Certification Report

(revised 27 March 2009)

The certification of
Conventional
And
MON 88017
Corn Certified Reference Materials

Certified Reference Materials

AOCS 0406-A
AOCS 0406-D

G. Clapper and R. Cantrill
AOCS, Champaign, IL, USA

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Abstract

This report describes the preparation and certification of the corn CRMs AOCS 0406-A and AOCS 0406-D produced by AOCS Technical Services in 2006. These CRMs have been prepared according to ISO Guides 30-35 and are intended to serve as control material for third party testing of corn or grain for transformation events. The purity of the conventional and genetically modified corn was verified using DNA and protein-based detection methods. AOCS 0406-Aand AOCS 0406-D are packaged in 10 g aliquots and are available in 27 -mL glass headspace vials. The conventional (line EXP 258B), was clean seed quality provided by Monsanto Company, St. Louis, MO, USA. The material was prepared by grinding the bulk source of seed according to standard corn processing protocols and was then packaged under a Nitrogen environment. The MON 88017 (Lot GLP-0409-15525-S) corn was provided by Monsanto Company, St. Louis, MO, USA, already ground, from a clean seed source and was then packaged under a Nitrogen environment.

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Glossary

AOCS	American Oil Chemists' Society
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 *Agrobacterium tumefaciens* strain CP4.
Cry1Ab	*Bacillus thuringiensis* subsp. Kurstaki. The genetic modification affords resistance to attack by the European corn borer.
Cry 3Bb1	*Bacillus thuringiensis subsp. Kumamotoensis.* The genetic modification affords resistance to attack by corn root worm.
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 transgenically 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
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 beneficial 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 biotech 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 0406-A and AOCS 0406-D were manufactured from corn seed according to ISO Guides 30-35 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

Materials and Methods

Monsanto Company (St. Louis, MO) delivered 25 kg conventional corn (line EXP 258B) to AOCS. The material was of appropriate seed quality, rather than grain. Twenty-five kilograms of MON 88017 (Lot GLP-0409-15525-S) was delivered to AOCS already ground.

AOCS received the bulk conventional material from Monsanto Company and made arrangements for Texas A&M University to process. The International Seed Testing Association's (ISTA) Seed Science and Technology Rules state a minimum of 5 primary samples (small portion taken from one point in the lot) be taken from batches up to 500 kg. Before the material was shipped to Texas A&M University, primary samples were taken from randomly selected areas and depths in the container to form a 5 kg

composite sample. Five working samples of 100 g each were prepared from the composite sample and sent to GeneScan USA, New Orleans, LA (ISO 17025 Accredited) for qualitative PCR analysis, followed with quantitative PCR if qualitative results for conventional seed indicated that MON 810, MON 863, NK603, or MON 88017 were present. The analyses performed by Genescan were used to assess thepurity and homogeneity of the seed lots. Three hundred grams of MON 88017 seed was provided to AOCS by Monsanto in May 2006 from which 500 seeds were randomly selected for testing with Strategic Diagnostics Inc's Trait √ Triple Trait (CP4 EPSPS, Cry 3Bb, and Cry1Ab) Corn Test Kit to verify seed-lot purity.

The conventional material 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 0406-A: 3, 126, 159, 296, 418, 638, 670, 741, 928, and 997 were sent to GeneScan USA (New Orleans, LA) for qualitative PCR analysis, followed with quantitative PCR if qualitative results indicated that MON 810, MON 863, NK603, or MON 88017 were presence in the sample.

After the non-modified seed packaging was completed, the genetically modified corn line 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 0406-D: 76, 321, 616, 658, 705, 767, 792, 890, 986, 987 were sent to GeneScan USA (New Orleans, LA) for qualitative PCR analysis to screen for MON 810, MON 863, NK603, and MON 88017 presence in the samples.

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 non-modified corn (line EXP 258B) is presented in Table 1. Two samples were negative for indicators of genetic modification; two samples were found to contain quantifiable MON 810 DNA relative to total corn DNA (sample 2 at 0.1 % and sample 5 at 0.05%); and one sample was found to contain < 0.2% quantifiable MON 810 DNA relative to total corn DNA and < 0.05 % NK603 DNA relative to total corn DNA . Results for the genetically modified corn material are presented in Table 2 and 3.

The CRMs were prepared solely as either identity preserved conventional or identity preserved genetically modified corn. Sample heterogeneity was not considered because there was no blending of conventional and genetically modified corn into defined mixtures.

Table 1. Results from GeneScan for the homogeneity of non-modified corn (line EXP 258B).

Sample	MON 810	MON 863	NK603	MON 88017	biotech DNA / total corn DNA
Monsanto Corn-1	-	-	-	-	n/a
Monsanto Corn-2	+	-	-	-	0.1 %
Monsanto Corn-3	-	-	-	-	n/a
Monsanto Corn-4	+	-	+	-	< 0.2% (MON 810) < 0.05 % (NK603)
Monsanto Corn-5	+	-	-	-	0.05%

Table 2. Results from administering Strategic Diagnostics Inc's Trait √ Triple Trait (CP4 EPSPS, Cry 3Bb, and Cry1Ab) Corn Test Kit to 500 MON 88017 corn kernels.

Kernels Tested	Results
Conventional	1
MON 88017	499
99.05% of the seeds in this line exhibit the MON 88017 trait (499/500 seeds with 95% confidence).	

Table 3. Results from GeneScan for the homogeneity of MON 88017 ground corn (Lot GLP 0409-15525-S) qualitative PCR screening.

Sample	MON 88017
Monsanto Corn-16	+
Monsanto Corn-17	+
Monsanto Corn-18	+
Monsanto Corn-19	+
Monsanto Corn-20	+

Prepared Sample Verification

Once the ground corn was packaged, 10 samples of each variety were identified by the Microsoft Excel 2003 Random Number Generator and sent to GeneScan USA (New Orleans, LA) for qualitative PCR analysis. The conventional samples were also subjected to quantitative PCR if the qualitative result was positive for MON 810, MON 863, NK603 or MON 88017.

Table 4 verifies that no contamination was introduced during the packaging phase of AOCS 0406-A. MON 810 is found in the conventional samples (line EXP 258B), though at the low level seen in the homogeneity samples (Table 1). The results for AOCS 0406-D [MON 88017(Lot GLP-0409-15525-S) corn] are presented in Table 5. These results are in agreement with the homogeneity data presented in Tables 2 and3.

Table 4. Results for the verification of non-modified corn (line EXP 258B) as tested by GeneScan USA by Qualitative PCR screening.

AOCS 0406-A	MON 810	MON 863	NK603	MON 88017	biotech DNA / total corn DNA
3	+	-	-	-	< 0.05 %
126	+	-	-	-	0.05 %
159	+	-	-	-	0.05 %
296	+	-	-	-	0.05 %
418	+	-	-	-	< 0.05 %
638	+	-	-	-	0.05 %
670	+	-	-	-	0.05 %
741	+	-	-	-	0.05 %
928	+	-	-	-	0.10 %
997	+	-	-	-	0.05 %

Table 5. Results for the verification of MON 88017 modified corn (Lot GLP-0409-15525-S) as tested by GeneScan USA by Qualitative PCR screening.

AOCS 0406-D	MON 88017
76	+
321	+
616	+
658	+
705	+
767	+
792	+
890	+
986	+
987	+

References

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

GeneScan USA
2315 N Causeway Blvd, Suite 200
Metairie, LA 70001, USA
Telephone: +1 504 297 4330 Fax: +1 504 297 4335
http://www.gmotesting.com

Illinois Crop Improvement Association
3105 Research Road
Champaign, IL 61826, USA
Telephone : +1 217 359 4053 Fax : +1 217 359 4075
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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
http://foodprotein.tamu.edu/

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