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Certified Reference Materials

AOCS 0306-I9

Report of the certification process for

LLRice62

Rice Certified Reference Materials

Ninth Batch

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ISO 17034:2016
A2LA Certificate 3438.01

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Table of Contents

Abstract.....	4
Acknowledgements.....	5
Glossary.....	6
Introduction	8
Materials and Methods.....	8
Stability	9
Results and Discussion.....	10
Sample Homogeneity	10
Prepared Sample Verification	10
References.....	12

Abstract

This report describes the preparation and certification of the rice CRM AOCS 0306-I9 produced by AOCS Technical Services in 2016. 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 presence of LLRice62 in the rice was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory). AOCS 0306-I9 is available in 0.5 ml skirted screw-cap self-sealing tubes. The rice LLRice62 DNA was provided by Bayer CropScience. The rice LLRice62 leaf DNA extract was extracted from clean leaves provided by Bayer CropScience. The leaf DNA extract 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 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
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: LLRice62	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium

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 0306-19 was manufactured from rice according to ISO 17034:2016 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 1 mg of LLRice62 rice 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, LLRice62. This testing was for presence confirmation as well as homogeneity purposes.

The leaf used to manufacture the LLRice62 materials was shown to contain the LLRice62 event as well as the absence of LLRice06 event and cp4EPSPS sequences (LOD<0.05%) using PCR protocols at Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory). The LLRice62 rice 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 0306-I9: 105, 111, 125, 153, 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 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 LLRice62 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 LLRice62 bulk material 0306-I9 provided by Bayer CropScience	
Sample	LLRice62 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 LLRice62 gene after the packaging of AOCs 0306-I9. These results are consistent with the homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0306-I9 LLRice62 rice 0306-I9 material as tested by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) with LLRice62 event-specific, qualitative PCR analysis.	
Sample	LLRice62 Presence
AOCS 0306-I9 105	Positive
AOCS 0306-I9 111	Positive
AOCS 0306-I9 125	Positive
AOCS 0306-I9 153	Positive
AOCS 0306-I9 170	Positive

The AOCS 0306-I9 CRM was prepared solely from an identity preserved rice produced by transgenic technology. Sample heterogeneity was not considered because there was no blending of conventional and transgenic technology derived rice into defined mixtures.

References

Center for Environmental Risk Assessment GM Database

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

Eurofins-GeneScan; 2219 Lakeshore Drive, Suite 400, Metairie, LA 70122; Telephone: +1 504 297 4330 Toll Free: +1 866 535 2730 Fax: +1 504 297 4335
<http://www.gmotesting.com>

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 174034:2016 (E) General Requirements for the Competence of Reference Material Producers

ISO Guide 35:2017 (E) Reference Materials – Guidance for Characterization and Assessment of Homogeneity and Stability

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