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Certified Reference Materials

AOCS 0809-B

Report for the certification process for
MON87769

Soybean Certified Reference Materials

First Lot

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Abstract

This report describes the preparation and certification of the soybean CRM AOCS 0809-B produced by AOCS Technical Services in 2009. The CRMs have been prepared according to ISO Guides 30-35 and are intended to serve as control material for third party testing of soybeans for transformation events. The purity of the MON87769 soybean was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory). AOCS 0809-B is available in 27 -mL glass headspace vials. The soybeans (MON87769; Orion ID 11225858) were clean seed quality provided by Monsanto Company, St. Louis, MO. The soybeans were prepared 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 David Grothaus and Manali Shah, Monsanto Company, 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
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 is the linear, double-helix 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 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 full set of genes and associated DNA characteristic of an organism
IRMM	Institute for Reference Materials and Measurement
ISO	International Organisation for Standardisation
Product of Modern Biotechnology	Organism that has had genetic sequences modified using molecular-level techniques

MON87769	MON87769 has an enhanced SDA fatty acid profile. SDA is an omega-3 fatty acid which is a normal metabolic precursor to the long chain, poly-unsaturated omega-3 fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans and animals.
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 reproducible. 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

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 conventionally bred matrix. The European Commission 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 analytical requirements for GM determination, AOCS 0809-B was manufactured from soybeans according to ISO Guides 30-35 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

Materials and Methods

The Monsanto Company, St. Louis, MO, delivered 25 kg of coarsely milled MON87769 soybeans (MON87769; Orion ID 11225858) to AOCS. The materials were clean seed quality. Before the materials were shipped to Texas A&M University for uniform processing, primary samples were taken from randomly

selected areas and depths to form a 5 kg composite sample in accordance with the International Seed Testing Association's (ISTA) Seed Science and Technology Rules for batches up to 500 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 event-specific, qualitative PCR analysis. The analyses performed by Eurofins-GeneScan were used to assess the purity and homogeneity of the lot.

The MON87769 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 0809-B: 244, 270, 290, 334, 368, 398, 399, 471, 620, and 682 were sent to Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for MON87769 presence in the samples.

Stability

Stability of these CRMs has been listed as 1 year from the introduction date. The materials were processed and are stored frozen, under nitrogen gas, in glass headspace vials. These materials are expected to be stable for longer than the estimated expiration date. The stability of the powder material will be reevaluated at time of expiration. If the samples are still representative of the certified value, the certificates will be extended.

Results and Discussion

Sample Homogeneity

The purity data for the MON87769 homogeneity samples is presented in Table 1.

Table 1. Results of the homogeneity testing performed by Eurofins-GeneScan on the MON87769 bulk material (MON87769; Orion ID 11225858) provided by Monsanto Company, St. Louis, MO.	
Sample	MON87769 Presence
Homogeneity Sample 1	Positive
Homogeneity Sample 2	Positive
Homogeneity Sample 3	Positive
Homogeneity Sample 4	Positive
Homogeneity Sample 5	Positive
Homogeneity Sample 6	Positive
Homogeneity Sample 7	Positive
Homogeneity Sample 8	Positive
Homogeneity Sample 9	Positive
Homogeneity Sample 10	Positive

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 event-specific, qualitative PCR analysis. These results are presented in Table 2. These data show no contamination occurred during the packaging of AOCS 0809-B. These results are in agreement with the homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0809-B [MON87769 soybean (MON87769; Orion ID 11225858)] material as tested by Eurofins-GeneScan with MON87769 event-specific, qualitative PCR analysis.

Sample	MON87769 Presence
AOCS 0809-B 244	Positive
AOCS 0809-B 270	Positive
AOCS 0809-B 290	Positive
AOCS 0809-B 334	Positive
AOCS 0809-B 368	Positive
AOCS 0809-B 398	Positive
AOCS 0809-B 399	Positive
AOCS 0809-B 471	Positive
AOCS 0809-B 620	Positive
AOCS 0809-B 682	Positive

The AOCS 0809-B CRMs were prepared solely as either identity preserved conventional soybeans or identity preserved soybeans derived from modern biotechnology. Sample heterogeneity was not considered because there was no blending of conventional and modern biotechnology derived soybeans into defined mixtures.

References

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

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.gmotesting.com>

Illinois Crop Improvement Association, 3105 Research Road, Champaign, IL 61826; Telephone: +1 217 359 4053 Fax: +1 217 359 4075; <http://www.ilcrop.com/index.htm>

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