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Certified Reference Materials

AOCS 0306-E4

Report of the certification process for

LLCotton25

Cotton Certified Reference Materials

Fourth Batch

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ISO 17034:2016
A2LA Certificate 3438.01

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Table of Contents

Abstract

This report describes the preparation and certification of the cotton CRM AOCS 0306-E4 produced by AOCS Technical Services in 2017. The CRMs have been prepared according to ISO 17034:2016 and are intended to serve as control material for third party testing of cotton for transformation events. The presence of LLCotton25 in the cotton was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory). AOCS 0306-E4 is available in 0.5 ml skirted screw-cap self-sealing tubes. The cotton LLCotton25 DNA was extracted from clean leaves by BASF Agricultural Solutions Seed US LLC. 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: LLCotton25	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-E4 was manufactured from cotton according to ISO 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 were 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.

BASF Agricultural Solutions Seed US LLC delivered 2.0 mg of LLCotton25 cotton 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 presence of the intended event, LLCotton25. This testing was for presence confirmation as well as homogeneity purposes.

The leaf used to manufacture the LLCotton25 materials was shown to contain the LLCotton25 event as well as the absence of LL117, LLCSIRO, 2mEPSPS, NPTII, and Cry1Ab by PCR protocols at BASF Agricultural Solutions Seed US LLC. The LLCotton25 cotton 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 0306-E4: 64, 75, 99, 117, and 175 were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for LLCotton25 presence in the samples.

Stability

Stability of these CRMs has been listed as 1 year from the certification 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 LLCotton25 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 LLCotton25 bulk material 0306-E4 provided by BASF Agricultural Solutions Seed US LLC

Sample	LLCotton25 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 LLCotton25 gene after the packaging of AOCS 0306-E4. These results are consistent with the homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0306-E4 LLCotton25 cotton 0306-E4 material as tested by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) with LLCotton25 event-specific, qualitative PCR analysis.

Sample	LLCotton25 Presence
AOCS 0306-E4 64	Positive
AOCS 0306-E4 75	Positive
AOCS 0306-E4 99	Positive
AOCS 0306-E4 117	Positive
AOCS 0306-E4 175	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; Telephone: +1 504 297 4330 Toll Free: +1 866 535 2730 Fax: +1 504 297 4335 http://www.gmotesting.com

ISO 17034:2016 (E) General requirements for the competence of reference material producers

ISO 17025:2005 and ISO 17025:2017, General Requirements for the Competence of Testing and Calibration Laboratories

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R1829&from=en)

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