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## **Certified Reference Materials**

### **AOCs 0707-A7**

Report of the certification process for

Non-Modified

Soybean Certified Reference Materials

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ISO 17034:2016  
A2LA Certificate 3438.01

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# Table of Contents

<b>Abstract.....</b>	<b>4</b>
<b>Acknowledgements.....</b>	<b>5</b>
<b>Glossary.....</b>	<b>6</b>
<b>Introduction .....</b>	<b>8</b>
<b>Materials and Methods.....</b>	<b>8</b>
<b>Stability .....</b>	<b>9</b>
<b>Results and Discussion.....</b>	<b>10</b>
<b>Sample Homogeneity .....</b>	<b>10</b>
<b>Prepared Sample Verification .....</b>	<b>11</b>
<b>References.....</b>	<b>12</b>

## **Abstract**

This report describes the preparation and certification of the soybean CRM AOCS 0707-A7 produced by AOCS Technical Services in 2016. The CRMs have been prepared according to ISO Guide 17034:2016 and are intended to serve as control material for third party testing of soybean for transformation events. The absence of A2704-12, A5547-127, and FG72 in the soybean were verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory). AOCS 0707-A7 is available in 0.5 ml skirted screw-cap self-sealing tubes. The non-modified soybean DNA was provided by BASF Agricultural Solutions Seed US LLC. The non-modified soybean leaf tissue genomic DNA was extracted from clean leaves. The leaf tissue genomic DNA samples 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 Ray Shillito and Benoit Maes, BASF Agricultural Solutions Seed US LLC, for offering AOCS the opportunity to manufacture and distribute these products; to Heather Waxdahl, SGS-Midwest 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	A related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food
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 be detected in a sample.
EC	European Commission
Genome	The full set of genes and associated DNA characteristic of an organism
GMO	Genetically modified/engineered organism: an organism in which the genetic material has been changed through modern biotechnology in a way that does not occur naturally by multiplication and/or natural recombination.
ISO	International Organisation for Standardisation
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 bind to a particular target DNA sequence and a special DNA-copying enzyme

(DNA polymerase) that exponentially amplifies 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 quantitated

Quantitative PCR PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules

## **Introduction**

Plant genetic modification 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 transgenic technology and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new crop derived from transgenic technology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above regulatory requirements for GMO determination, AOCS 0707-A7 was manufactured from conventional soybean according to ISO Guide 10734:2016 and in accordance with EC No 1829/2003. The CRM is available from AOCS.

## **Materials and Methods**

BASF Agricultural Solutions Seed US LLC prepared the bulk material by taking source leaf material from plants which had been tested individually using several quality standards and was grown from seeds harvested from plants that had themselves passed the same criteria. Plants not meeting the quality standards were removed and destroyed. Leaf material was harvested from the plants which met the quality standards and frozen immediately and stored at -70 °C. The genomic DNA was extracted from leaves of one or more plants according to CTAB-based (Doyle JJ and Doyle JL, 1987) protocol. The integrity and concentration of the genomic DNA was determined by electrophoresis in a 1.0% agarose gel and ethidium bromide-staining and compared to lambda molecular weight standards by digital imaging quantification. The concentration measurement was done in triplicate, repeated in three different gels. No indications for physical degradation were apparent and the DNA migrated at positions higher than 40 Kb.

BASF Agricultural Solutions Seed US LLC delivered 2.0 mg of non-modified soybean leaf DNA to AOCS. Five (5) working samples of DNA, 10 µg each, were prepared from the composite and sent to Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for the absence of the intended event(s), A2704-12, A5547-127, and FG72. This testing was for absence confirmation as well as homogeneity purposes.

The leaf used to manufacture the non-modified materials was shown to have the absence of A2704-12, A5547-127, and FG72 using PCR protocols at Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory). The non-modified soybean leaf DNA was packaged by SGS-Midwest Seed Services in sterile, 0.5 ml skirted screw-cap self-sealing tubes in aliquots of 10 µg.

AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of gene absence, homogeneity, and to rule out degradation during packaging. Sample numbers AOCS 0707-A7: 131, 64, 165, 105, and 120 were sent to Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for A2704-12, A5547-127, and FG72 absence in the samples.

## **Stability**

Stability of these CRMs has been listed as 1 year from the introduction date. The materials were sealed and stored in the dark at 4 °C, therefore not exposed to air and are expected to be stable for longer than the estimated expiration date. The stability of the leaf tissue genomic DNA material will be reevaluated annually. If the samples still test negative for the presence of the trait(s), the certificate validity dates will be extended.

## Results and Discussion

### Sample Homogeneity

The PCR data for the non-modified soybean homogeneity samples is presented in Table 1.

<b>Table 1. Results of the homogeneity testing performed by Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) on the non-modified material 0707-A7 provided by BASF Agricultural Solutions Seed US LLC</b>	
<b>Sample</b>	<b>A2704-12, A5547-127, and FG72 Presence</b>
Homogeneity Sample 1	Negative
Homogeneity Sample 2	Negative
Homogeneity Sample 3	Negative
Homogeneity Sample 4	Negative
Homogeneity Sample 5	Negative

## Prepared Sample Verification

Five (5) samples were identified by the Microsoft Excel 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. This data confirms the absence of the A2704-12, A5547-127, and FG72 gene(s). These results are consistent with the homogeneity data presented in Table 1.

<b>Table 2. Results for the verification of AOCS 0707-A7 non-modified soybean material as tested by Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) with event-specific, qualitative PCR analysis.</b>	
<b>Sample</b>	<b>A2704-12, A5547-127, and FG72 Presence</b>
AOCS 0707-A7 131	Negative
AOCS 0707-A7 64	Negative
AOCS 0707-A7 165	Negative
AOCS 0707-A7 105	Negative
AOCS 0707-A7 120	Negative

## References

Center for Environmental Risk Assessment GM Database

[http://www.cera-gmc.org/?action=gm\\_crop\\_database](http://www.cera-gmc.org/?action=gm_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>

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

ISO Guide 30:2015 (E/F), Reference Materials – Selected Terms and Definitions

ISO Guide 31:2015 (E), Reference Materials- Contents of Certificates, Labels and Accompanying Documentation

ISO Guide 174034:2016 (E) General Requirements for the Competence of Reference Material Producers

ISO Guide 35:2017 (E) Reference Materials – Guidance for Characterization and Assessment of Homogeneity and Stability

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