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Certified Reference Materials

AOCS 1108-A8

Report of the certification process for

GHB614

Cotton

Eighth 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 cotton CRM AOCS 1108-A8 produced by AOCS Technical Services in 2022. 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 and for no other purpose. The purity of the GHB614 cotton was verified using event-specific, qualitative PCR analysis by FoodChain ID, Chantilly, VA (an ISO 17025-accredited laboratory). AOCS 1108-A8 is available in 0.5 ml skirted screwcap self-sealing tubes. The GHB614 cotton DNA was extracted from clean leaves that were individually tested and confirmed positive for GHB614 cotton by BASF Agricultural Solutions US LLC. The leaf DNA extract sample shall be stored in the self-sealing tube at +4 ˚C in the dark.

Acknowledgements

The authors would like to express sincere appreciation and gratitude to several individuals and their companies for support and guidance throughout this project. Thanks go to Benoit Maes and Ray Shillito, BASF Agricultural Solutions US LLC, for offering AOCS the opportunity to manufacture and distribute these products; to Heather Waxdahl, SGS Midwest Seed Services for packaging the samples; and to Dan Smith, Bernd Schoel and Joy Bolster, FoodChain ID, for event-specific, qualitative PCR analysis including the provision of information on running the analyses and interpreting the results.

Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Crop variety with no history of modern biotechnology and is produced through 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
Detection Limit	Lowest level at which target DNA can be detected in a sample and be reliably tested by PCR methods. It is typically expressed as a percentage: the ratio of the number of modern biotechnology derived genomes to the number of crop genomes times 100 percent
EC	European Commission
Genome	The complete set of genes and DNA associated with an organism
GMO	Organism that has had genetic sequences modified using molecular-level techniques
ISO	International Organization for Standardization
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 target DNA sequence in a sample can be reliably quantified. It is typically expressed as the ration of the number of transgenic genomes to the number of crop genomes times 100 percent
Quantitative PCR	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules
Trait: GHB614	Tolerance to the herbicide glyphosate

Introduction

Plant biotechnology 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 (CRM) created from leaf, seed, or grain containing the new trait as well as a CRM created from the conventionally bred matrix. The European Commission (EC) has mandated that from 18 April 2004, a method for detecting a new event derived from modern biotechnology and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new crop derived from modern biotechnology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level ranging from 0.90 to 5% of authorized biotech events before accepting a shipment.

To meet the above regulatory requirements for measurement standards, AOCS 1108-A8 was manufactured according to ISO 17034:2016 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

Materials and Methods

BASF Agricultural Solutions US LLC prepared the bulk material by taking plants that had been individually tested using several quality standards and were grown from cotton seeds harvested from plants that had passed the same criteria. Only the leaf material confirmed as positive for the GHB614 event and meeting all quality requirements were harvested, immediately frozen, and stored at -70 °C. Plants not meeting the quality standards were removed and destroyed.

Genomic DNA was extracted from GHB614-positive leaf tissue obtained from one or more plants using a CTAB-based protocol (Doyle and Doyle, 1987). DNA integrity and concentration were evaluated by electrophoresis on a 1.0% agarose gel stained with ethidium bromide and compared against lambda molecular weight standards by digital imaging quantification. Concentration measurements were performed in triplicate across three independent gels. No evidence of physical degradation was observed, and the DNA migrated at positions greater than 40 kb. The leaf tissue used to produce the

AOCS 1108-A8 material was confirmed to contain the GHB614 event and to be absent of nptII, cp4epsps, P35S, cry2Ae, and GHB623 (LOD < 0.05%) using PCR protocols at BASF Agricultural Solutions US LLC.

BASF Agricultural Solutions US LLC delivered 3.0 mg of GHB614 cotton genomic DNA extract to AOCS. The A2704-12 soybean leaf DNA was packaged by SGS North America Inc., Brookings, SD in sterile, 0.5 ml skirted screw-cap self-sealing tubes in aliquots of 10 µg.

For the verification of purity and homogeneity purposes, 5 working samples were of DNA (10 µg) each were prepared from the composite and submitted to FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis for GHB614 cotton. For the verification of trait presence, homogeneity, and to rule out degradation during packaging, 10 random samples were collected and submitted for event-specific, qualitative PCR analysis for GHB614 cotton: 37, 92, 119, 128, 152, 179, 185, 268, 286, and 307. Vial numbers were obtained by the Random Number Generator function of Microsoft Excel and the testing was conducted by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory). The results confirm the presence of the GHB614 after the packaging (*Table 1*).

Stability

Stability of these CRMs has been listed as 1 year from the certification date. The materials were sealed and stored under refrigerated conditions, 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 and Prepared Sample Verification

Once the bulk material was processed and packaged, ten (10) samples were identified by the Microsoft Excel Random Number Generator and sent to FoodChain ID, Chantilly, Va (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis. These results are presented in *Table 1*. The results show that no contamination occurred during the packaging of AOCS 1108-A8.

***Table 1**. Results on the verification and homogeneity of AOCS 1108-A8, GHB614 cotton, event-specific qualitative PCR analysis conducted by FoodChain ID Testing*

Sample	GHB614 Presence
AOCS 1108-A8 #37	Positive
AOCS 1108-A8 #92	Positive
AOCS 1108-A8 #119	Positive
AOCS 1108-A8 #128	Positive
AOCS 1108-A8 #152	Positive
AOCS 1108-A8 #179	Positive
AOCS 1108-A8 #185	Positive
AOCS 1108-A8 #268	Positive
AOCS 1108-A8 #286	Positive
AOCS 1108-A8 #307	Positive

References

Center for Environmental Risk Assessment GM Database http://www.cera-gmc.org/?action=gm_crop_database

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical bulletin.

FoodChain ID Testing, LLC, 4150 Lafayette Center Drive, Suite 600, Chantilly, VA, 20151 Telephone: 1 703-222-8700 www.foodchainid.com

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

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

ISO 17034:2016, General Requirements for the Competence of Reference Material Producers

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed; eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A32003R1829&from=en

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