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Certified Reference Material AOCS 0911-A

Report for the Certification Process for Non-Modified Soybean Certified Reference Material

First Lot

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Revised: 7 March 2012

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Abstract

This report describes the preparation and certification of the soybean certified reference material (CRM) AOCS 0911-A produced by AOCS Technical Services in 2011. The CRM has been prepared according to ISO Guides 30 through 35 and is intended to serve as control material for third-party testing of soybeans for biotechnology-derived events. The purity of the Non-Modified soybeans was verified using BPS-CV127-9 event-specific, qualitative PCR analysis by Eurofins GeneScan, Metairie, LA (an ISO 17025 accredited laboratory). AOCS 0911-A is available in 27-mL glass headspace vials. The soybeans ("Non-Modified") were provided by BASF Plant Science L.P. and were clean grain. AOCS devitalized the bulk soybeans at BASF and then transferred the coarsely milled material to AOCS. The soybeans were further processed by grinding the bulk sources according to standard soybean processing protocols by Texas A&M University and were then packaged under a nitrogen gas environment at Illinois Crop Improvement Association. The powder sample shall be stored dry in a sealed container 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 Angela McKean, BASF Plant Science L.P., for offering AOCS the opportunity to manufacture and distribute these products; to Richard Clough, Texas A&M University, for providing expertise for milling/processing the soybeans into a uniform blend; to John McKinney, Sandra Harrison, and Charlie Drennan at Illinois Crop Improvement Association for packaging the samples; and to Frank Spiegelhalter, Greg Ditta, E. Pearce Smith, and Daniel Thompson, Eurofins GeneScan 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
BPS-CV127-9	Imidazolinone herbicide-tolerant soybean plants derived from a single transformation event and produced by the introduction of an imidazolinone-tolerance-conferring acetohydroxyacid synthase large subunit gene from *Arabidopsis thaliana* (L.) Heynh. into the soybean plant genome
DNA	Deoxyribonucleic acid is the linear, double-helical macromolecule that makes up the genetic material of most organisms
Detection Limit	Lowest level at which target DNA can exist in a sample and be reliably detected by PCR methods; often abbreviated as “LOD”
EC	European Commission
ISO	International Organization for Standardization
Non-Modified Crop	Crop variety with no history of modern biotechnology modification and which 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

Product of Modern Biotechnology	Organism that has had genetic sequences modified using molecular-level techniques
PCR	Polymerase Chain Reaction is a technique used to determine whether a sample 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 generates copies of the target sequence.
Qualitative PCR	PCR methods that determine the presence or absence of a specific target DNA sequence at a particular limit of detection

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 created from leaf, seed, or grain containing the new trait as well as a CRM created from the corresponding non-modified crop. The European Commission (EC) has mandated that from 18 April 2004, a method for detecting a new event derived from modern biotechnology and Reference Material must be available before the EC will consider authorizing a new food or feed derived from modern biotechnology. Several nations outside of Europe also require grain and ingredients to be labeled if authorized biotechnology-derived events are present above a threshold level ranging from 0.90 to 5%.

To meet the above analytical requirements for biotechnology-derived event determination, AOCS 0911-A was manufactured from soybeans according to ISO Guides 30 through 35 and in accordance with EC No 1829/2003, EC No 641/2004, and EC No 619/2011. The CRM is available from AOCS.

Materials and Methods

BASF Plant Science L.P. delivered 14 kg of Non-Modified soybeans, devitalized by AOCS on the BASF premises, to AOCS. The materials were clean grain. Before the materials were shipped to Texas A&M University for processing to a uniform particle size, primary samples were taken from randomly selected areas and depths to form a 3 kg composite sample in accordance with the

International Seed Testing Association's (ISTA) Seed Science and Technology Rules for batches up to 100 kg. Ten (10) working samples of 100 g each were prepared from the composite sample and sent to Eurofins GeneScan, Metairie, LA (an ISO 17025 accredited laboratory) for BPS-CV127-9 event-specific, qualitative PCR analysis. The analyses performed by Eurofins GeneScan were used to assess the purity and homogeneity of the seed lot.

These Non-Modified soybeans were processed according to industry-standard soybean processing procedures, packaged in 27-mL glass headspace vials, and sealed under a nitrogen gas environment. AOCS used the Random Number Generator function of Microsoft Excel 2003 to select samples for verification of purity, homogeneity, and to rule out contamination during packaging. Sample numbers AOCS 0911-A: 34, 58, 59, 161, 175, 233, 331, 373, 401, and 466 were sent to Eurofins GeneScan, Metairie, LA (an ISO 17025 accredited laboratory) for BPS-CV127-9 event-specific, qualitative PCR analysis to screen for BPS-CV127-9 presence in the Non-Modified samples. Each selected bottle was sampled twice and each sample subjected to BPS-CV127-9 event-specific qualitative PCR analysis to check for intra-bottle homogeneity.

Stability

This CRM has been certified for 1 year from the introduction date. This material was processed and is stored frozen, under nitrogen gas, in glass headspace vials. This material is expected to be stable for longer than the estimated expiration date. The stability of the powder material will be reevaluated at the time of expiration. If the samples are still representative of the certified value, the certificate will be extended.

Results and Discussion

Sample Homogeneity

The homogeneity test results for the Non-Modified soybean bulk material are presented in Table 1.

Table 1. Results of the homogeneity testing performed by Eurofins GeneScan on the Non-Modified bulk material provided by BASF Plant Science L.P.

Sample	BPS-CV127-9 Presence (LOD ≤ 0.04 %)
Homogeneity Sample 1	Negative
Homogeneity Sample 2	Negative
Homogeneity Sample 3	Negative
Homogeneity Sample 4	Negative
Homogeneity Sample 5	Negative
Homogeneity Sample 6	Negative
Homogeneity Sample 7	Negative
Homogeneity Sample 8	Negative
Homogeneity Sample 9	Negative
Homogeneity Sample 10	Negative

Prepared Sample Verification

Once the bulk material was processed and packaged, ten (10) samples were identified by the Microsoft Excel 2003 Random Number Generator and sent to Eurofins GeneScan, Metairie, LA (an ISO 17025 accredited laboratory) for BPS-CV127-9 event-specific, qualitative PCR analysis. These results are presented

in Table 2. These data show that no contamination occurred during the packaging of AOCS 0911-A. These prepared sample results, including the intra-bottle homogeneity, are in agreement with the bulk material homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0911-A [Non-Modified soybean] material as tested by Eurofins GeneScan with BPS-CV127-9 event-specific, qualitative PCR analysis.

Sample	BPS-CV127-9 Presence (LOD ≤ 0.04 %)
AOCS 0911-A 34	Negative
AOCS 0911-A 58	Negative
AOCS 0911-A 59	Negative
AOCS 0911-A 161	Negative
AOCS 0911-A 175	Negative
AOCS 0911-A 233	Negative
AOCS 0911-A 331	Negative
AOCS 0911-A 373	Negative
AOCS 0911-A 401	Negative
AOCS 0911-A 466	Negative

The AOCS 0911-A CRM was prepared from Non-Modified soybeans. Sample heterogeneity was not considered because there was no blending of Non-Modified and modern biotechnology-derived soybeans into defined mixtures.

References

Center for Environmental Risk Assessment GM Crop Database. Available at: http://www.cera-gmc.org/?action=gm_crop_database, accessed January 31, 2012

Eurofins GeneScan; 2315 N Causeway Blvd, Suite 200, Metairie, LA 70001; Telephone: +1 504 297 4330; Toll Free: +1 866 535 2730; Fax: +1 504 297 4335. http://www.eurofinsus.com/locations/genescan.html, accessed February 23, 2012

Illinois Crop Improvement Association; 3105 Research Road, Champaign, IL 61822; Telephone: +1 217 359 4053; Fax: +1 217 359 4075.
http://www.ilcrop.com, accessed January 31, 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 32:1997 (E) Calibration in analytical chemistry and use of certified reference materials

ISO Guide 33:2000 (E) Uses of certified reference materials

ISO Guide 34:2009 (E) General requirements for the competence of reference material producers

ISO Guide 35:2006 (E) Reference materials- General and statistical principles for certification

International Seed Testing Association; International rules for seed testing: Seed science and technology rules, Volume 21, Supplement, Rules, 1993

Texas A&M University; Food Protein Research and Development Center; 373 Olsen Blvd; College Station, TX 77843; Telephone: +1 979 862 2262; Fax: +1 979 845 2744; http://foodprotein.tamu.edu/, accessed January 31, 2012

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