AOCS
STREET ADDRESS
3356 Big Pine Trail, Ste. C&D,
Champaign, IL 61822 USA
MAILING ADDRESS
PO Box 7230,
Champaign, IL 61826 USA
+1 217-359-2344
general@aocs.org
aocs.org

Certified Reference Materials

AOCS 0911-C2

Report for the certification process for

CV127

Soybean

First Batch

OECD Unique ID BPS-CV127-9

Zoe Serelis
Technical Services Manager

Tiffanie West
Technical Services Director

A2LA ACCREDITED
ISO 17034:2016
A2LA Certificate 3438.01

Legal Notice

Neither AOCS nor any person acting on behalf of AOCS is responsible for the use which might be made of the following information.

AOCS Mission Statement

AOCS advances the science and technology of oils, fats, proteins, surfactants, and related materials, enriching the lives of people everywhere.

More information regarding AOCS is available at www.aocs.org

Table of Contents

Abstract

This report describes the preparation and certification of the soybean certified reference material (CRM) AOCS 0911-C2 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 soybeans was verified using CV127 event-specific, qualitative PCR analysis by Eurofins GeneScan, Metairie, LA (an ISO 17025-accredited laboratory). AOCS 0911-C2 is available in 27-mL glass headspace vials. The soybeans (CV127) 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 ambient or lower temperature and 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
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
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: CV127	Resistance to the herbicide Imidazolinone

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 above a threshold level before accepting a shipment.

To meet the above analytical requirements for biotechnology-derived event determination, AOCS 0911-C2 was manufactured from soybeans according to ISO 17034:2016 and in accordance with EC No 1829/2003, EC No 641/2004 and EC No 619/2011. This CRM is available from AOCS.

Material Processing

BASF Plant Science L.P. delivered 16 kg of CV127 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 CV127 event-specific, qualitative PCR analysis. The analyses performed by Eurofins GeneScan were used to assess the purity and homogeneity of the seed lot.

These CV127 soybeans were processed according to industry-standard soybean processing procedures, packaged in 27-mL glass headspace vials, and sealed under a nitrogen gas environment.

Trait Verification

The presence of the CV127 trait was assessed on ten (10) random vials of 0911-C2. AOCS used the Microsoft Excel Random Number Generator function of Microsoft Excel to select samples for verification of the trait presence. Sample numbers that were randomly selected were sent to Eurofins GeneScan, Metairie, LA (an ISO 17025-accredited laboratory) for CV127 event-specific, qualitative PCR analysis. These results are presented in Table 1.

Table 1. Results for the verification of AOCS 0911-C2 [CV127 soybean] material as tested by Eurofins GeneScan with CV127 event-specific, qualitative PCR analysis.

Sample	CV127 Presence
AOCS 0911-C2 26	Positive
AOCS 0911-C2 175	Positive
AOCS 0911-C2 196	Positive
AOCS 0911-C2 203	Positive
AOCS 0911-C2 251	Positive
AOCS 0911-C2 299	Positive
AOCS 0911-C2 392	Positive
AOCS 0911-C2 432	Positive
AOCS 0911-C2 443	Positive
AOCS 0911-C2 445	Positive

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0911-C2 was assessed by AOCS. A total of 80 soybean seeds were subjected to testing for the presence of CV127 by qualitative event-specific PCR. 80 of the 80 seeds tested positive for the presence of CV127. Purity estimation was calculated using SeedCalc8 (Remund et al., 2008). The % purity in the samples was 100%, when 80 seeds were tested. Based on the upper bound of true % purity and with 95% confidence, the true certified value is ≥ 963 g/kg.

The measurement uncertainty (U_{CRM}) is the expanded uncertainty with a coverage factor of 1.65 and a confidence level of 95%. It is obtained by combining the uncertainties from the purity assessment ($u_{char,rel}$), the homogeneity assessment ($u_{bb,rel}$), the transport stability assessment ($u_{sts,rel}$) and the long-term stability assessment ($u_{lts,rel}$):

$$u_{CRM,rel} = \sqrt{{u_{char,rel}}^2 + {u_{bb,rel}}^2 + {u_{sts,rel}}^2 + {u_{lts,rel}}^2}$$

$$U_{CRM} = 1.65 \times u_{CRM,rel} \times 1000\ g/kg$$

The expanded measurement uncertainty for this CRM is -37 g/kg.

Homogeneity

The homogeneity of AOCS 0911-C2 is related to the purity of the seeds. 80 out of 80 seeds tested positive for the CV127 soybean trait by event-specific PCR. Based on the sample purity of 100%, as determined using SeedCalc8, the batch was expected to be homogenous.

After the CV127 soybean seed was ground and bottled as described above, ten samples of AOCS 0911-C2 were randomly selected using the Microsoft Excel Random Number Generator function and were sent to Eurofins GeneScan, Metairie, LA (an ISO 17025-accredited laboratory) for CV127 event-specific, qualitative PCR analysis. The test results for the CV127 soybean bulk material are presented in Table 2 and further confirm the homogeneity of AOCS 0911-C2.

Table 2. Results of the homogeneity testing performed by Eurofins GeneScan on the 0911-C2 non-modified soybean material.

Sample	CV127 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

Stability

Time, temperature and light are regarded as the most relevant influences on the stability of CRM (Linsinger, et al., 2001). The influence of light is mitigated by shipping and storing the vials in boxes, thus minimizing the possibility of degradation due to light. The influence of temperature is mitigated by storing the vials in a temperature-controlled room, and shipping vials at ambient temperature.

The effect of temperature and time are investigated.

A transport (short-term) stability study is conducted to assess the stability of maize CRM during transport. The temperature and time conditions in the study cover the typical conditions and the not so rare situations. The outcome of the study is considered transferable to other CRMs of similar property. Samples were subject to 3 different temperatures (4 ℃ (fridge), 25 ℃ (ambient), 60 ℃ (oven)) for 4 different durations (0, 1, 2, and 4 weeks). The study concluded that samples are stable at 4 ℃ (fridge) and 25 ℃

(ambient) for 4 weeks. The estimated uncertainty contribution from transport (short-term) stability is 1.0%.

A long-term stability study is conducted to assess the stability of maize CRM during storage. Samples are stored at 25 ℃ (ambient) and the stability of the sample is monitored as long as the samples is available. The storage temperate studied is 25 ℃ and the length of time to be studied is 10 years. The outcome of the study is considered transferable to other CRMs of similar property. In the initial 1-year stability study, samples were subject the storage condition for 4 different durations (0, 1, 3, 6 and 12 months). The study concluded that samples are stable at 25 ℃ (ambient) for 12 months. The estimated uncertainty contribution from long-term stability is 0.42%.

Stability of these CRMs has been listed as 1 year from the certification date. This material was processed and are stored at ambient or lower temperature, under nitrogen, 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.

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 61826; Telephone: +1 217 359 4053 Fax: +1 217 359 4075; https://www.ilcrop.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

ISO Guide 30:1992 (E/F), Reference Materials – Selected Terms and Definitions

ISO Guide 31:2000 (E), Reference Materials- Contents of Certificates, Labels and Accompanying Documentation

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

Linsinger, T., Pauwels, J., van der Veen, A. *et al.* Homogeneity and stability of reference materials. *Accred Qual Assur* 6**,** 20–25 (2001). doi.org/10.1007/s007690000261

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:32003R1829&from=en

Remund K, Simpson R, Laffont J-L, Wright D, and Gregoire S. Seedcalc8. 2008 https://www.seedtest.org/en/services-header/tools/statistics-committee/statistical-tools-seed-testing.html

Texas A&M University; Food Protein Research and Development Center; 373 Olsen Blvd; College Station, TX 77845, USA; Telephone: +1 979 862 2262 Fax: +1 979 845 2744; perdc.tamu.edu/