standardization
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 specific DNA sequence. PCR relies on primer sets that attach to a specific target DNA sequence and DNA polymerases that produces 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 or above the level of detection provided by the official method
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

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 0306-D5 was manufactured from rice DNA according to ISO 17034 and in accordance with EC No 1829/2003. The CRM is available from AOCS.

Materials and Methods

Bayer CropScience 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 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.

Bayer CropScience delivered 2 mg of nonodified rice leaf tissue genomic DNA 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 absence of LLRice62. This testing was for absence confirmation as well as homogeneity purposes.

The leaf used to manufacture the non-modified materials was shown to contain the absence of LLRice62 using PCR protocols at Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory). The non-modified rice 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 DNA.

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 0306-D5: 24, 112, 149, 166, and 170 were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for LLRice62 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 tissue genomic DNA material will be reevaluated annually. If the samples still test negative for the presence of LLRice62, the certificates will be extended.

Results and Discussion

Sample Homogeneity

The PCR data for the non-modified 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 non-modified bulk material 0306-D5 provided by Bayer CropScience

Sample	LLRice62 Presence
Homogeneity Sample 1	Negative
Homogeneity Sample 2	Negative
Homogeneity Sample 3	Negative
Homogeneity Sample 4	Negative
Homogeneity Sample 5	Negative

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 absence of LLRice62 after the packaging of AOCS 0306-D5. These results are consistent with the homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0306-D5 Non-Modified Rice 0306-D5 material as tested by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) with Non-Modified event-specific, qualitative PCR analysis.	
Sample	**LLRice62 Presence**
AOCS 0306-D5 24	Negative
AOCS 0306-D5 112	Negative
AOCS 0306-D5 149	Negative
AOCS 0306-D5 166	Negative
AOCS 0306-D5 170	Negative

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.gmotesting.com

ISO Guide 30:2015 (E/F), Terms and definitions used in connection with reference materials

ISO Guide 31:2015 (E), Reference Materials- Contents of certificates and labels

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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