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## **Certified Reference Material**

### **AOCS 0906-D**

Report of the certification process for

MON 88913

Cotton Certified Reference Materials

First Batch

OECD Unique ID MON-88913-8

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ISO 17034:2016  
A2LA Certificate 3438.01

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## Abstract

This report describes the preparation and certification of the ground cottonseed CRM AOCS 0906-D produced by AOCS Technical Services in 2006. This CRM has been prepared according to ISO Guides 30-35 and is intended to serve as control material for third party testing of cotton seed or grain for transformation events. The purity of the genetically modified cottonseed was verified using DNA and protein-based detection methods. AOCS 0906-D is packaged in 10 g aliquots and is available in 27 -mL glass headspace vials. The Roundup Ready Flex<sup>®</sup> <sup>1</sup>cotton [MON 88913 (Line ST 4664 RF)] was clean seed quality provided by Monsanto Company, St. Louis, MO, USA. The cottonseed was prepared by grinding the bulk sources according to standard cotton processing protocols and was then packaged under a Nitrogen environment. The ground sample shall be stored dry in a sealed container at ambient or cooler conditions in the dark.

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<sup>1</sup> Roundup Ready Flex<sup>®</sup> is the next generation of Monsanto's Roundup Ready<sup>®</sup> cotton technology.

## **Acknowledgements**

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## Glossary

AOCS	American Oil Chemists' Society ( <a href="http://www.aocs.org">www.aocs.org</a> )
Conventional Variety	Crop variety with no history of genetic engineering and are 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
CP4 EPSPS	Glyphosate tolerance derived by inserting a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) encoding gene from <i>Agrobacterium tumefaciens</i> strain CP4
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 trans-genetically 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

Genome	The full set of genes and associated DNA characteristics of an organism
ISO	International Organisation for Standardisation
ISTA	International Seed Testing Association
MON 88913	Also known as Roundup Ready® Flex. This cotton was developed to allow the use of glyphosate, the active ingredient in the herbicide Roundup®, as a weed control option in cotton production. This genetically engineered cotton variety contains a glyphosate-tolerant form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), isolated from the soil bacterium <i>Agrobacterium tumefaciens</i> strain CP4, hereafter termed CP4 EPSPS. Compared to previously approved glyphosate tolerant lines of cotton, which will tolerate glyphosate applications only to the fifth true leaf stage, MON 88913 was developed to tolerate glyphosate applications beyond the fifth true leaf stage. MON 88913 cotton was developed by introducing two CP4 EPSPS coding sequences into the cotton variety 'Coker 312' using <i>Agrobacterium</i> -mediated transformation.
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.

## **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 biotechnology-derived event and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new genetically modified crop. 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 0906-D was manufactured from cotton seed according to ISO Guides 30-35 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

## **Materials and Methods**

AOCS received appropriate seed quality bulk material [20 kg of MON 88913 cotton (Line ST 4664 RF)] from Monsanto Company (St. Louis, MO) and made arrangements for Texas A&M University to process the material. Before the materials were shipped to Texas A&M University, 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 working samples of 100 g each were prepared from the composite sample and sent to Eurofins GeneScan, Metairie, LA (ISO 17025 Accredited laboratory) for qualitative PCR analysis. The analysis performed by Eurofins GeneScan was used to assess the purity

and homogeneity of the seed lot. Five hundred seeds were randomly selected from the MON 88913 primary sample and analyzed with EnviroLogix' QuickStix™ Kit for Roundup Ready® Cotton Seed (CP4 EPSPS) to verify seed-lot purity.

The genetically modified cotton, MON 88913, was packaged in 27 -mL headspace vials and sealed under a Nitrogen 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 0906-D: 107, 232, 239, 251, 335, 400, 471, 524, 548, and 549 were sent to Eurofins GeneScan (New Orleans, LA) for qualitative PCR analysis to screen for MON 88913 presence in the samples.

## **Stability**

Stability of these CRMs has been listed as 1 year from the introduction date. The materials have been ground and are stored frozen under Nitrogen gas in a sealed, glass vial. These materials are expected to be stable for longer than the estimated expiration date. The stability of the ground 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 following tables are the purity data for the homogeneity samples. The MON 88913 Cotton (line ST 4664 RF) PCR data are presented in Table 1. Table 2 includes the data generated from EnviroLogix' QuickStix™ Kit for Roundup Ready® Cotton Seed (CP4 EPSPS). Five hundred seeds were tested to verify seed-lot purity.

Samples were prepared as identity preserved genetically modified cotton. Sample quantitative heterogeneity is not a factor due to the fact that conventional and genetically modified cotton were not mixed into prepared blends.

**Table 1. Results from Eurofins GeneScan for the homogeneity of MON 88913 Cotton (line ST 4664 RF) by qualitative PCR.**

Sample	MON 88913 Presence
Monsanto Cotton D-1	+
Monsanto Cotton D-2	+
Monsanto Cotton D-3	+
Monsanto Cotton D-4	+
Monsanto Cotton D-5	+
Monsanto Cotton D-6	+
Monsanto Cotton D-7	+
Monsanto Cotton D-8	+
Monsanto Cotton D-9	+
Monsanto Cotton D-10	+

**Table 2. Results from administering EnviroLogix' QuickStix™ Kit for Roundup Ready® Cotton Seed (CP4 EPSPS) to 500 MON 88913 Cotton seeds.**

Kernels Tested	Results
Conventional	0
MON 88913	500
Unreacted Lateral Flow Strips	0
99.40% of the seeds in this line exhibit the MON 88913 trait (500/500 seeds with 95% confidence)	

**Prepared Sample Verification**

Once the ground cotton was packaged, 10 samples were identified by the Microsoft Excel 2003 Random Number Generator and sent to Eurofins GeneScan (New Orleans, LA) for qualitative PCR analysis. Table 3 verifies that no contamination was introduced during the packaging phase of AOCS 0906-D [MON 88913 (Line ST 4664 RF)]. These results are in agreement with the homogeneity data presented in Tables 1 and 2.

**Table 3. Qualitative PCR results for the verification of MON 88913 modified cotton (Line ST 4664 RF) as tested by Eurofins GeneScan.**

AOCS 0906-D	MON 88913
107 of 600	+
232 of 600	+
239 of 600	+
251 of 600	+
335 of 600	+
400 of 600	+
471 of 600	+
524 of 600	+
548 of 600	+
549 of 600	+

## References

agbios database: <http://www.agbios.com/dbase.php>

Eurofins GeneScan  
2315 N Causeway Blvd, Suite 200  
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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 34:2000 (E) General requirements for the competence of reference material producers

ISO Guide 35:1989 (E) Certification of reference materials-General and statistical principles

International Seed Testing Association, International Rules of Seed Testing: Seed Science and Technology Rules, Volume 21, Supplement, Rules, 1993

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