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Certified Reference Material

AOCS 0911-C

Report for the Certification Process for
BPS-CV127-9 Soybean
Certified Reference Material

First Lot

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Abstract

This report describes the preparation and certification of the soybean certified reference material (CRM) AOCS 0911-C 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 BPS-CV127-9 soybeans was verified using BPS-CV127-9 event-specific, qualitative PCR analysis by Eurofins GeneScan, Metairie, LA (an ISO 17025 accredited laboratory). AOCS 0911-C is available in 27-mL glass headspace vials. The soybeans (BPS-CV127-9) 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.

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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 <i>Arabidopsis thaliana</i> (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

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-C 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 16 kg of BPS-CV127-9 soybeans, coarsely-milled by AOCS on the BASF premises, to AOCS. The materials were clean grain. Prior to devitalization of the bulk material, AOCS selected, crushed, and sent 80 individual seeds for single-seed BPS-CV127-9 event-specific, qualitative PCR analysis at Eurofins GeneScan, Metairie, LA (an ISO 17025 accredited laboratory). 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.

The BPS-CV127-9 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 homogeneity and consistency with the BPS-CV127-9 bulk material homogeneity test results. Sample numbers AOCS 0911-C: 26, 175, 196, 203, 251, 299, 392, 432, 443, and 445 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 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. The 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 Purity and Homogeneity

To determine the trait purity of the BPS-CV127-9 soybean seed lot used to generate the CRM, the genetic identity of eighty (80) randomly-selected seeds was checked. Each individual seed was crushed and sent to Eurofins GeneScan where they were tested for the presence of BPS-CV127-9 using an event-specific qualitative PCR assay. A statistical analysis of the distribution of the probability of finding a negative seed in the BPS-CV127-9 soybean material was used to determine the trait purity with a 95% confidence level. Each of the 80 individually-tested seeds was "Positive" for BPS-CV127-9.

The homogeneity test results for the ten 100-gram samples of BPS-CV127-9 soybean bulk material are presented in Table 1.

Table 1. Results of the homogeneity testing performed by Eurofins GeneScan on the BPS-CV127-9 bulk material provided by BASF Plant Science L.P.

Sample	BPS-CV127-9 Presence (LOD ≤ 0.04 %)
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 BPS-CV127-9 event-specific, qualitative PCR analysis. These results are presented in Table 2. These data confirmed the homogeneity of the AOCS 0911-C prepared samples, including the intra-bottle homogeneity, and were in agreement with the bulk material homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0911-C [BPS-CV127-9 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-C 26	Positive
AOCS 0911-C 175	Positive
AOCS 0911-C 196	Positive
AOCS 0911-C 203	Positive
AOCS 0911-C 251	Positive
AOCS 0911-C 299	Positive
AOCS 0911-C 392	Positive
AOCS 0911-C 432	Positive
AOCS 0911-C 443	Positive
AOCS 0911-C 445	Positive

The AOCS 0911-C CRM was prepared from BPS-CV127-9 soybeans. Uncertainty of the certified value for AOCS 0911-C due to heterogeneity of the prepared samples 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

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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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